BIOCHEMICAL ANALYSIS OF FOSSIL AND LIVING PLANTS.

by

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ABSTRACT.

Lignin oxidation products from a variety of living and fossil plants were obtained using the alkaline cupric oxide method. The aldehydes and ketones obtained were separated by gas chromatography and gas chromatography/mass spectroscopy was used to verify the structures of the lignin oxidation products.

Vanillin remained the major lignin oxidation product obtained from the lignin of gymnosperms. However small amounts of p-hydroxybenzaldehyde and syringic aldehyde were obtained from all the gymnospermous lignins examined. Immature dicotyledons and the woodmeal of the monocotyledons examined gave varying amounts of p-hydroxybenzaldehyde when oxidized. No p-hydroxybenzaldehyde was detected from the lignin of the monocotyledon fibres examined. Syringic aldehyde remained the major lignin oxidation product obtained from the angiosperms.

All three types of lignin nuclei were obtained in small amounts from several lower plants including the mosses and liverworts.

No correlation between the presence of syringic aldehyde and the occurrence of vessels was found. The suggestion that it is the fibres in plants which contribute to the presence of syringic aldehyde is made.

All three types of lignin nuclei were observed from certain fossil wood, fossil compressions and coals. Syringic aldehyde remained as a lignin oxidation product in bituminous coals of ~300 million years old.

Lignin derivatives can be used as supporting evidence for anatomical and morphological studies. However lignin derivatives cannot be used as characteristics for fossil compressions and coals because chemical changes which occur alter the original phenolic aldehyde ratios. Only speculations can be made.

Contents.

ABSTRACT. 2

CHAPTER 1 : GENERAL INTRODUCTION. 11

- General Introduction. 12
- Uses of wood. 15
- Chemical composition of lignin. 16
- Biosynthesis of lignin. 26
- Aims. 33

CHAPTER 2 : MATERIALS AND METHODS. 37

- Materials. 38
- Factors influencing the type of material sampled. 42
- The identification of fossil wood. 44
- Preparation of the spores from the Carboniferous coals. 45
- Oxidation methods. 46
- Oxidation methods used in this investigation. 49
- Principles of gas chromatography. 51
- Chromatographic methods used for lignin oxidation products. 54
- Calibration curves. 60

CHAPTER 3 : LIGNIN CHEMOTAXONOMY IN SPERMATOPHYTES. 73

- Lignin chemotaxonomy in Spermatophytes. 74
- Results. 82
- Results with Gymnosperms. 84
- Results with Angiosperms. 89
- Bark. 93
- Discussion: Spermatophytes. 94
  - Gymnosperms. 94
  - Angiosperms: Monocotyledons. 105
  - Angiosperms: Dicotyledons. 112
  - Bark. 118
- Summary. 120

CHAPTER 4 : LIGNIN CHEMOTAXONOMY IN CRYPTOGAMS. 124

Lignin chemotaxonomy in Cryptogams. 125
Results with Cryptogams. 128
Tree ferns. 128
Sphenopsids and Lycopsids. 130
Bryophytes. 132
Discussion: Cryptogams. 135
Tree ferns. 135
Equisetum and the Lycopods. 140
Mosses and Liverworts. 142

CHAPTER 5 : LIGNIN DERIVATIVES FROM FOSSILS AND COALS. 144

Lignin derivatives from fossils and coals. 145
Results with Quaternary fossil wood. 150
Results with Tertiary fossil wood. 176
Results with Coals. 223
Results with Compression Fossils. 231
Discussion: Lignin derivatives from fossils and coals. 240
Quaternary wood. 240
Tertiary wood. 243
Coal and fossil plants. 247

GENERAL CONCLUSIONS. 263

REFERENCES. 267

APPENDICES. 291

ACKNOWLEDGEMENTS. 300

List of Tables.

Table 1 : Dependence of Lignification on Age.
Table 2 : The major lignin oxidation products from the Cycadales.
Table 3 : The major lignin oxidation products from the sapwood of Gymnosperms.
Table 4 : The major lignin oxidation products from the fibres and wood of Monocotyledons.
Table 5 : The major lignin oxidation products from the sapwood of Dicotyledons.
Table 6 : The lignin oxidation products from Barkmeal.
Table 7 : G.C./M.S. of oxidation products from lignin of *Sequoia semperivirens*.
Table 8 : G.C./M.S. of oxidation products from lignin of *Agave sisalana*.
Table 9 : G.C./M.S. of oxidation products from lignin of a *Bambusa* species.
Table 10: G.C./M.S. of oxidation products from lignin of *Magnolia soulangeana*.
Table 11: The major lignin oxidation products from stems of tree ferns.
Table 12: The major lignin oxidation products from shoots of the Sphenopsids and Lycopsids.
Table 13: The major lignin oxidation products from the Musci and Hepaticae.
Table 14: G.C./M.S. of oxidation products from lignin of *Dennstaedtia bipinnata*.
Table 15: The major lignin oxidation products from the fossil wood of the Quaternary period.
Table 16: The major lignin oxidation products from the fossil wood of the Tertiary period.
Table 17: The lignin oxidation products from a range of coals.
Table 18: Percentage occurrence of the species found in dull and bright coals from the Middle Coal Measures.
Table 19: The lignin oxidation products from the bituminous coal from the Middle Coal Measures of the British Carboniferous.

Table 20: The lignin oxidation products from compression fossils.

Table 21: G.C./M.S. of oxidation products from lignin of Konin Patnów.

Table 22: G.C./M.S. of oxidation products from lignin of Hengistbury.

Table 23: G.C./M.S. of oxidation products from lignin of *Lepidodendron*.

List of Figures.

Figure 1 : Constitutional model of spruce lignin.

Figure 2 : Shikimic acid pathway from glucose to prephenic acid.

Figure 3 : Pathway from the amino acid pools to primary lignin precursors.

Figure 4 : A chromatogram of the authentics (3mg/ml) using a 3% OV 101 on Diatomite CQ 80-100 mesh column.

Figure 5 : A chromatogram of the authentics (3mg/ml) using a 5% Pdegs on Diatomite CLQ 80-100 mesh column.

Figure 6 to 29 : Calibration curves

Figure 30: Infra-red spectra of milled wood lignin.

Figure 31: A typical chromatogram of the lignin oxidation products of a member of the Cycadales.

Figure 32: A typical chromatogram of the lignin oxidation products of one of the Coniferales.

Figure 33: A typical chromatogram of the lignin oxidation products of one of the monocotyledon fibres.

Figure 34: A chromatogram from the wood of a palm.

Figure 35: A chromatogram of an immature dicotyledon.

Figure 36: A chromatogram of a mature dicotyledon.

Figure 37 to 40 Mass spectra of the authentics.

Figure 41 to 43: Mass spectra of the oxidation products from Sequoia semperivirens.

Figure 44 to 47: Mass spectra of the oxidation products from Agave sisalana.

Figure 48 to 51: Mass spectra of the oxidation products from Magnolia soulangeana.

Figure 52: A chromatogram of the lignin oxidation products of a tree fern.

Figure 53: A chromatogram of the lignin oxidation products of one of the Selaginellas.

Figure 54: A chromatogram of the lignin oxidation products of a Lycopodium species.

Figure 55: A chromatogram of the lignin oxidation products of one of the mosses.

Figure 56: A chromatogram of the lignin oxidation products of _Polytrichum commune_ with the added authentics.

Figure 57 to 60: Mass spectra of the oxidation products from _Dennstaedtia bipinnata_.

Figure 61: A chromatogram of the lignin oxidation products of a gymnospermous wood from the Quaternary.

Figure 62: A chromatogram of the lignin oxidation products of an angiospermous wood from the Quaternary.

Figure 63: A chromatogram of the lignin oxidation products of a gymnospermous wood from the Tertiary.

Figure 64: A chromatogram of the lignin oxidation products of an angiospermous wood from the Tertiary.

Figure 65: A chromatogram of the lignin oxidation products of a brown coal.

Figure 66: A chromatogram of the lignin oxidation products of a bituminous coal.

Figure 67: A chromatogram of the lignin oxidation products of the Daw Mill Coal with the added authentics.

Figure 68: A chromatogram of the lignin oxidation products of _Ginkgo huttoni_.

Figure 69: A chromatogram of the lignin oxidation products of _Sigillaria ovata_.

Figure 70: A chromatogram of an angiospermous wood from the Tertiary.

Figure 71 to 72: Mass spectra of the oxidation products from Konin Patnów.

Figure 73 to 75: Mass spectra of the oxidation products from _Lepidodendron_.

List of Plates.

Plate 1 :Sample 1, *Pinus sylvestris*, T.S.
Plate 2 :Sample 1, *Pinus sylvestris*, T.S.
Plate 3 :Sample 1, *Pinus sylvestris*, R.L.S.
Plate 4 :Sample 1, *Pinus sylvestris*, T.L.S.
Plate 5 :Sample 2, *Betula pendula*, T.S.
Plate 6 :Sample 2, *Betula pendula*, R.L.S.
Plate 7 :Sample 3, *Quercus* species, T.S.
Plate 8 :Sample 4, *Ulmus procera*, T.S.
Plate 9 :Sample 4, *Ulmus procera*, T.S.
Plate 10:Sample 5, *Pinus sylvestris*, T.S.
Plate 11:Sample 5, *Pinus sylvestris*, R.L.S.
Plate 12:Sample 5, *Pinus sylvestris*, R.L.S.
Plate 13:Sample 5, *Pinus sylvestris*, R.L.S.
Plate 14:Sample 6, Salixaceae?, T.S.
Plate 15:Sample 6, Salixaceae?, T.S.
Plate 16:Sample 6, Salixaceae?,L.S.
Plate 17:Sample 6, Salixaceae?,L.S.
Plate 18:Sample 7, ~~*Pinus*~~ species, T.S. Reidentified as
Plate 19:Sample 7, ~~*Pinus*~~ species, T.S. Taxodiaceae.
Plate 20:Sample 7, ~~*Pinus*~~ species, T.L.S.
Plate 21:Sample 7, ~~*Pinus*~~ species, R.L.S.
Plate 22:Sample 7, ~~*Pinus*~~ species, R.L.S.
Plate 23:Sample 7, ~~*Pinus*~~ species, R.L.S.
Plate 24:Sample 7, ~~*Pinus*~~ species, R.L.S.
Plate 25:Sample 7, ~~*Pinus*~~ species, R.L.S.
Plate 26:Sample 8, Taxodiaceae, T.S.
Plate 27:Sample 8, Taxodiaceae, T.L.S.
Plate 28:Sample 8, Taxodiaceae, R.L.S.
Plate 29:Sample 8, Taxodiaceae, R.L.S.
Plate 30:Sample 8, Taxodiaceae, R.L.S.
Plate 31:Sample 9, Taxodiaceae, T.S.
Plate 32:Sample 9, Taxodiaceae, T.L.S.
Plate 33:Sample 9, Taxodiaceae, R.L.S.
Plate 34:Sample 9, Taxodiaceae, R.L.S.
Plate 35:Sample 9, Taxodiaceae, R.L.S.
Plate 36:Sample 9, Taxodiaceae, R.L.S.

List of Plates(cont.)

Plate 37:Sample 10, L. endiandroides, T.S.
Plate 38:Sample 10, L. endiandroides, T.L.S.
Plate 39:Sample 10, L. endiandroides, R.L.S.
Plate 40:Sample 11, coniferous wood, T.L.S.
Plate 41:Sample 11, coniferous wood, T.L.S.
Plate 42:Sample 11, coniferous wood, T.L.S.
Plate 43:Sample 11, coniferous wood, R.L.S.
Plate 44:Sample 12, angiospermous wood, T.L.S.
Plate 45:Sample 12, angiospermous wood, T.L.S.
Plate 46:Sample 12, angiospermous wood, T.L.S.
Plate 47:Sample 12, angiospermous wood, T.L.S.
Plate 48:Sample 12, angiospermous wood, R.L.S.
Plate 49:Sample 12, angiospermous wood, R.L.S.
Plate 50:Sample 12, angiospermous wood, R.L.S.
Plate 51:Sample 13, Taxodiaceae?, R.L.S.
Plate 52:Sample 13, Taxodiaceae?, R.L.S.
Plate 53:Sample 13, Taxodiaceae?, T.L.S.
Plate 54:Sample 13, Taxodiaceae?, R.L.S.
Plate 55:Sample 13, Taxodiaceae?, R.L.S.
Plate 56:Sample 14, coniferous wood, T.S.
Plate 57:Sample 14, coniferous wood, T.S.
Plate 58:Sample 14, coniferous wood, T.L.S.
Plate 59:Sample 14, coniferous wood, R.L.S.
Plate 60:Sample 14, coniferous wood, R.L.S.
Plate 61:Sample 14, coniferous wood, R.L.S.
Plate 62:A Lycospora type of miospore.
Plate 63:A Densosporites type of miospore.
Plate 64:Pseudofrenelopsis parceramosa.
Plate 65:Ginkgo huttoni.
Plate 66:Ptilophyllum pectinoides.
Plate 67:Pachypteris lanceolata.
Plate 68:Lepidodendron species.
Plate 69:Lepidophlois species
Plate 70:Sigillaria ovata.

CHAPTER 1.

GENERAL INTRODUCTION.

General Introduction.

Vascular plants possess a specialised conducting system of xylem and phloem tissue. All vascular plants contain a primary xylem and certain living plants notably the gymnosperms and the majority of angiosperms develop secondary xylem which often results in what is termed wood in the mature plant. This wood is anatomically and chemically a heterogenous material. Despite its complexity and diversity all wood is a typically lignified tissue. Lignification is not confined to the cell walls of the xylem tissue in plants but occurs in sclerenchyma cells too.

The primary cell walls of plants are composed of cellulose and polysaccharides. According to Esau, Cheadle and Gill (1966) lignin penetrates the wall from the outside primary wall inwards at a very early stage of secondary thickening of the cell wall and finally encrusts the microfibrils and the matrix polysaccharides of the primary wall. Within those cells which are lignified other complex substances such as tannins, resins, oils and starch can occur.

The phenomenon of lignification is associated with the evolution of plants possessing a conducting system and it is thought to be significant as a factor associated with their adaptation to a terrestrial habit. Its association with other wall constituents and its location in the cell wall and the time of its development during cell wall ontogeny are consistent with such a view. In fact the existence of lignins as an essential cell wall constituent is considered amongst botanists to be unambiguously proven for all vascular plants including the most primitive living species. Plants must have developed the ability to synthesize lignin either concurrently with or prior to the development of conducting tissues. Banks (1975a) regards the presence of xylem tissue as an important anatomical criterion for affirming a vascular plant habit within the evolutionary context. Edwards and Davies (1976) have

reported the first macrofossils with tracheids in situ from the Ludlow series. There is however still some dispute over the first macrofossils. The previous first vascular land plant is the well documented Cooksonia Lang (1937) from the pre-Pridolian, Upper Silurian. However Gray and Boucot (1977) consider that pre-Ludlovian vascular plant evolution must have occurred. They came to this conclusion using Lang's information and their own interpretations of Silurian microfossils. However these interpretations have been disputed by other palaeobotanists (Banks, 1975a; Schopf, 1978 and Edwards and Rogerson 1979).

Structural preservation and critical evidence of the histology of organised cambial activity and secondary growth is known in disappointingly few cases in the middle Devonian. Barghoorn (1964) considers that the middle Devonian genus Schizopodium shows the probable nature of the primitive secondary growth. Radially aligned xylem elements which are peripheral to the lobate primary xylem cone of the stem appears to represent rudimentary cambial activity. By the Upper Devonian extensive secondary growth occurs as in Paracalamites. Extant plants which possess a secondary xylem include a very few members of the Cryptogams such as species of Isoetes, and the fern Botrychium virginianum and the Spermatophytes. Only the seed bearing vascular plants, the Spermatophytes, are used as a commercial source of wood because they gradually form a massive trunk consisting of secondary xylem. The Spermatophytes are divided into the gymnosperms and the angiosperms. The wood obtained from the gymnosperms is termed softwood where as that obtained from angiosperms is termed hardwood. Spermatophytes generally have pycnoxylic wood but the Cycadales have soft loose secondary wood which is called manoxylic wood. Manoxylic wood is unsuitable as a lumber source. Gymnosperms form a smaller part of the present day vegetation, some 650 species in comparison with the angiosperms 150,000 species yet the Coniferales occupy a unique position commercially as a lumber source

and subsequently more chemical knowledge has been obtained from this group. This is due to conifers being monopodial in growth as well as covering wide areas with almost pure stands. Conifer forests develop in temperate zones where the demand for wood is heavy.

Uses of wood.

Since prehistoric times wood has been one of man's basic raw materials with the advantage of being a perpetually renewable crop with the modicum of human care. Wood is widely distributed in nature, undergoes slow biological decomposition, could become very important as a source of fuel as oil and gas reserves become depleted and it is the present source of the world's paper.

The variety of uses for wood reflects the different anatomical properties species have. *Pinus sylvestris* is used for railway sleepers and *Salix* species are used for cricket bats. Both woods are light and can stand the temporary deformation that occurs which is largely due to the amount of air present in those woods.

Most uses require the strength confered by lignin. In paper making however, it is deliberately removed to make a less rugged flexible material. The pulp and paper industry has emerged as an important chemical industry which is continually expanding as the world consumption of paper products increases. The industry is engaged in intensive research on the chemistry of wood and its by-products. Large amounts of lignin waste are discarded and therefore the wood pulping industries are consequently attempting to utilize this material. Lignin is abundant in terms of weight and is probably second only to cellulose among renewable organic materials and therefore research on lignin and its biodegradation has increased dramatically. The pulp industry now supplies cellulose for the world's rayon and increasing amounts of cellulose from the wood as well as some lignin are used for plastics. Other chemically synthesized products derived from wood include simulated leather, adhesives, synthetic rubber and a synthetic flavouring agent called vanillin. One of the reasons why greater utilization of lignin has not yet been achieved is because our knowledge of its chemical structure is still incomplete.

Chemical Composition of Lignin.

Isolation methods.

The incompleteness of lignin chemistry is attributed to the difficulty in separating the lignin from the other components of the cell wall. Northcote (1972) has reviewed the chemistry of the cellulose and polysaccharides of the plant cell wall. Although lignin is in a chemical combination with the plant polysaccharides it belongs to a completely different chemical class. Nevertheless, separation of the lignin in the wood from the other components is difficult and has only been achieved by the application of rather vigorous chemical reagents which generally have the effect of modifying the lignin chemically.

Methods for the isolation of lignin are of two types. The first is designed to remove the cellulose to give a residue of crude lignin. In this class are included sulphuric acid or Klason lignin, (Klason, 1920) and cuprammonium hydroxide lignin, (Freudenberg, 1956). The second approach is designed to extract the lignin and leave the cellulose as a residue. Such methods involve, for example strong acids and alkalis and are dealt with in detail by Schubert (1965) and Pearl (1967).

Attempts to isolate lignin in the unchanged form, example,'native lignin' (Brauns, 1939), enzymatically liberated lignins (Nord and Schubert, 1951) and milled wood lignins (Bjorkman, 1954) have been described. However these methods still all alter the lignin to some degree either chemically or physically.

To avoid contamination of the isolated lignin the extraneous material (waxes, resins, organic acids and pigments) are removed by extraction with suitable solvents such as ether, benzene, ethanol or a mixture of these solvents. The most commonly employed mixture is ethanol and benzene.

Chemical criteria.

Extensive investigations of lignin chemistry were made by Klason in the nineteenth century by studying one of the wood-pulping commercial by-products lignosulphonic acid. Klason (1897) was the first to suggest that the parent structure of lignin might be a phenylpropane derivative of the coniferyl type. This hypothesis is still widely accepted.

(1) coniferyl structure.

Other lignin characterizations rely on its elementary composition, derivatives and degradation properties. Characters include solubility, ultraviolet and infra-red absorption spectra, alkaline hydrolysis as well as those colour reactions which are employed in microscopic studies. One of the principle characteristics of lignin is its methoxyl content (Brauns, 1952).

If lignin is degraded by ethanolysis a mixture of water soluble monomeric phenyl derivatives known as Hibbert's ketones are produced in low yield, (Cooke, McCarthy and Hibbert, 1941). The ketones produced are thought to represent specific structural units of the lignin structure.

Hibbert's ketones.

(2) vanillin

CHO, OH, OCH_3

(3) ethoxypropioguaiacone

CH_3, $CH(OC_2H_5)$, C=O, OCH_3, OH

(4) vanilloyl acetyl

CH_3, C=O, C=O, OCH_3, OH

(5) guaiacyl acetone

CH_3, C=O, CH_2, OCH_3, OH

Freudenberg, Lautch and Engler (1940) found that lignins when subjected to mild oxidation with nitrobenzene in an alkaline medium gave vanillin. This initially provided proof that lignin was aromatic in nature. Numerous alkaline oxidations have since been widely used and this practice has been firmly established and accepted as an analytical approach to lignin composition. The major products from alkaline oxidation reactions are the phenolic aldehydes p-hydroxybenzaldehyde (4-hydroxybenzaldehyde), vanillin (4-hydroxy 3 methoxybenzaldehyde) and syringic aldehyde (4-hydroxy 3,5,dimethoxybenzaldehyde). The mechanisms of this reaction will be dealt with later.

(6) p-hydroxybenzaldehyde

CHO
OH

(7) vanillin

CHO
OCH_3
OH

(8) syringic aldehyde

CHO
CH_3O OCH_3
OH

The significance of the phenolic aldehydes to the complete structure of the lignin is that a linkage capable of yielding C_6-C_1 fragments under these oxidizing conditions must exist. Phenolic aldehydes are now used mainly in an analytical sense to determine the nature and abundance of the lignin under study.

<u>Definition of lignin</u>.

Lignin is a difficult substance to define because lignin preparations which have been isolated by different methods vary extensively in their chemical composition and chemical characterization relies on various criteria.

Lignins are polymeric natural products arising from an enzyme initiated dehydrogenative polymerisation of three primary precursors <u>trans</u>-coniferyl, <u>trans</u>-sinapyl and <u>trans</u>-<u>p</u>-coumaryl alcohols (Sarkanen and Ludwig, 1971).

(9) <u>trans-coniferyl alcohol</u>

(10) <u>trans-sinapyl alcohol</u>

(11) <u>trans-p-coumaryl alcohol</u>

$CHCH_2OH$ / CH / OCH_3 / OH

$CHCH_2OH$ / CH / CH_3O / OCH_3 / OH

$CHCH_2OH$ / CH / OH

Manskaya and Drozdova (1968) further define lignin as a high polymer that is highly variable in structure depending upon the nature of the plant and on its ontological development. It is clear that lignin occurs in the lowest vascular plants having weak woody tissue where it has an especially low methoxyl content and gives a low yield of aromatic aldehydes on alkaline nitrobenzene oxidation. Lignin from woody coniferous plants is derived from units of the phenylpropane series of the coniferyl type, with sinapyl and coumaryl units making, generally, a less important contribution.

It is generally agreed amongst lignin chemists that the term lignin should not be used to define one compound but rather it should be used for similar high molecular amorphous compounds. Much work on lignin has been done by chemists for well over one hundred years. However a

precise structure for lignin is unknown, only deduced structures have been made for the lignin macromolecule.

Combination of phenylpropane units in lignin.

In the lignin macromolecule the monomeric phenylpropane units are joined together three dimensionally by both ethereal linkages and by carbon to carbon bonds. Chemical evidence supporting these linkages has been reviewed by Sarkanen (1963). The carbon-carbon linkages include 5-5'bond (12), α-α' (13) or β-β' (14) link and a β-5' (15) combination.

(12)

(13)

(14)

(15)

Ethereal linkages can unite phenylpropane units at one or more than one point. They include α-alkyl ether (16), β-4'-ethereal linkage (17) and the widely recognised guaiacylglycerol-β-aryl ether structure (18).

(16)

(17)

(18)

Multiple points of attachment involving both ethereal and carbon-carbon linkages are represented in 'benzofuran' (19) and 'pinoresinol' (20) structures.

(19) (20)

Chemical proof for the existence of many of these linkages is still lacking but they have gained support from biochemical and degradation studies.

Suggestions for the structure of lignin using many of the above linkages have been made by various authors including Adler (1961), Brauns (1962), Ludwig, Nist and McCarthy (1964) and Freudenberg (1965). Figure 1 suggested by Freudenberg (1965) shows the types of linkages which could arise by known biosynthetic methods.

Figure 1.

Constitional model of spruce lignin. Freudenberg (1965).

The structural formulae of lignin proposed by Freudenberg is made up of 18 units interlinked in a fashion corresponding to the biochemical growth of the naturally occuring lignin molecule.

Biosynthesis of lignin.

Lignin is ultimately derived from carbohydrates which are formed from carbon dioxide by photosynthesis. Numerous studies have demonstrated that the majority of aromatics in higher plants are generated through the shikimic acid pathway (Conn,1940 and Neish, 1964). Shikimic acid was first recognised as being an important precursor of several aromatic metabolites in mutants of the bacterium *Escherichia coli* (Davis, 1951). Subsequently shikimic acid was found to be widely distributed in higher plants by Hattori, Yoshida and Hasegawa (1954) and it seems to act as a precursor of aromatics in general, in higher plants.

The biogenesis of lignin can be subdivided into three main stages 1) aromatization of the carbohydrates by way of the shikimic acid and prephenic acid pathway, 2) transformation of these C_6-C_3 acids into the alcohols p-coumaryl, coniferyl and sinapyl alcohol and their glucosides which are assumed to be the precursors of lignin and 3) the oxidative condensation of these monomers into the polymer called lignin.

(21)
R=H p-coumaryl alc.
R=$C_6H_{11}O_5$
glucocoumaryl alc.

(22)
R=H coniferyl alc.
R=$C_6H_{11}O_5$
coniferin.

(23)
R=H sinapyl alc.
R=$C_6H_{11}O_5$
syringin.

H_2COH
HC
HC
OR

H_2COH
HC
HC
OCH_3
OR

H_2COH
HC
HC
CH_3O
OCH_3
OR

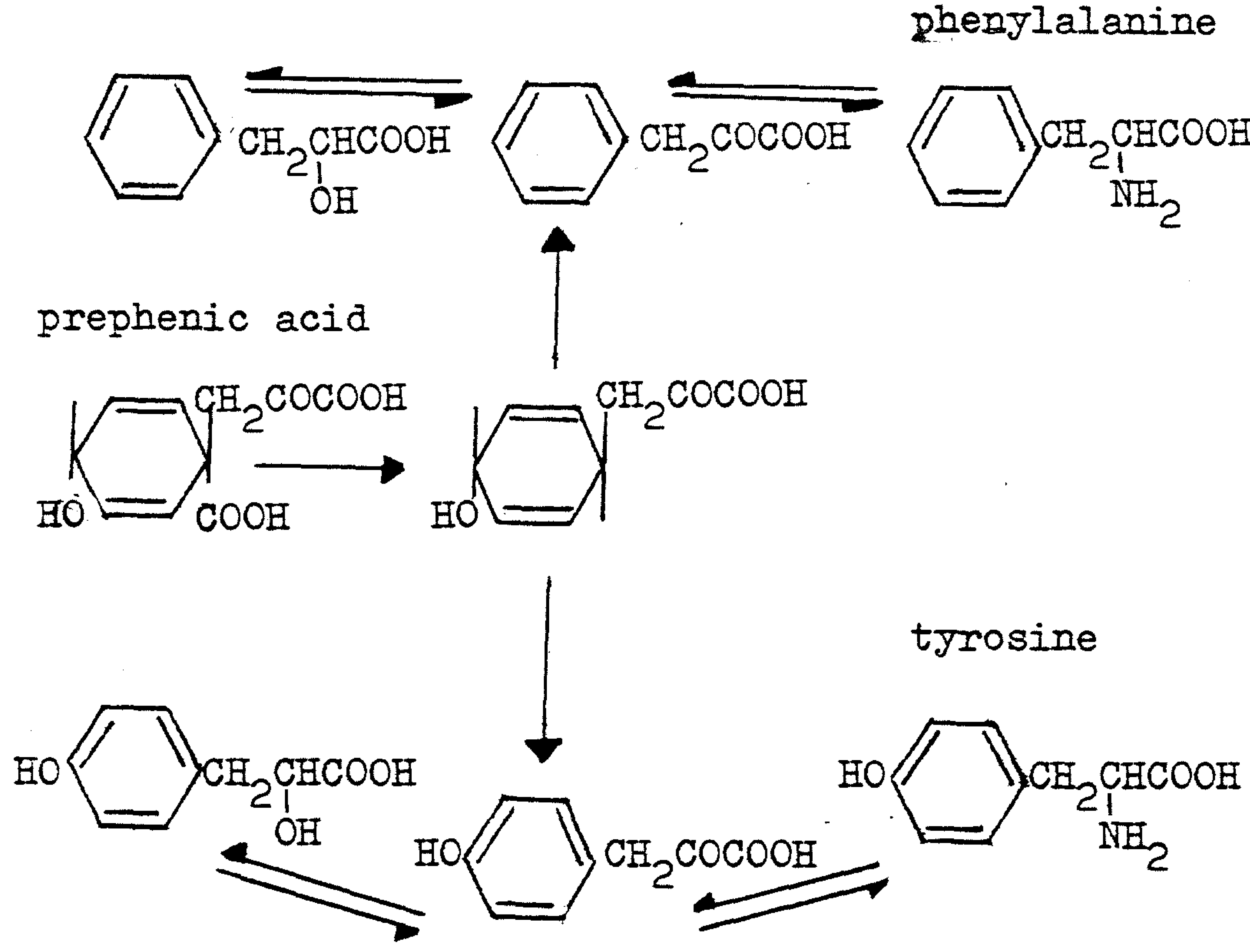
Figure 2.

1) Shikimic acid pathway from glucose to prephenic acid. (Pearl, 1967).

A D-glucose
B 2-phosphoenolpyruvic acid
C D-erythrose 4 phosphate
D 7-phospho-3 deoxy-D arabinoheptulosonic acid (DAHP)
E 5-dehydroquininic acid
F quininic acid - may be utilised as an intermediate in this sequence
G 5-dehydroshikimic acid
H shikimic acid
I 5 phosphoshikimic acid
J 3-O-(α-carboxyvinyl)5-phosphoshikimic acid
K chlorismic acid
L prephenic acid

The first evidence for the operation of the shikimic acid pathway in lignin formation was provided by Brown and Neish (1955). They demonstrated that randomly labelled shikimic acid and phenylalanine were outstandingly efficient precursors for lignin in Triticum aestimum and Acer negundo. In 1956 Ebehart and Schubert fed (2, 6-^{14}C) shikimic acid to sugar cane. The resulting lignin was converted to vanillin by nitrobenzene oxidation and the pattern of the labelling in vanillin corresponded to the labelling in the original shikimic acid.

2) <u>Transformation into cinnamyl alcohols</u>. The conversion of prephenic acid to phenylalanine and tyrosine occurs as follows:-



Brown (1961) found that grasses possessed the ability to use L tyrosine as efficiently as L phenylalanine, but limited ability to use L tyrosine was observed in some other plants. Neish (1961) concluded that the ability to utilise L tyrosine was dependent upon the presence of tyrase (L tyrosine ammonia-lyase) an enzyme capable of converting tyrosine to <u>trans</u>-<u>p</u>-coumaric acid.

$$HO\text{-}C_6H_4\text{-}CH_2CH(NH_2)COOH \longrightarrow HO\text{-}C_6H_4\text{-}CH{=}CHCOOH$$

The reduction of cinnamic acid intermediates to primary lignin precursors is only partially understood. The information which is available conforms with the idea of the reduction of p-coumaric, ferulic and sinapic acids to the corresponding alcohols.

HO-⟨benzene ring⟩-CH=CHCOOH ⟶ (MeO, HO, MeO)-⟨benzene ring⟩-CH=CHCHO ⟶ (MeO, HO, MeO)-⟨benzene ring⟩-$CH=CHCH_2OH$

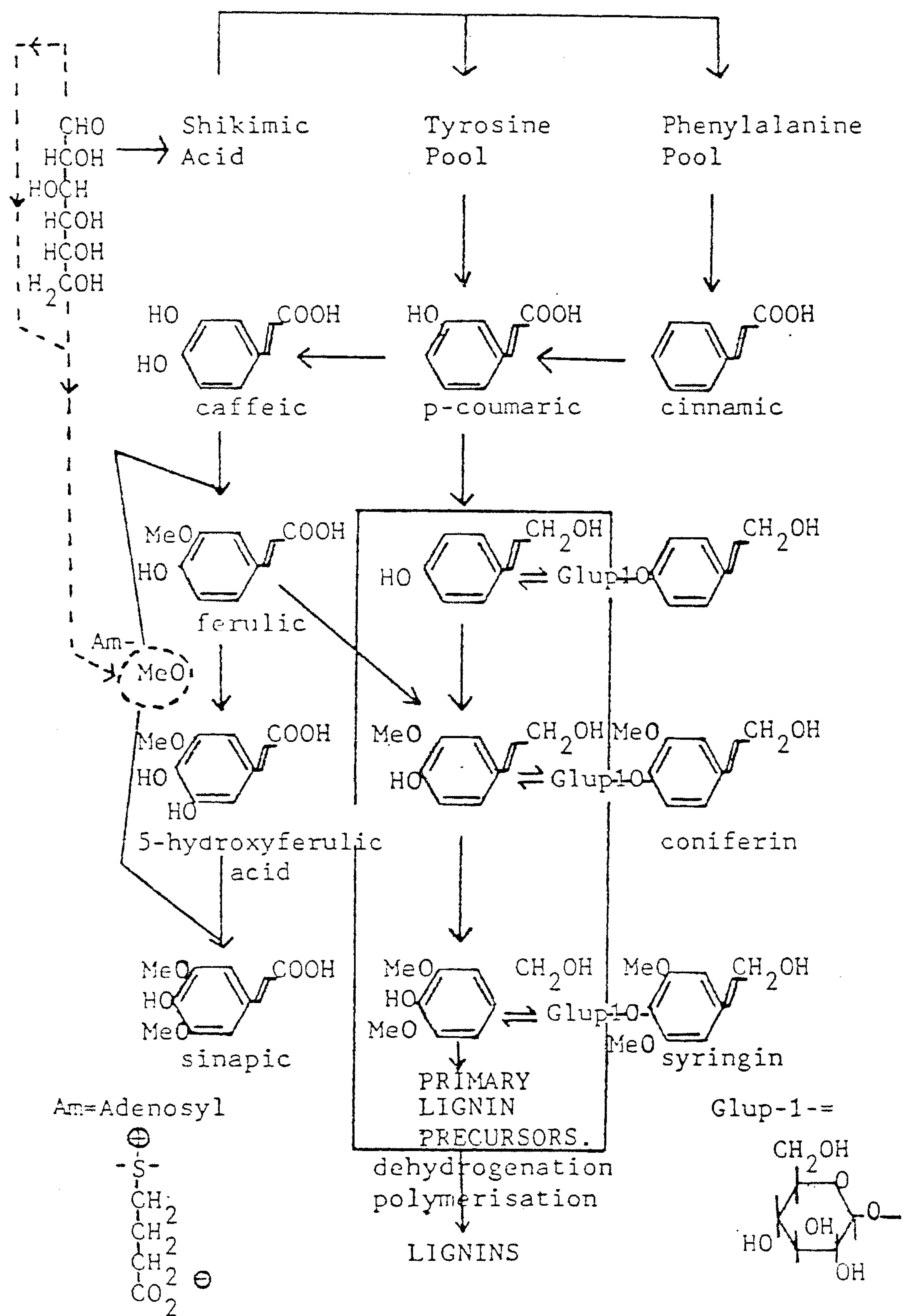
The participation of the phenolic glucosides of the cinnamyl alcohol precursors in the biogenesis of lignin has been suggested by Freudenberg (1965) but the question does not appear to have been resolved. Isherwood (1976) has recently summarised the evidence concerning the role of the cinnamyl alcohols in lignification.

3) Oxidative condensation.

The oxidative condensation or dehydrogenative polymerisation of primary lignin monomers to lignin is a complex process. Freudenberg (1965) found that a lignin-like oxidative polymerizate was produced in vitro by treating coniferyl alcohol under aerobic conditions with a mushroom *Agaricus campestris* extract which was later characterised as a laccase, and with commercial pure horseradish peroxidase. The linkages between monomers to form macromolecules and ultimately a lignin polymer have already been discussed (page 22). Freudenberg (1965) discusses lignin growth mechanisms and possible intermediates in detail. His views, modified by Sarkanen and Ludwig, are summarized in schematic form in figure 3.

Figure 3.

Pathway from the amino acid pools to primary lignin precursors. (Sarkanen and Ludwig, 1971).



Deamination of phenylalanine to trans-cinnamic acid is mediated by phenylalanine deaminase. The conversion of cinnamic acid intermediates to lignin is shown in figure 3. The sequence, which consists of successive hydroxylation and o-methylation steps, is suggested by numerous biosynthetic experiments with labelled precursors, (Brown and Neish 1956, Higuchi and Brown 1963).

Aims.

Phytochemistry has made great contributions to our understanding of relationships amongst living plants as discussed in Chapter 3. Attempts are being made to use a biochemical approach with fossils although such work is at a very early stage of development. The potential of palaeophytochemistry is great including the origin of life and the elucidation of the origin, evolution and diversification of the vascular plants. Fossils often offer little anatomical detail but it may be that the fossil in question will have chemical characteristics which could aid in identification.

Two factors , the preservation and the microorganisms will ultimately affect what biochemical information will be obtained from the fossils. Lignified and suberised tissues found in organs such as stems and seeds are more likely to be preserved than delicate tissues such as mesophyll, cortex and pith found in organs such as leaves and flowers. Lignin and suberin are not only more resistant to mechanical destruction but are also more resistant to attack by microorganisms, only certain fungi and possibly certain strains of bacteria are capable of efficiently decomposing lignin (Iwahara, 1980; Fukuzumi, 1980; Crawford and Sutherland, 1980 and Balkinoja-Salonen and Sundman, 1980). An enclosed pool of water in which fine grained sediments are rapidly accumulating , with a low oxygen content is an ideal environment for good preservation to occur.

Compression fossils are preserved due to the compaction of the sediment and the plant,with some microbial breakdown of the plant tissues usually occurring. Chemically inert compounds such as lignin and suberin are more likely to survive in anaerobic sedimentary environments and are less likely to be subjected to microbial attack than cellular material. Such substances

can therefore be used as a means for comparing a number of fossils. The cellular plant material becomes carbonaceous when subjected to burial, heat and pressure resulting ultimately in the formation of lignite, bituminous coal and anthracite coal.

Petrified fossils have their internal cell structure preserved in the form of remaining cell walls and are therefore invaluable for anatomical studies. The precise chemistry of petrifactions are not completely known but methods of artificially petrifying wood have been developed and are reviewed by Leo and Barghoorn (1976). It is generally agreed that in natural conditions the dissolved mineral material in the water in which the plants were immersed,gradually crystallize out of solution filling the interior of each cell. Minerals such as silica, calcium carbonate, magnesium carbonate and pyrite are the most common. As the remains of the cell walls are often preserved, sometimes even coalified, they have the potential for biochemical investigations.

Impressions and casts do not offer much biochemical detail because usually the plant material has decayed. An impression occurs when the coaly compression is completely destroyed leaving an imprint. A cast is a type of three dimensional impression which may be formed if hollow plant parts are filled with sediments either during burial or when sediments are settling over them. Sometimes casts have a carbon compression attached to them.

During and after fossilization, contamination of the original plant components such as its amino-acids or hydrocarbons can occur from overlying sediments through percolating water. It is important to acknowledge, in the process of decay, the extensive role of microorganisms. Lignin exists as a complex phenylpropane polymer and is therefore more resistant to microbial attack and can remain relatively unchanged. The decomposition of wood

by fungi is of three main types, which have been described as 'brown rots' and 'white rots' by Cartwright and Findlay (1962) and "soft rots" by Savory (1954 a and b). The cellulose and associated carbohydrates are attacked preferentially by the brown rot fungi. Brown rot fungi primarily demethylate and oxidize the lignin as they decompose wood (Kirk, 1971). White rot fungi frequently can be found as the sole organisms decaying wood in nature. They are the most studied lignin degraders and are largely oxidative (Cain, 1980 and Hall, Glasser and Drew, 1980). Rapid bacterial degradation, in comparison to white rot fungi has not been observed yet, but studies with adequate techniques are only beginning. Low molecular weight, lignin-related, aromatic compounds are degraded by bacteria (Cain, 1980; Iwahara, 1980 and Kuwahara, 1980). Plant tissue is nearly always extensively invaded by microorganisms soon after death and any biochemical constituent extracted from the fossil cannot always be regarded as the original attribute of the source plant.

Many workers have used lignin derivatives chemotaxonomically in living plants. They have been used to assess phylogenetic relationships particularly those referring to common ancestral evolutionary sequences and are reviewed in Chapter 3 and Chapter 4. A re-examination of the lignin oxidation products of the Spermatophytes and Cryptogams is presented here in Chapters 3 and 4. Lignin oxidation products from compression and partially petrified fossils are examined in Chapter 5 as characters for consideration as a means of identification and elucidation of phylogenetic relationships. The process of coalification is also discussed in respect to the types of coal possessing lignin oxidation products.

The lignin oxidations using the alkaline cupric oxide method and analytical work using gas-liquid chromatography was carried out at the Chemistry Department of Queen Mary College. Work using the technique of gas chromatography/

mass spectrometry was carried out at the Chemistry Department of Queen Elizabeth College. Scanning electron microscopy and light microscopy studies were carried out at the Biological Sciences Department of Goldsmiths' College.

CHAPTER 2.

MATERIALS AND METHODS.

MATERIALS.

Classification and source of the plant material used in this thesis.

* fossil plant material	Ascension no./Source.
Division BRYOPHYTA	
Conocephalum conicum (L.)Underw.	West Wales.
Plagiochila aspleniodes (L.)Dum.	West Wales.
Polytrichum commune L.	West Wales.
Division LYCOPHYTA	
**Lepidophlois* species	Kilmersdon Colliery, Radstock.Carboniferous.
**Lepidodendron* species	Kilmersdon Colliery, Radstock.Carboniferous.
**Sigillaria ovata* Sauveur.	104 Frickley Colliery, Yorkshire.Carboniferous.
**Sigillaria ovata* Sauveur.	116 Frickley Colliery, Yorkshire.Carboniferous.
Lycopodiella cernua (L.)Pic.Ser.	Ghana.
Lycopodium squarrosum Forst.	314-71-03175 Kew Gardens,
Selaginella species	214-70-01983 Kew Gardens.
Selaginella wildenovii (Desv.)Baker.	000-73-13802 Kew Gardens.
Selaginella myosurus (Sw.)Alston.	Ghana.
Division SPHENOPHYTA	
Equisetum fluviatile L.	Loring Kent.
Division PTERIDOPHYTA	
Cyathea arborrea	175-76-01-327 Kew Gardens.
Cibotium barometz (L.)J.Sm.	Kew Gardens.
Dicksonia squarrosa Swz.	036-64 Kew Gardens.
Dennstaedtia bipinnata (Caj.)Maxon	625-58 Kew Gardens.
Pteris podophylla	303-62-30302 Kew Gardens.

Division CYCADOPHYTA

*Ptilophyllum pectinoides (Phillips)Morris. — Hasty Bank, Yorkshire, Jurassic.

*Pachypteris lanceolata Brongniart. — Hasty Bank, Yorkshire. Jurassic.

Zamia furfuraceae L.fil. — Kew Gardens.

Cycas revoluta Thunb. — Kew Gardens.

Eucephalartos lebomboensis Verdoorn. — Kew Gardens.

Division GINKGOPHYTA

*Ginkgo huttoni (Sternberg)Heer. — Scalby Ness, Yorkshire. Jurassic.

Ginkgo biloba L. — Kew Gardens

Division CONIFEROPHYTA

*Pseudofrenelopsis parceramosa (Fontaine)Watson. — Isle of Wight, Wealden, Cretaceous.

*Chierolepidaceae — Isle of Wight, Wealden, Cretaceous.

*Sample 8(Taxodiaceae) — Vilettwitz, Senftenberg, East Germany. Miocene.

*Sample 9(Taxodiaceae) — East Germany. Miocene.

*Sample 13(Taxodiaceae?) — Lower Headon Bed, Hordle, Hampshire. Eocene.

*Sample 1 Pinus sylvestris L. — Far Black Clough, Southern Pennines. Flandrian.

*Sample 5 Pinus sylvestris L. — Freshwater Bed, West Runton, Norfolk.Cromerian.

*Sample 7 Pinus species. — Bees Nest Pit, Derby. Brassington Formation, Miocene.

*Sample 11 coniferous — Bovey Basin, South Devon. Eocene.

*Sample 14 coniferous — Lower Headon Bed, Hordle, Hampshire. Paleocene.

Araucaria araucana(Mol.)K.Koch. — Bedgebury Pinetum, Kent.

Podocarpus andinus ex.Endl. — Bedgebury Pinetum, Kent.

Juniperis communis L. — Bedgebury Pinetum, Kent.

Division CONIFEROPHYTA(continued).

Sequoia sempervirens(D.Don.)Endl.	Bedgebury Pinetum, Kent.
Sciadopitys verticillata Thunb.	Bedgebury Pinetum, Kent.
Taxodium distichum Rich.	Bedgebury Pinetum, Kent.
Cryptomeria japonica(L.f.)D.Don.	Bedgebury Pinetum, Kent.
Abies alba Mill.	Bedgebury Pinetum, Kent.
Picea abies Karst.	Bedgebury Pinetum, Kent.
Larix decidua Mill.	Bedgebury Pinetum, Kent.
Pinus sylvestris L.	Bedgebury Pinetum, Kent.
Taxus baccata L.	Bedgebury Pinetum, Kent.
Gnetum scandens Roxb.	Kew Gardens.

Division ANTHOPHYTA

Agave sisalana Perrine.	Tropical Products Institute, London.
Phormium tenax J.R.&G Forst.	Tropical Products Institute, London.
Furcraea gigantea Vent.	Tropical Products Institute, London.
Cocos nucifera Mill.	Tropical Products Institute, London.
Sabal palmetto Lodd.	Florida, U.S.A.
Bambusa species	Ghana.
*Laurinoxylon endiandroides Süss.	East Germany. Miocene.
*Sample 6 (Salixaceae?)	Freshwater Bed, West Runton, Norfolk. Cromerian.
*Sample 4 Ulmus species	Broxbourne, Hertfordshire. Radiocarbon dated as 8120 $\pm$ 160 B.P.
*Sample 2 Betula pendula Roth.	Ringinglow Bog Southern Pennines. Flandrian.
*Sample 3 Quercus species	Mildenhall Fen, Cambridge. Radiocarbon dated as 4204 $\pm$ 60 B.P.
*Sample 12 angiospermous	Chara Shell Bed, Lower Headon, Hordle, Hampshire. Eocene.

Division ANTHOPHYTA (continued).

Liriodendron chinese Sargent.	Kew Gardens.
Liriodendron tulipiraflora L.	Kew Gardens.
Magnolia kobus	Kew Gardens.
Magnolia grandiflora L.	Kew Gardens.
Magnolia soulangeana	Kew Gardens.
Tilia x europeae L.	Kew Gardens.
Ulmus procera Salisb.	Kew Gardens.
Betula pendula Roth.	Kew Gardens.
Quercus robur L.	Kew Gardens.

COALS.

Hengistbury coal.	Hengistbury Head, Hampshire.
German coal.	Germany.
Konin Patnów coal.	Poland.
Canakkale coal.	Turkey.
Daw Mill coal.	South Midlands.
Kiverton Park coal.	British, Middle Carboniferous.
Gelding Top coal.	British, Middle Carboniferous.
Peckfield coal.	British, Middle Carboniferous.
Gelding Top coal.	British, Middle Carboniferous.
Flockton Orgreave coal.	British, Middle Carboniferous.
Bettws Beans coal.	Wales.

Factors influencing the type of material sampled.

1. Age of the wood.

As early as 1951 Stone, Blundell and Tanner showed that the maximum lignification occurred at maturity in wheat plants. However El-Basyouni and Towers (1964) found no marked difference in the phenolic acids of wheat during development, although they reached a maximum concentration after nine days germination. Kawamura and Higuchi (1962) showed that the lignin in younger trees contained less than the normal amounts of methoxyl groups.

Table (I) Dependence of Lignification on Age.

From Kawamura and Higuchi (1962).

Lignin contents of samples and methoxyl contents of Klason lignin.

SAMPLE	LIGNIN IN WOOD %		METHOXYL IN WOOD %	
Robina pseudacacia				
1 year old	9.9	10.9	10.7	12.7
2 year old	23.8	17.1	15.7	17.7
mature	24.1	21.1	17.1	18.3
Pinus thunbergii				
1 year old	24.5	24.4	14.7	12.5
2 year old	25.4	27.3	14.9	13.4
mature	28.3	27.6	15.0	14.3
Acacia mollisima				
1 year old	22.6		15.2	
2 year old	24.0		15.6	
mature	25.4		15.9	

More recently Renate (1971) has investigated the polysaccharides and lignin in Taxodium distichum branch-wood at different ages and found more lignin in older wood.

2. Reaction wood.

Reaction wood of softwood is known as compression wood while that of hardwood is known as tension wood. Reaction wood shows eccentric growth largely due to stress. In compression wood the tracheids are characteristically rounded and wide growth rings are composed mainly of late wood tracheids so that the contrast between the early and late wood is reduced. Tension wood is more compact than normal wood with fewer and smaller vessels but more fibres. Not surprisingly therefore the lignin concentration, compared to that in normal wood, is higher in compression wood and lower in tension wood. Hagglund and Ljundren (1933) first established low methoxyl contents of compression wood in *Pinus excelsa*. This was subsequently confirmed by Bland (1958), who also found that a large proportion of the aldehyde obtained in the nitrobenzene oxidation consisted of p-hydroxybenzaldehyde.

3. Sapwood and Heartwood.

Sarkanen, Chang and Allan (1967) recommended that lignin should be isolated from the sapwood because heartwood lignin contains large amounts of polyphenolic impurities which may be linked chemically with the lignin.

In the light of these considerations, in the present work only mature plant material was used unless otherwise stated. Diseased material, reaction wood and heartwood were scrupulously avoided.

The Identification of Fossil Wood.

Transverse, tangential longitudinal and radial longitudinal sections of fossil wood were hand sectioned and examined under the light microscope. Certain pieces of wood were bleached using hydrogen peroxide because it was often very darkly stained. Hydrogen peroxide was also used to soften and swell some of the compressed wood enabling hand sections to be made. All sections were mounted in glycerine jelly.

Macerations of the fossil wood were made using Schultze's method. That is small slivers of wood were placed in a test-tube with concentrated nitric acid, (approximately 5ml.) and 1gm. of potassium chlorate and gently warmed for 1-2 hours. The oxidation process was stopped after this time by adding cold water to the mixture and the ragged wood fragments were removed. The wood fragments were then washed in distilled water, taken through an alcohol series and mounted in glycerine jelly.

The sections and macerations obtained were examined using the Zeiss photomicroscope and photographs were taken using an Ilford FP4 film.

An ISI Super III A scanning electron microscope was also used for examining the fossil wood. 1cm^3 blocks of wood, hand sectioned using a new blade for each side, were made and mounted on stubs and coated with gold. Wood that was brittle and coalified was fragmented, using razor blades and placed on stubs for the scanning electron microscope and coated with gold. Photographs were taken using an Ilford FP4 film.

Preparation of the spores from the Carboniferous coals.

The method of Smith (1962) was used for the isolation of spores from the Carboniferous coals. Approximately 1gm. of coal was ground to a fine powder using a pestle and mortar. Spores were extracted from the powdered coal by treatment with fuming nitric acid. A maceration period of eighteen hours was employed for all the coals. The spores were washed with quantities of fuming nitric acid, concentrated nitric acid, dilute nitric acid and finally distilled water. A portion of the miospores from each residue was prepared for microscope examination by mounting in glycerine jelly on a microscope slide. A count of 1,000 miospores per coal sample was made, which was regarded as sufficient for purposes to establish the general character of the assemblage.

Oxidation Methods.

The lignin macromolecule is aromatic in nature and can be oxidized to fragments which retain the aromatic (benzenoid) nucleus. Such oxidations result in aromatic carbonyl type compounds which are important in the characterization of lignin and in the commercial production of aromatic compounds within the paper and pulp industries. Oxidizing agents include nitrobenzene, molecular oxygen and metal oxides all in an alkaline medium, as well as permanganate after methylation of the lignin (Richtzenhain, 1950) and using permanganate directly (Read and Purves, 1952). Lignin oxidation methods have been reviewed in detail by Schubert (1965), Pearl (1967) and Chang and Allan (1971). The alkaline oxidation by nitrobenzene in the presence of a metal oxide (cupric oxide) was selected for use in the present work.

The alkaline nitrobenzene oxidation of lignin was first reported by Freudenberg, Lautch and Engler (1940) and has now become one of the principle methods for lignin investigations. The oxidation of the lignin by alkaline nitrobenzene is a two electron transfer process, resulting in the production of phenolic aldehydes (6,7,8) together with some minor products. The isolation and abundance of these oxidation products was first used to provide proof of the aromatic nature of the lignin. They are now used increasingly in an analytical way to determine the nature and amount of the lignin being studied. Stone and Blundell (1951) developed a micromethod for the quantitave analysis of the phenolic aldehydes obtained fron the alkaline nitrobenzene oxidation of lignin using paper chromatography. This method has subsequently been modified by Pepper and Siddiqueullah (1961). Stone and Blundell's micromethod has been applied to the study of lignin in a botanical context where it has been adapted for taxonomical purposes, in coalification processes and more recently in geochemical studies (Leo and Barghoorn, 1970 and Hedges and Parker, 1976).

Alkaline suspensions of some metal oxides also oxidatively degrade lignin. Such oxides include cupric, mercuric, silver and cobalt oxides. Aromatic aldehydes or aromatic

carboxylic acids or mixtures of both are the major products, with their ratio depending on the particular oxidant used. Cupric oxide is a weak oxidant resulting in aldehydes with lignin while silver oxide gives acids as the major oxidants because it has a higher oxidizing potential. Unlike the alkaline nitrobenzene oxidation the metallic oxides employed are known to be one electron transfer oxidants.

The use of cupric oxide as an oxidant for lignin materials was first reported by Pearl (1942) while investigating oxidations of lignosulphonates. Pearl and Beyer (1950) and Pepper, Casselman and Karapally (1967) found that the yields of oxidation products were comparable with those obtained when alkaline nitrobenzene was used and the products obtained from both methods of oxidation were similar. In the case of the oxidation of softwood lignins, acetovanillone was produced at the expense of vanillin. Acetosyringone was produced in significant amounts in hardwood lignin as a result of the cupric oxide oxidation but was not present when alkaline nitrobenzene was used, implying that cupric oxide is a milder and more selective oxidant than is nitrobenzene.

(24) acetovanillone

CH_3
C=O
OH OCH_3

(25) acetosyringone

CH_3
C=O
CH_3O OH OCH_3

Three other new products namely (26) 4,4' -dihydroxy -3,3'-dimethoxychalcone, (27) 4,4'-3,3'-dimethoxybenzil (vanillil) and (28) 4,4'-dihydroxy-3,3'-dimethoxybenzophenone were identified from this oxidation by Pearl

and Beyer (1950), Pearl and Dickey (1952) and Pearl and Beyer (1954) using lignosulphonate material.

(26) <u>4,4'-dihydroxy-3,3'-dimethoxychalcone</u>

(27) <u>4,4'-dihydroxy-3,3'-dimethoxybenzil</u>

(28) <u>4,4'-dihydroxy-3,3'-dimethoxybenzophenone</u>

The isolation of 4,4'-dihydroxy-3,3'-dimethoxychalcone and 4,4'-dihydroxy-3,3'-dimethoxybenzil led Pearl and Dickey (1952) to propose 'dimeric-type' structures in the lignin macromolecule mentioned previously. In their investigations on cupric oxide oxidations of fermented spent liquor of spruce origin they found that 4,4'-dihydroxy-3,3'-dimethoxychalcone was not a primary lignin derivative but an artifact produced by the interaction of vanillin and acetovanillone.

Pepper, Casselman and Karapally (1967) concluded that the isolation and identification of the products is simpler when cupric oxide rather than nitrobenzene is used as an oxidant.

Oxidation method used in this investigation.

The method used throughout is essentially that of Pepper, Casselman and Karapally (1967). This cupric oxide method, unlike the nitrobenzene method does not produce by-products which mask the lignin oxidation products.

The plant material was milled (2mm. mesh) in a wood grinder. The milled woodmeal was then extracted in a Soxhlet with a mixture of 300ml. of toluene and ethanol (1:1) for 48 hours to remove soluble organic constituents such as waxes, resins, organic acids and pigments. This extracted woodmeal was then air dried and used as the source of lignin.

All oxidations were performed in a small stainless pressure-tested reaction vessel of 16ml. capacity which was inserted into an electrically heated metal block. 1.0gm. of woodmeal and 1.2gm. of cupric oxide were added to the stainless steel vessel together with 10ml. of 2N sodium hydroxide. The vessel was sealed and the reaction mixture was heated up to 180°C ∓ 5° which took approximately 1 hour. The stainless steel vessel was kept at this temperature for 2 hours, removed from the heat and left to cool at room temperature. The contents of the vessel were filtered using a Buchner funnel and the residue was washed with 20ml. of 2N sodium hydroxide. The combined filtrate and washings were acidified to pH3 with 2N sulphuric acid. The acidified mixture was continuously extracted with 125ml. diethyl ether overnight for 18 hours using a small continuous ether extraction apparatus. The diethyl ether extract was then washed with distilled water in a separating funnel until the wash water was neutral. The diethyl ether extract was then dried with 1gm. of anhydrous magnesium sulphate and filtered into a clean flask. The separating funnel and flask containing the magnesium sulphate residue was rinsed with 10ml. diethyl ether, and added to the filtrate. The filtrate was evaporated at the rotary evaporator to remove the ether leaving a yellowish-brown oily residue. The residue was dissolved in either

diethyl ether or methanol and the final volume was adjusted to either 1ml. or 3ml. using volumetric flasks. In the case of some fossil and coal samples which were found to contain only trace amounts of aromatic aldehydes the final volume was adjusted to 0.25ml. with a syringe. To prevent the oxidation of the aldehyde to the corresponding acid the samples were kept under nitrogen and stored in the freezer, until they were chromatographed.

The coalified material from compression fossils was removed by chipping the material from the matrix with a small chisel. Rubber gloves were used and all vessels and tools were cleaned with chromic acid prior to use. The fossil and coal samples were ground to a fine powder using a pestle and mortar and stored in glass bottles. Both coal and fossil compression samples were subsequently treated in the same way as wood and other plant material.

Injection size.

The injection size of a sample to be chromatographed was adjusted in regard to the amount of lignin that was present in the original sample. Thus, smaller injection sizes were used for the Spermatophytes whereas larger injection sizes were used for the fossil and coal samples.

Principles of gas chromatography.

Grob (1977) defines chromatography as "a series of techniques having in common, the separation of components of a mixture by a series of equilibration operations which result in the entities being separated as a result of their partitioning (differential sorption), between two different phases; one stationary with a large surface and the other a moving phase in contact with the first. Chromatography is not restricted to analytical separations. It may be used in the preparation of pure substances, the study of the kinetics of reactions, structural investigations on the molecular scale and the determination of physicochemical constants."

In gas chromatography (g.c.) the mixture to be analysed must be stable and must interact with the column material (either a solid adsorbent in gas-solid chromatography (g.s.c.) or a liquid stationary phase in gas-liquid chromatography (g.l.c.)). The mixture to be analysed is carried in a gas flow (the moving phase) normally nitrogen or helium through a column containing the stationary phase. The result of this interaction is the differing distribution of the sample components between the two phases, resulting in the separation of the sample components. This separation is termed 'elution development' and the principle is as follows: Consider the sample to be made up of components A and B. The two components travel through the column at a rate determined by their retention on the stationary phase. If the differences in adsorption are sufficient a complete separation of A and B is possible.

The separating ability of a column and the speed with which the components are separated depends on many things, the type of packing material used, the stationary phase (organic liquid coated onto the support), how well the column is packed, the flow rate of the carrier gas, the temperature of the column and the length of the column. A long time interval may be required to remove a highly adsorbed component, but this can be overcome by increasing

the column temperature during the separation process. Increasing the column temperature results in the vapour pressure of the components being raised which therefore accelerates the adsorption, desorption process in the column. Column size is important: better separation occurs with a longer column because there are more theoretical plates.

In the present work a flame ionization detector (F.I.D.) was employed. The F.I.D. has high sensitivity and is sensitive to organic substances but is insensitive to inorganic gases and water. When sample molecules enter the flame, ionization occurs yielding a current flow which, after proper amplification, is displayed on a chart recorder. A chromatographic peak provides information on the elapsed time from the injection point or the difference in elution times of two or more peaks (qualitative information) and the peak size (quantitative information).

Qualitative analysis by g.c. involves the comparison of retention data of an unknown sample with that of a known sample. However it must be emphasised that because the retention time of an unknown and a known component are the same, the two components are not necessarily identical. In many fields of analysis, the mixtures frequently encountered contain a number of similar components that will possess the same, or nearly the same retention time. G.c. is not generally used alone for qualitative analysis because it cannot differentiate or identify indisputably the structure of the molecule. It is therefore better used with other techniques to determine what components are present and it is usually combined with mass spectrometry for this purpose.

Once the retention time of the peaks is known and compared with those of known standards the various components can be tentatively identified. The quantity of the components can also be calculated from the peak size by comparison with those of the standards. The size of a chromatographic peak is proportional to the quantity of the constituent causing the peak and may be considered

in two ways: peak height and peak area. Peak height is much more dependent on slight changes in column temperature and carrier gas flow rate than peak area, and because it cannot be used very successfully if peaks are broad, peak area is preferable. Peak area is commonly measured by using one of four methods: multiplying the peak height by the peak width at half height, that is triangulation; cutting out the peak and weighing, using a planimeter; or by using electronic integrators. Reproducibility of peak size for each sample is essential and depends not only on the operating conditions remaining constant but also on the injection technique of the operator. With practice replicate injections should yield identical peaks.

Chromatographic methods used for lignin oxidation products.

1. Previous methods used.

Initially lignin oxidation products were identified and quantitatively estimated using paper chromatography by Stone and Blundell (1951). The method was later modified by Pepper and Siddiquellah (1961). Subsequently Bland (1966) used thin layer chromatography to separate and to determine p-hydroxybenzaldehyde, vanillin and syringic aldehyde. In 1962 Pepper, Manolopoula and Burton effected the separation of p-hydroxybenzaldehyde, vanillin, acetovanillone and syringic aldehyde by g.l.c. using a 15% Apiezon N on Fluropak 80 column run at a temperature of 220°C.

2. Methods used in the present work.

p-Hydroxybenzaldehyde, vanillin, acetovanillone and syringic aldehyde (not less than 98% pure) were the authentics used throughout. p-Hydroxybenzaldehyde and acetovanillone were recrystallized before use.

Initially both paper chromatography (petroleum ether 100-120°: n-butyl ether: water=6:1:1, Stone and Blundell, 1951) and thin layer chromatography (benzene:acetic acid=9:1) were evaluated for the separation of the lignin oxidation products. However paper chromatography and thin layer chromatography were found to be unsuitable systems when dealing with such amounts as were expected in plant and fossil material in the present studies. G.l.c. was therefore evaluated. Several g.l.c. columns were tried because the separation of lignin oxidation products was found to be difficult.

Gas Liquid Chromatography Systems Used.

15% Apiezon N on Fluropak 80 column.

Apparatus.

Analyses were performed on a Perkin Elmer F11 equipped with a flame ionization detector using a 6'x $\frac{1}{8}$ " o.d. stainless steel column packed with 15% Apiezon N on Fluropak 80. The column was conditioned overnight at 280°C while nitrogen was passed through at a flow rate of 10ml./min.

Operating conditions.

The column was run isothermically at a temperature of 210°C with the flow rate of nitrogen at 40ml./min.

However the best peaks obtained using this column were not as well defined as those reported by Pepper, Manolopoula and Burton (1962) using the Apiezon column. This is because they used a larger column (6'x ¼" i.d. copper tubing) compared to the one used in these studies (6'x ⅛" o.d.). Therefore other columns with a range of differing stationary phases were tried.

3% OV 101 on Diatomite CQ 80-100 mesh. Column 1.

Apparatus.

Analyses were performed on a Hewlett Packard Model 5830A equipped with a flame ionization detector using a 2m. x ⅛" o.d. stainless steel column packed with 3% OV 101 (dimethylsilicone fluid) on Diatomite CQ 80-100 mesh. The column was conditioned overnight at 300°C, while nitrogen was passed through at a flow rate of 10ml./min.

Operating conditions.

Temperature of the injection block 180°C, temperature of the flame ionization detector 215°C, initial column temperature 140°C for 13 mins., final column temperature 200°C, temperature programming rate 10°C/min., and the flow rate of the nitrogen 9ml./min.

Electronic integration.

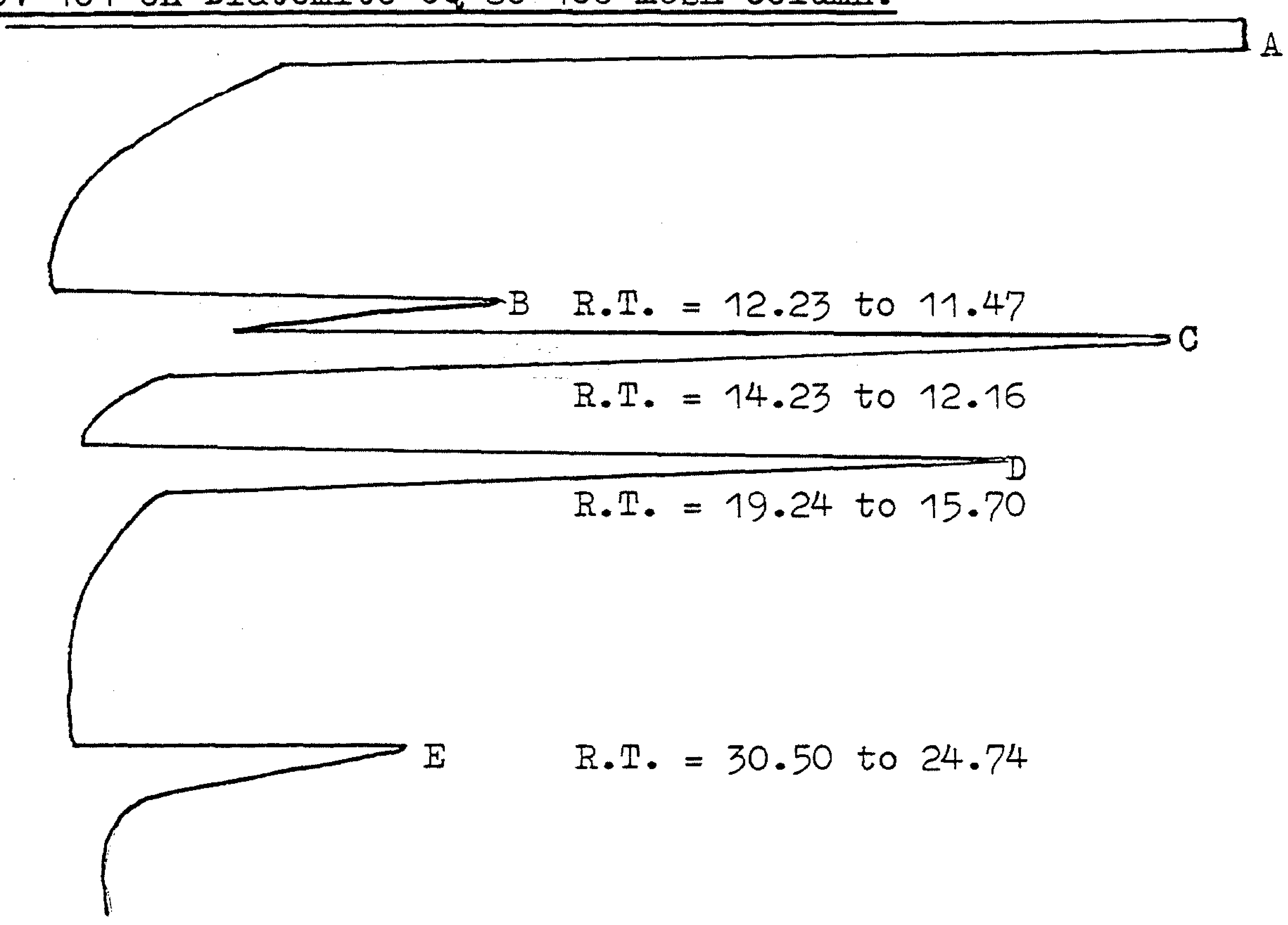
A Hewlett Packard 18850 A.G.C. terminal was used. Integrator parameters were attenuation 8, chart speed 0.3 cm./min. and slope sensitivity 0.3.

This column under the above operating conditions gave the separation shown in figure (4) which gave the sharpest peaks obtained with the columns that were available.

After some time this column began to bleed (that is loss of the liquid phase that coats the support occurred) and so it was replaced by a second 3% OV 101 column. Attempts were made to prevent further bleeding using silylating agents but they were unsuccessful.

Figure (4).

A chromatogram of the authentics (3mg./ml.) using a 3% OV 101 on Diatomite CQ 80-100 mesh column.



A solvent peak, ether.
B p-hydroxybenzaldehyde.
C vanillin.
D acetovanillone.
E syringic aldehyde.
R.T. retention time.

3% OV 101 on Diatomite CQ 80-100 mesh. Column 2.

All apparatus and conditions used for the first 3% OV 101 column were the same for the second column except for the following; the initial column temperature 140°C, final column temperature 200°C, temperature programming rate 1°C/min. and the flow rate of nitrogen was 10ml./min.

The retention times are as follows; p-hydroxybenzaldehyde R.T. = 10.96 to 12.23, vanillin R.T. = 12.16 to 14.23, acetovanillone R.T. = 16.83 to 18.23 and syringic aldehyde R.T. = 27.31 to 29.19. To compare retention times on two different columns of the same type is difficult. Differences in packing density, liquid loading activity of the support, age and variations in the composition of the column wall

can lead to large differences in retention measurements between the two columns.

After prolonged use of this column the p-hydroxybenzaldehyde showed an adsorption problem as shown in the calibration curve, figure (14), the calibration curve not extrapolating through the origin. It is possible to work with a calibration curve that does not pass through the origin, but this also requires that the calibrations be renewed quite frequently. Due to the absorption problems of p-hydroxybenzaldehyde and the fact that it was not completely separated from the vanillin it was felt that a better separation of the lignin breakdown products could be achieved using a different column to that of the 3% OV 101 on Diatomite CQ 80-100 mesh.

The following references; (Paluch and Stangret, 1972; Korolczuk, Daniewski and Mielnicuzuk, 1974 and Brauns and Hiecke, 1976) indicate that either derivatives should be made or a more polar stationary phase used. It was thought that the preparation of derivatives of trace amounts from fossil and coal samples would be extremely difficult and so a more polar stationary phase that of 5% Poly-(Diethylene Glycol Succinate) (Pdegs) on Diatomite CLQ 80-100 mesh was used.

<u>5% Poly-(Diethylene Glycol Succinate) on Diatomite CLQ 80-100 mesh. Column 1.</u>

<u>Apparatus</u>.

Analyses were performed on a Hewlett Packard Model 5830A equipped with a flame ionization detector using a 1.8m.x 6mm. o.d. x 3mm. i.d. glass column packed with 5% Pdegs on Diatomite CLQ 80-100 mesh. The column was conditioned overnight at 200°C, while nitrogen was passed through at a flow rate of 10ml./min.

<u>Operating conditions</u>.

The column was run isothermically at a temperature of 190°C, temperature of the injection block 250°C, temperature of the flame ionization detector 390°C and the flow rate of the nitrogen was 28ml./min.

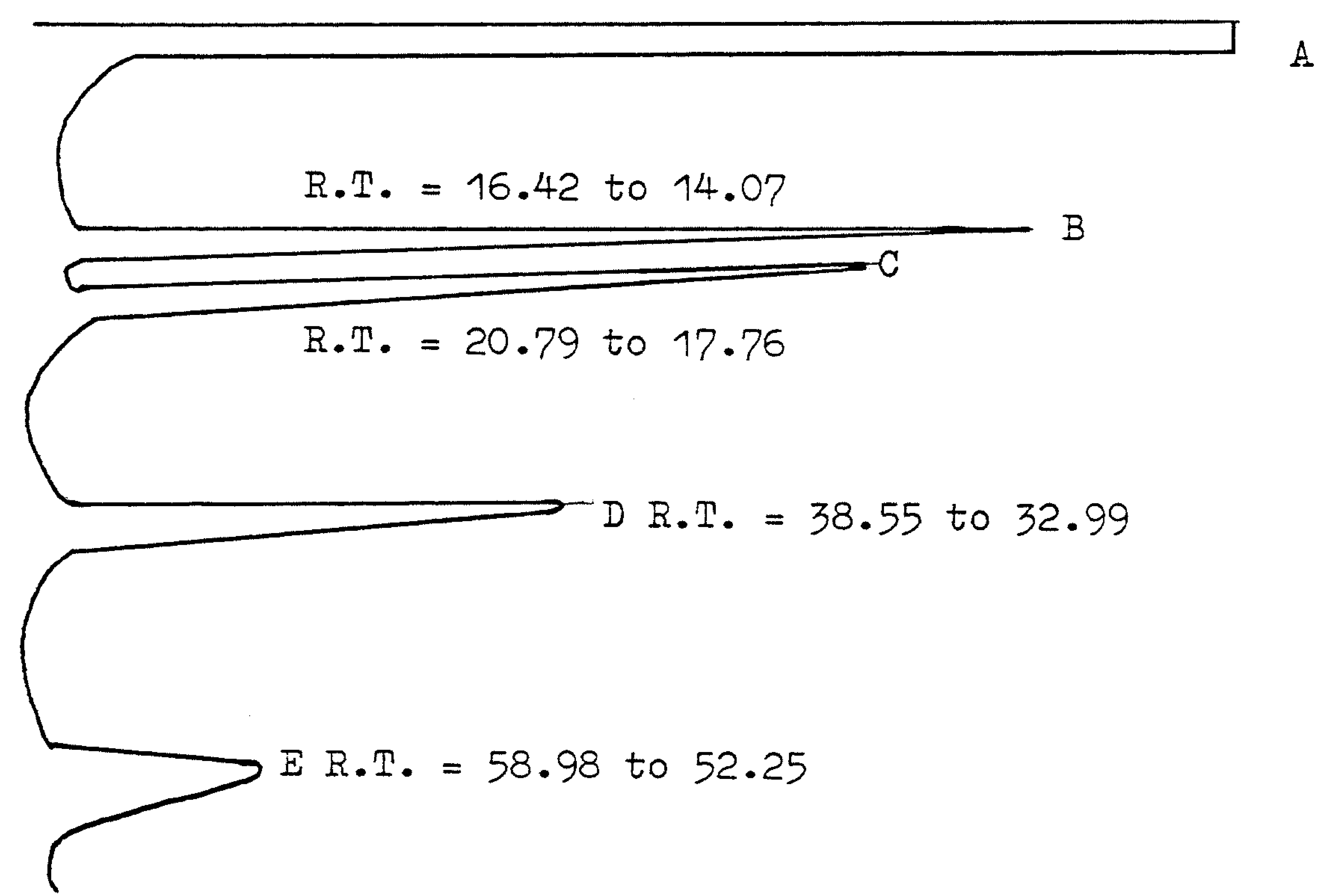
Electronic integration.

A Hewlett Packard 18850 A.G.C. terminal was used. Integrator parameters are as follows: attenuation 8-4, chart speed 0.14cm./min. and slope sensitivity 0.1.

The separation was obtained using this column under the above conditions as shown in figure (5).

Figure (5)

A chromatogram of the authentics (3mg./ml.) using a 5% Poly-(Diethylene Glycol Succinate) on Diatomite CLQ 80-100 mesh column.



A solvent peak, methanol.
B vanillin.
C acetovanillone.
D p-hydroxybenzaldehyde.
E syringic aldehyde.

It should be especially noted here that when using the 5% Pdegs column as compared to the 3% OV 101 column the p-hydroxybenzaldehyde has changed position from being the first compound to be eluted in the 3% OV 101 column to being the third compound eluted in the Pdegs column.

Also when using the Pdegs column complete separation is obtained of the major lignin oxidation products.

5% Poly-(Diethylene Glycol Succinate) on Diatomite CLQ 80-100 mesh. Column 2.

All apparatus and conditions used for the first 5% Pdegs column were the same for the second column.

The retension times are as follows: vanillin R.T. = 23.32 to 19.56, acetovanillone R.T. = 29.39 to 25.38, p-hydroxybenzaldehyde R.T. = 55.61 to 46.77 and syringic aldehyde R.T. = 84.98 to 71.74.

5% Poly-(Diethylene Glycol Succinate) on Diatomite CLQ 80-100 mesh. Column 3.

All apparatus and conditions used for the first Pdegs column were the same for the third column.

The retension times are as follows: vanillin R.T. = 21.60 to 17.89, acetovanillone R.T. = 23.77 to 21.45, p-hydroxybenzaldehyde R.T. = 44.68 to 40.56 and syringic aldehyde R.T = 67.45 to 63.29.

Calibration Curves.

Stock solutions of the authentics in diethyl ether were made up in volumetric flasks when using the OV 101 columns and in methanol when using the Pdegs columns. Calibration curves were made for each column. A dilution series from the stock solution was made in each case and 1ml. samples were injected onto the column. This sample size was used for all the calibrations made. Replicate injections were made. The standard solutions were run under the same conditions, for each individual column, as were used for plant and fossil samples. Conditions for each column have already been stated. The size of a chromatographic peak is proportional to the amount of material contributing to that peak and the size of this peak was measured. The peak unit area against mg/ml. of the authentics were plotted to give the following calibration curves, figures (6 - 29).

External standards were run chromatographically under identical conditions as the sample. In effect, one can recalculate the slope of the calibration curve with the new standard which acts as a correction for instrument variation. When necessary calibrations must be recalculated. Replicate injections of the actual plant and fossil samples were made as well as replicating the actual alkaline oxidation with cupric oxide of the plant and fossil material. The quantities of p-hydroxybenzaldehyde, vanillin, acetovanillone and syringic aldehyde were then calculated from the corresponding calibration curves.

Qualitative Analyses.

The tentative identification of the lignin oxidation products has been based upon a comparison of the retention data with those obtained from the authentic samples. However as has already been discussed gas chromatography does not identify indisputably the structure of the molecule and so other techniques have also been used.

Mixed chromatography was found to be particularly useful when only trace amounts were present in fossil material as will be shown in Chapter 5.

External standards, see Appendix 1.

Lignin oxidation products were more fully identified as will be shown later by using gas chromatography/mass spectrometry (g.c./m.s.). The instrument used for g.c./m.s. was an A.E.I. M.S. 30 double focusing mass spectrometer integrated with a Pye-Unicam model 104 gas chromatography and equipped with an A.E. I.D.S. 50 data processing system. A 5'x ¼" o.d. 3% OV 101 on Gas Chrom Q column was used under the same conditions as those given for the 3% OV 101 column 1. A 5% Poly-(Ethylene Glycol Adipate) on Diatomite CLQ 80-100 mesh was used under the same conditions as those given for the 5% Pdegs column 1. This work was carried out on the U.L.I.R.S. equip ment at Queen Elizabeth College.

Figures 6 to 29. Calibration curves using the authentics obtained from the columns used in this work.

Fig.6

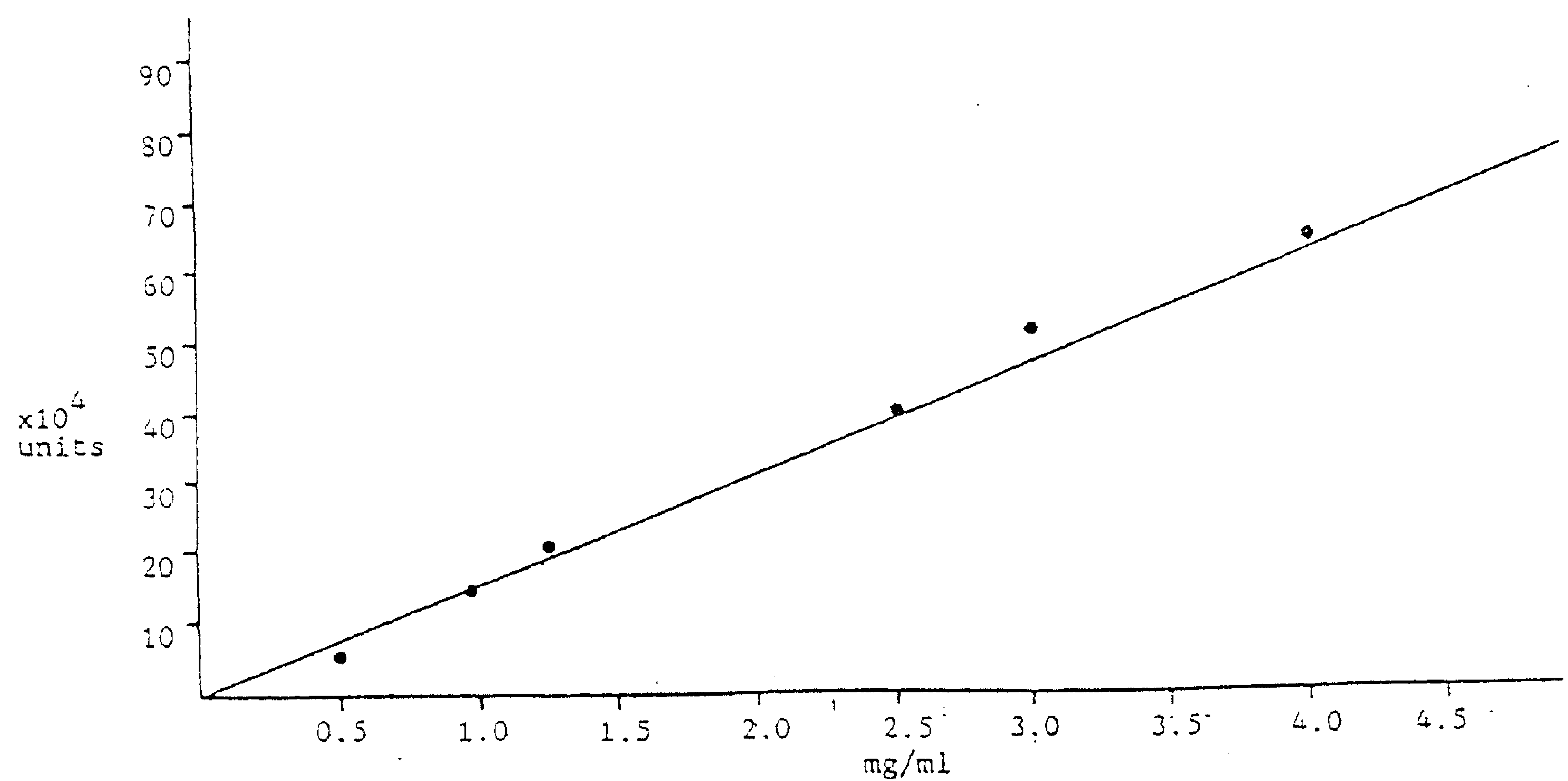


Fig.7

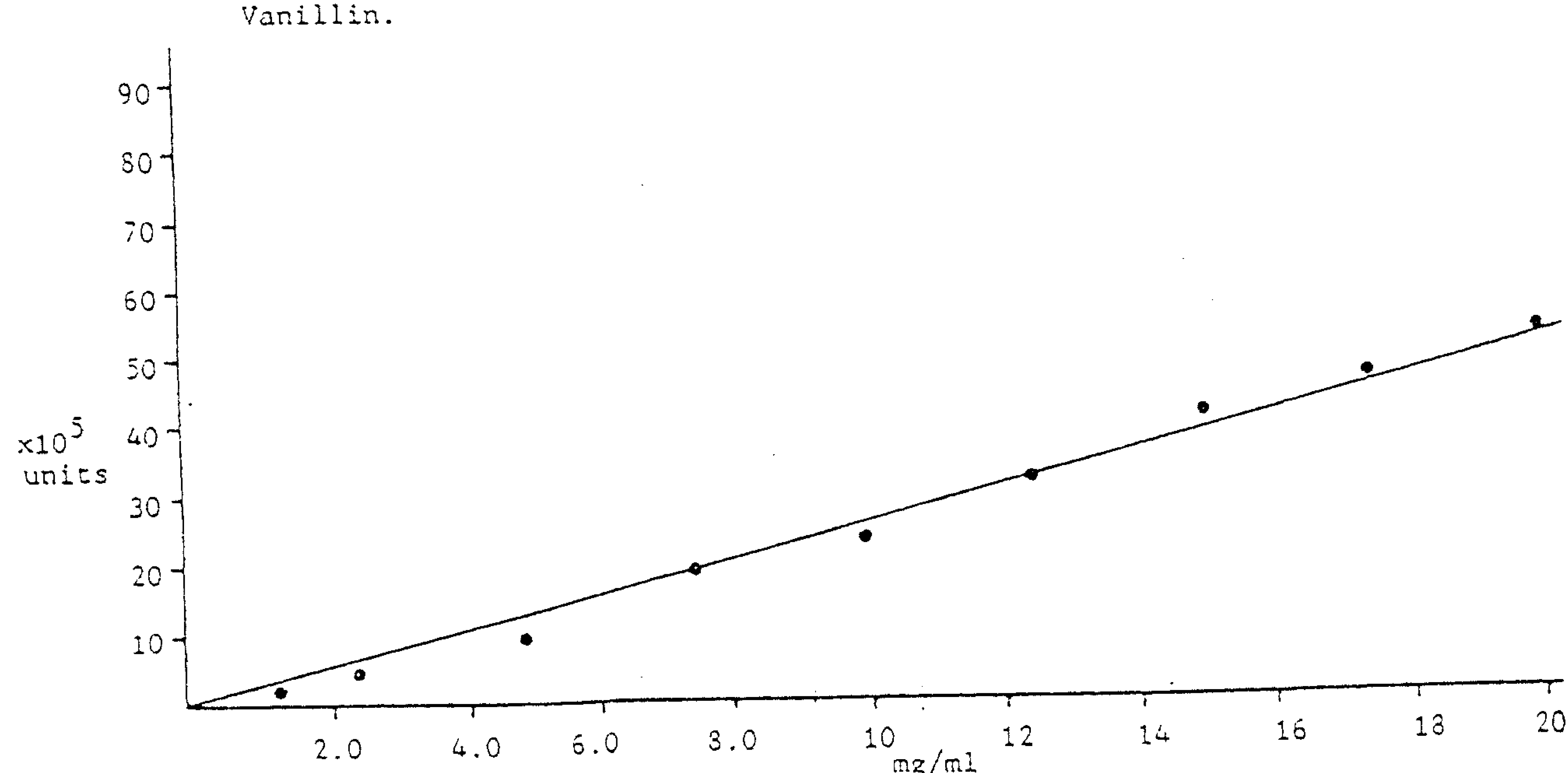


Fig.8

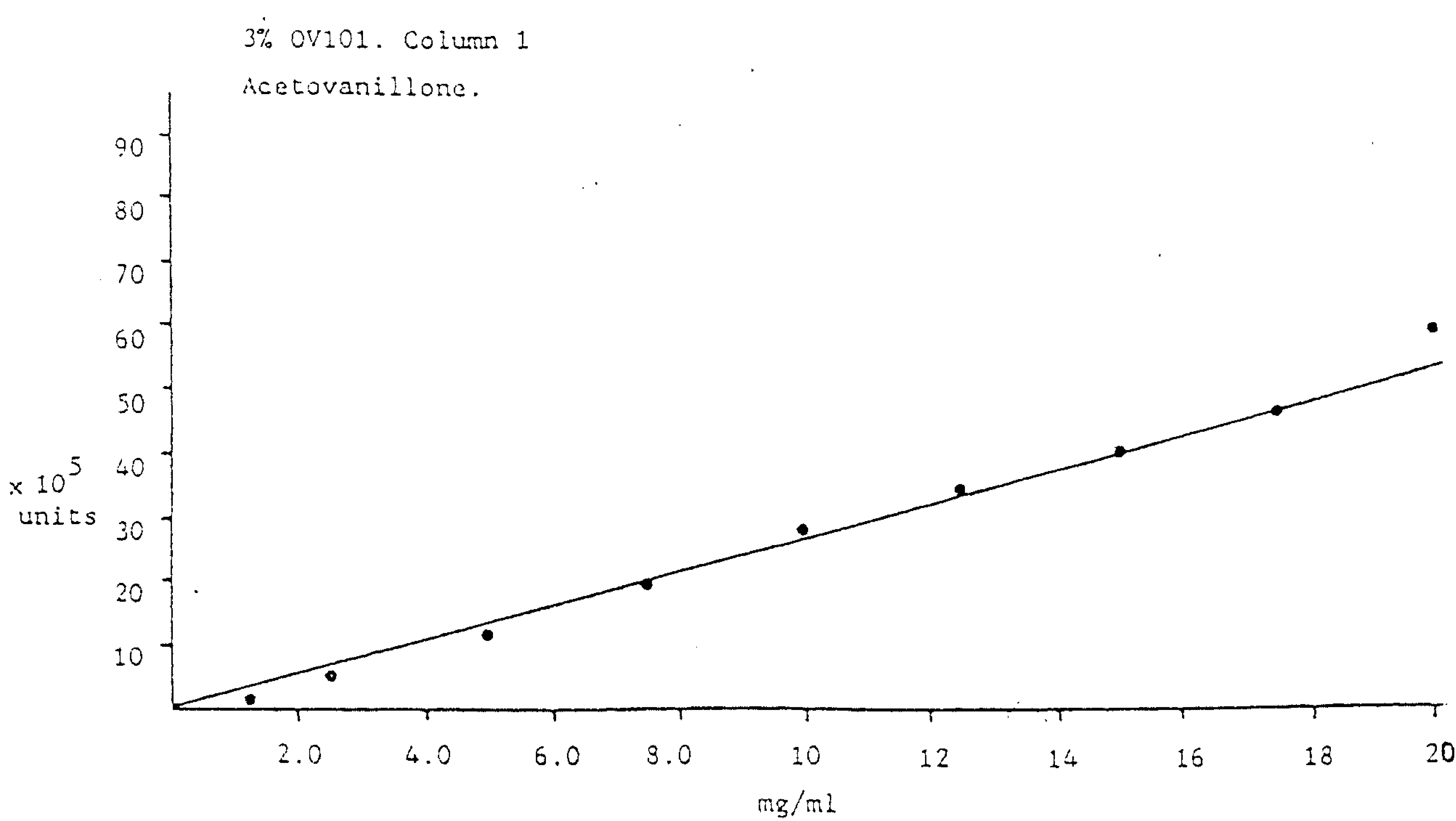


Fig.9

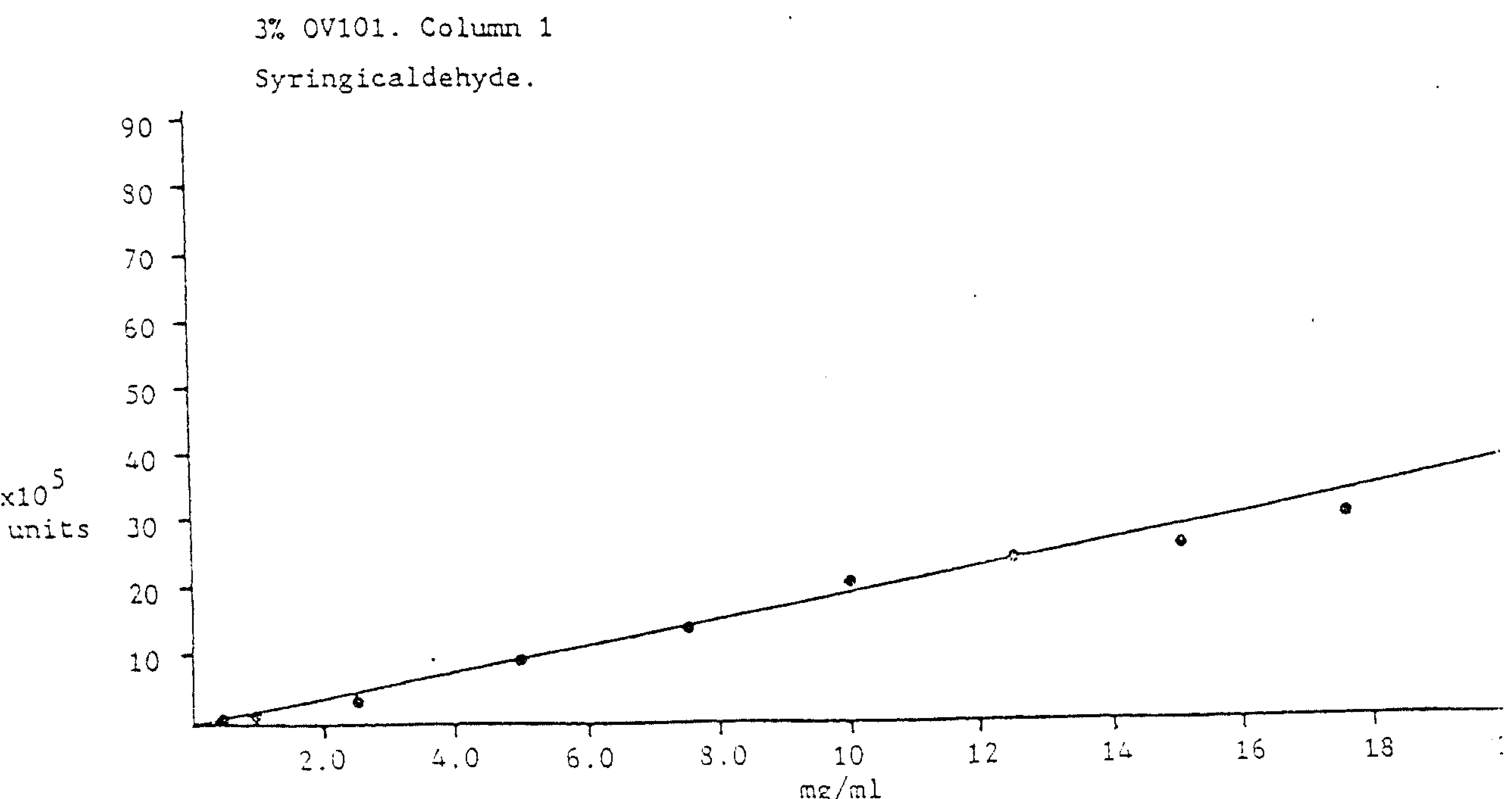
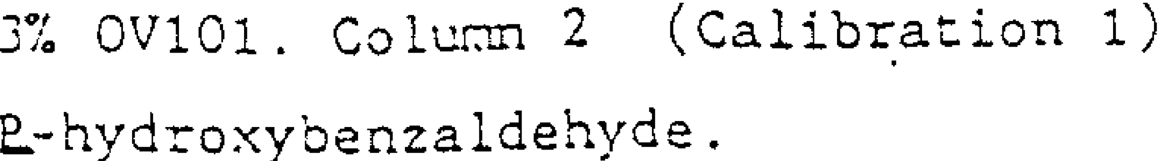


Fig. 10



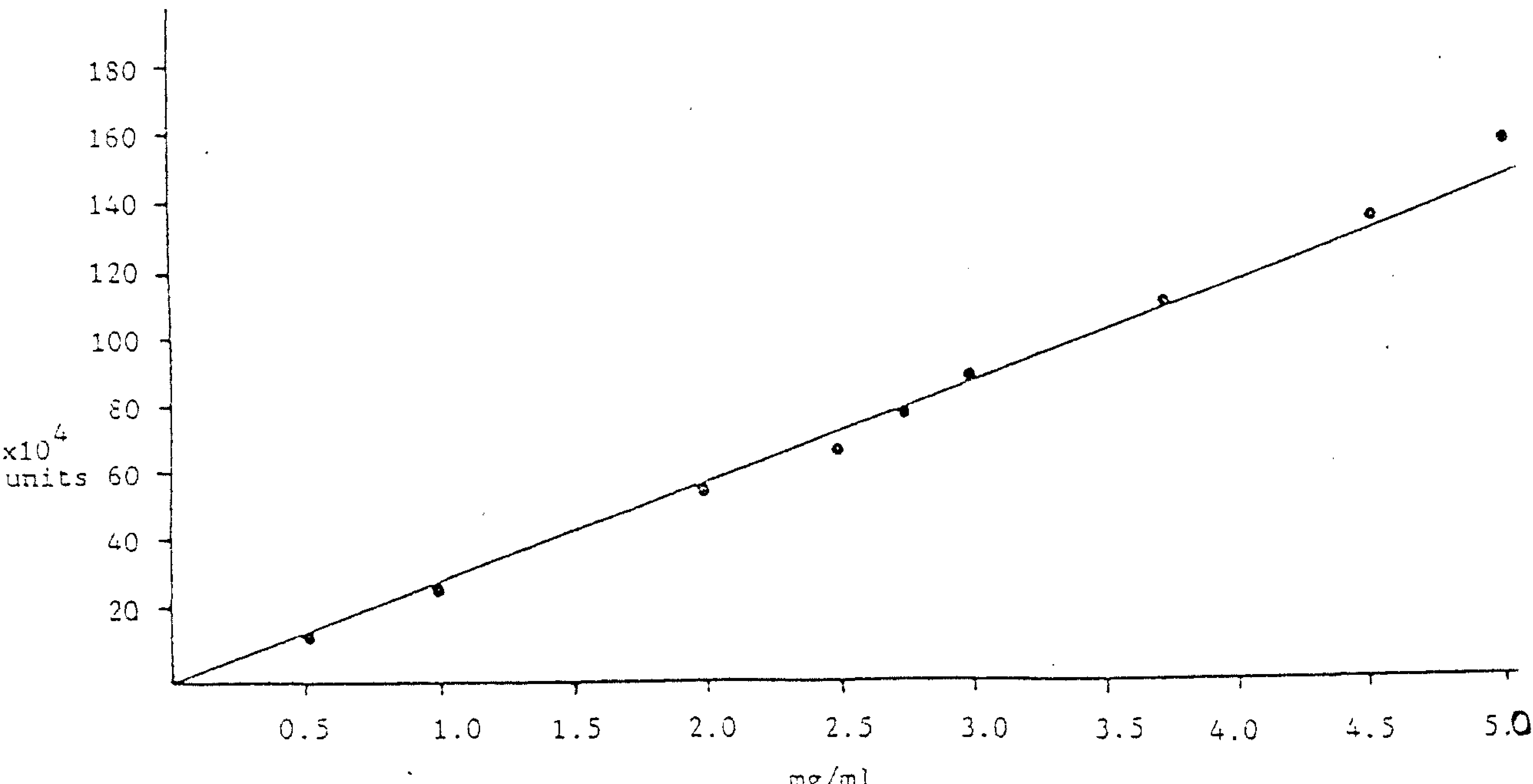


Fig.11

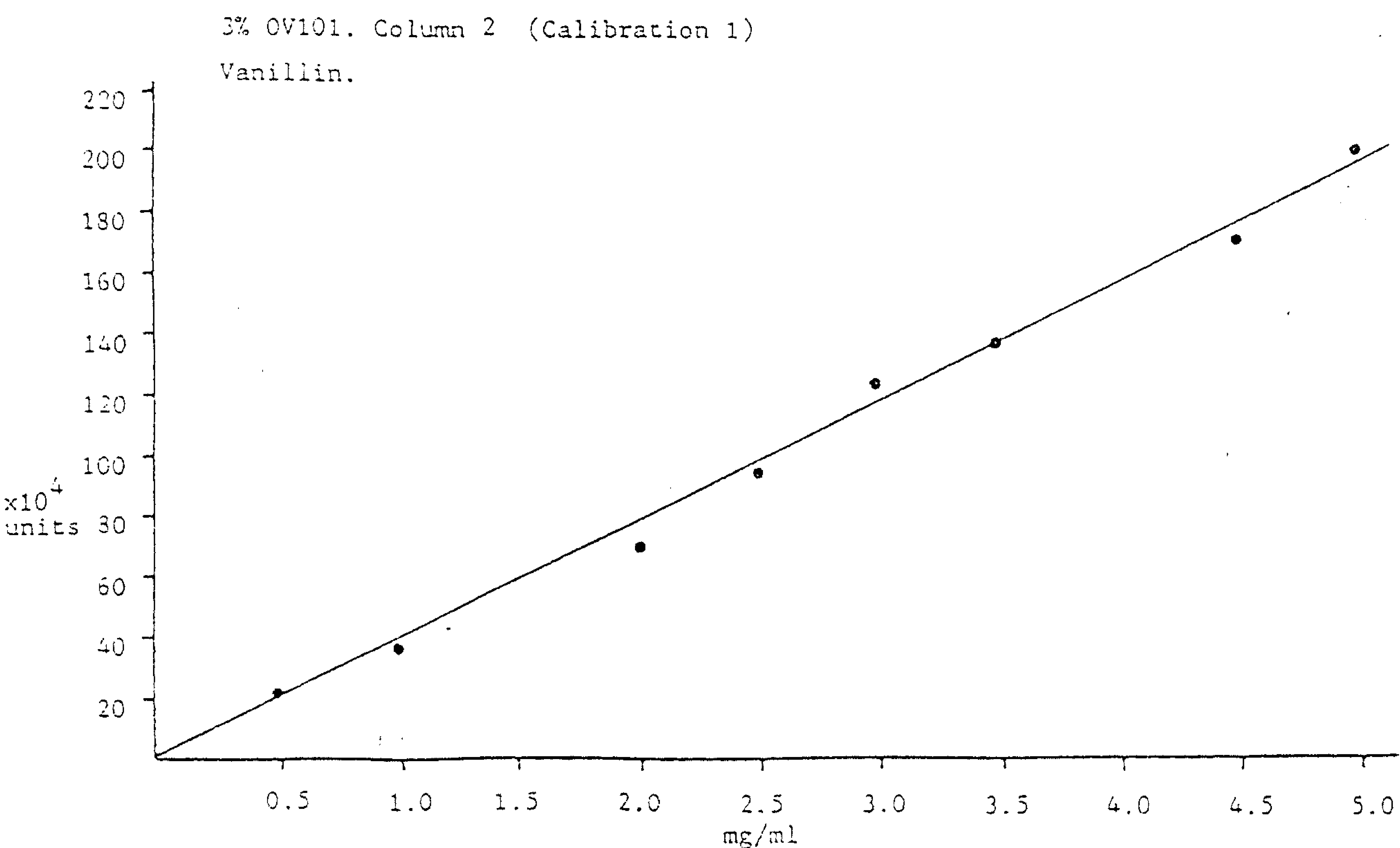


Fig.12

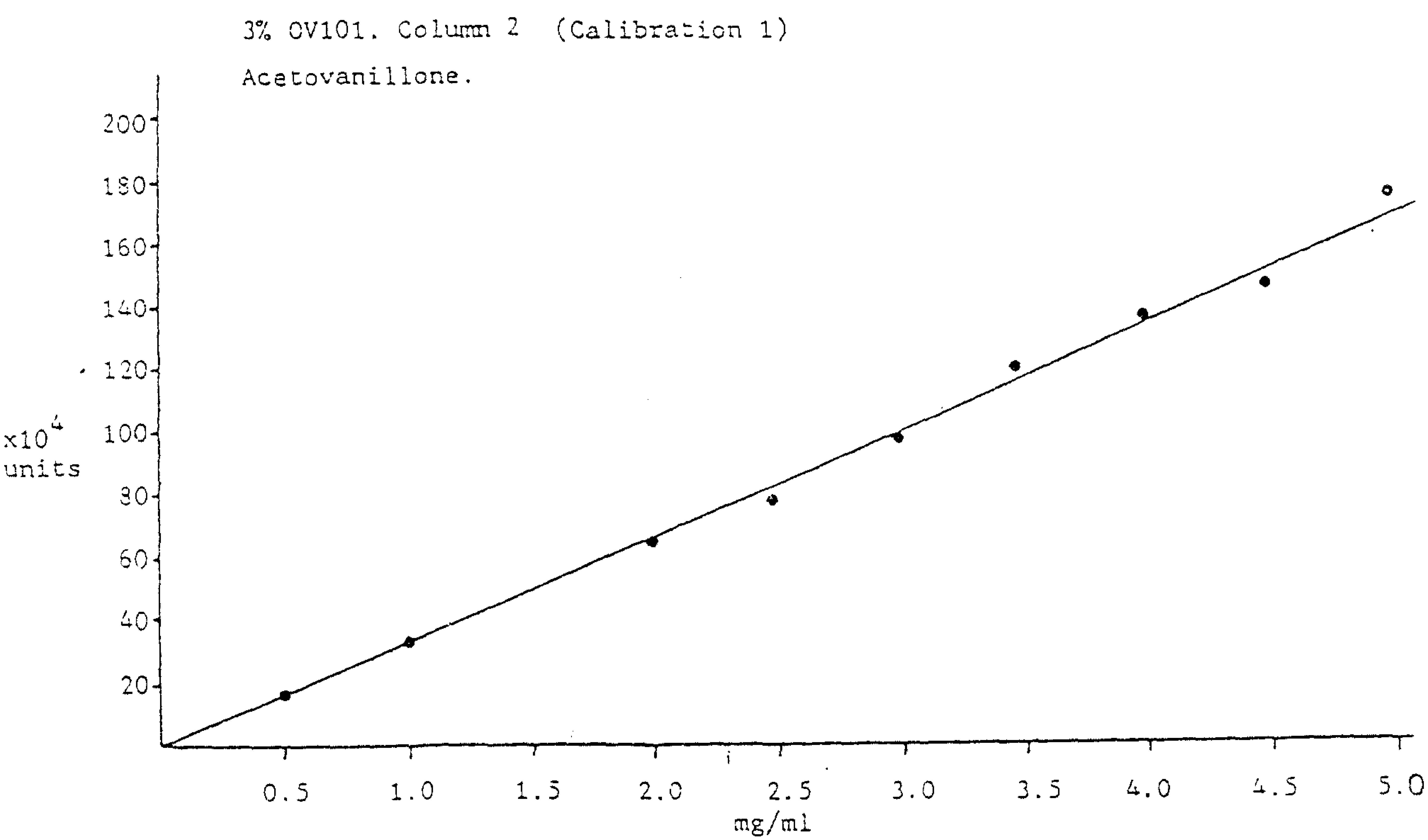


Fig.13

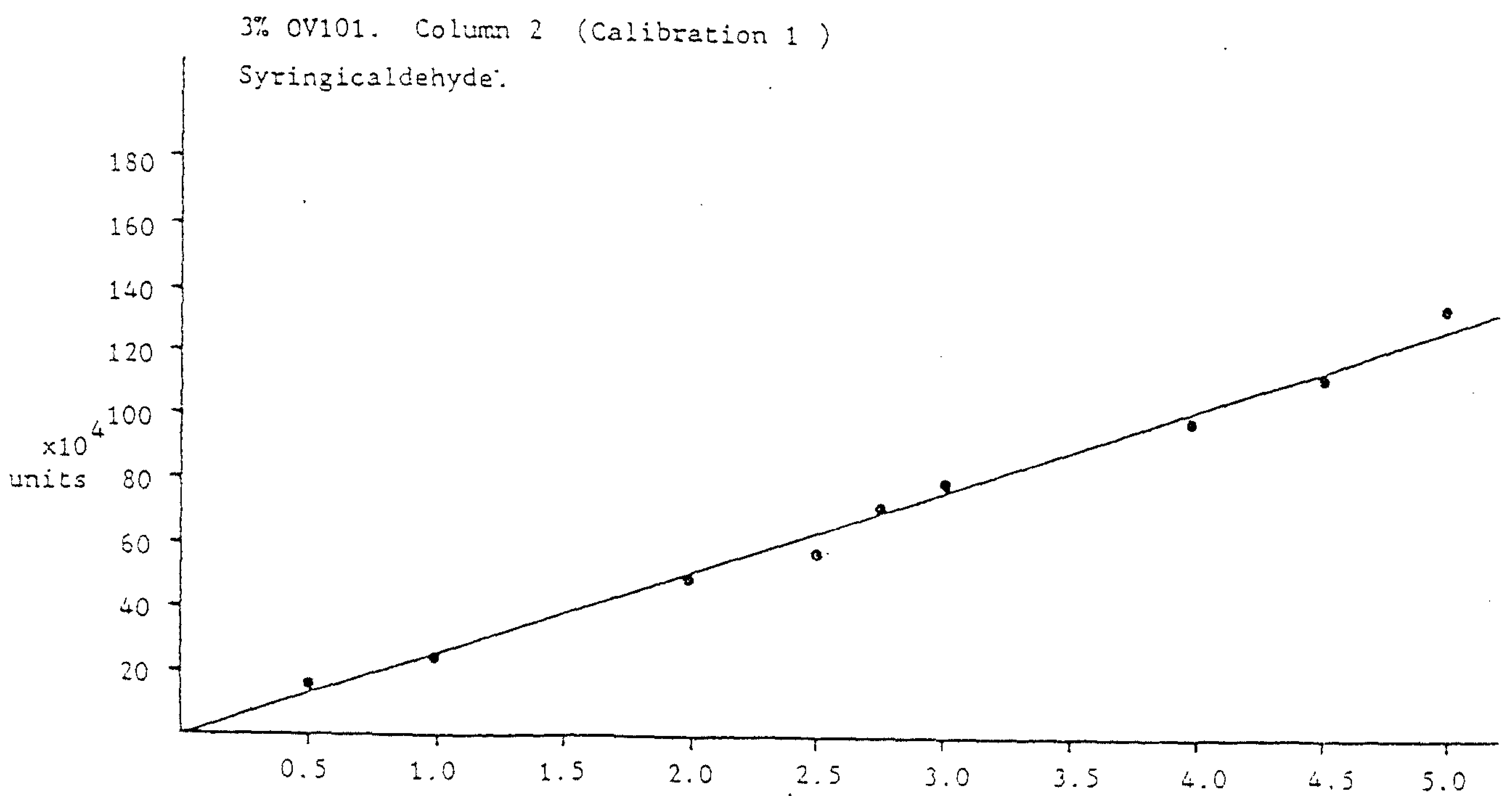


Fig.14

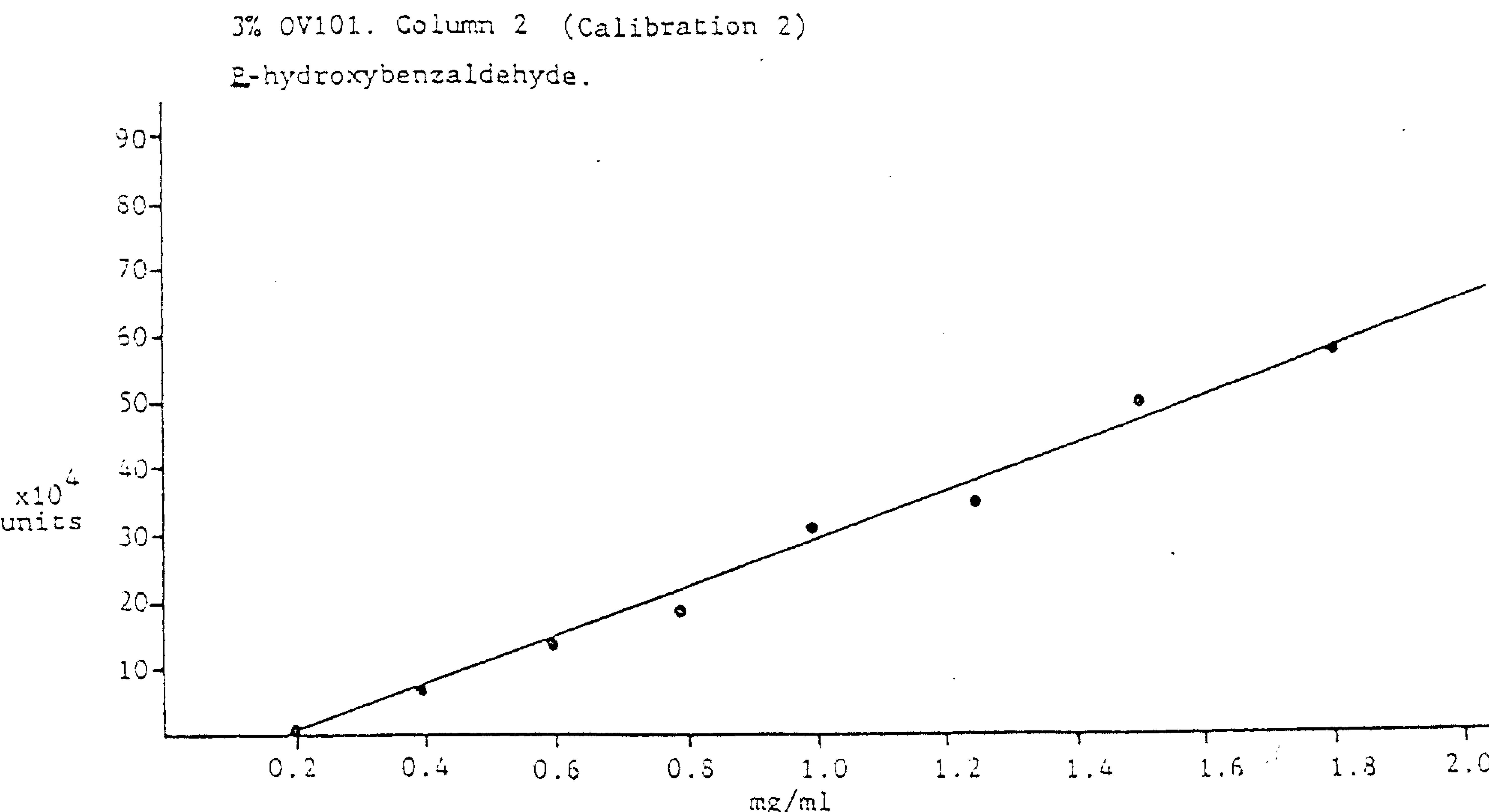


Fig.15

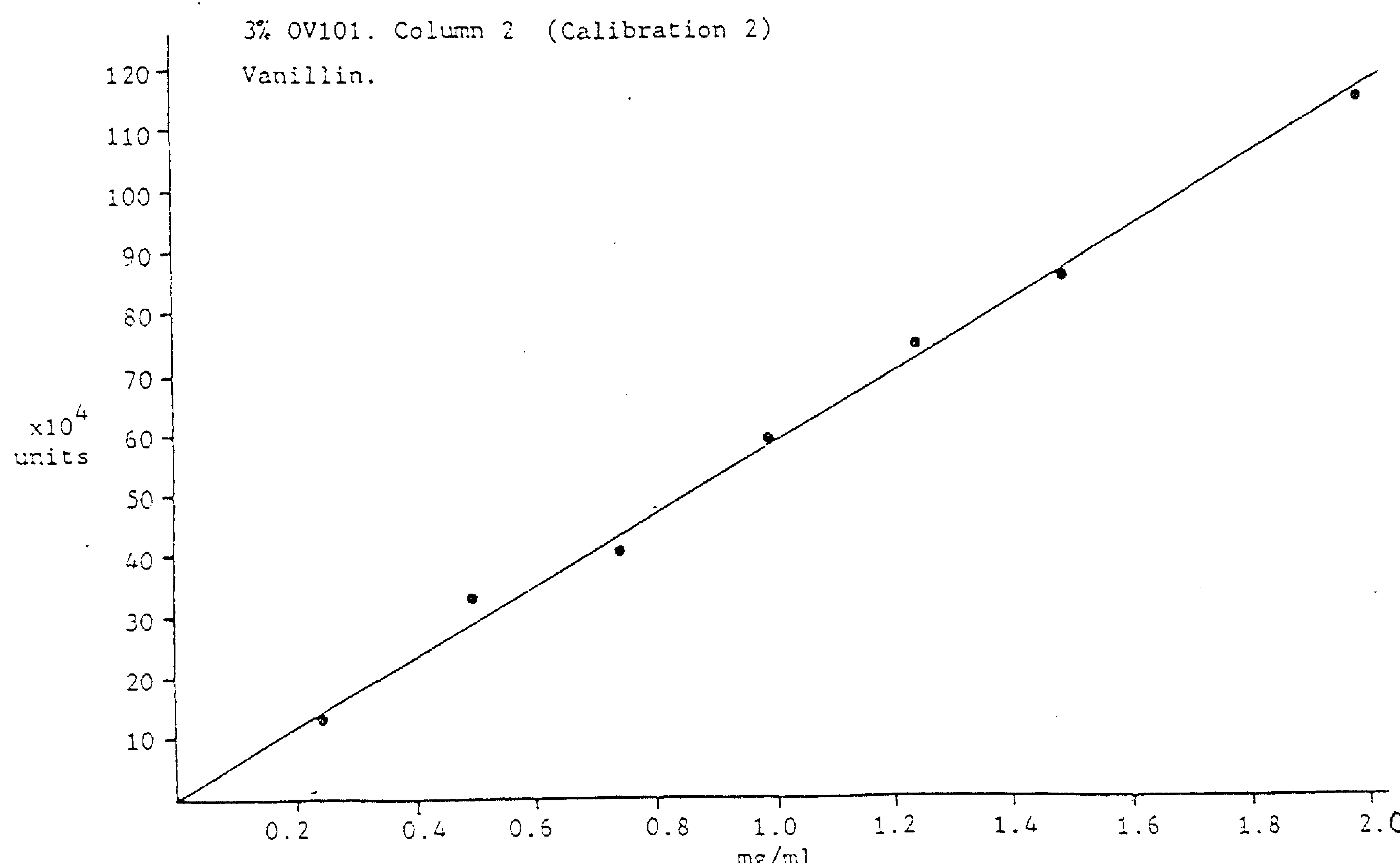


Fig.16

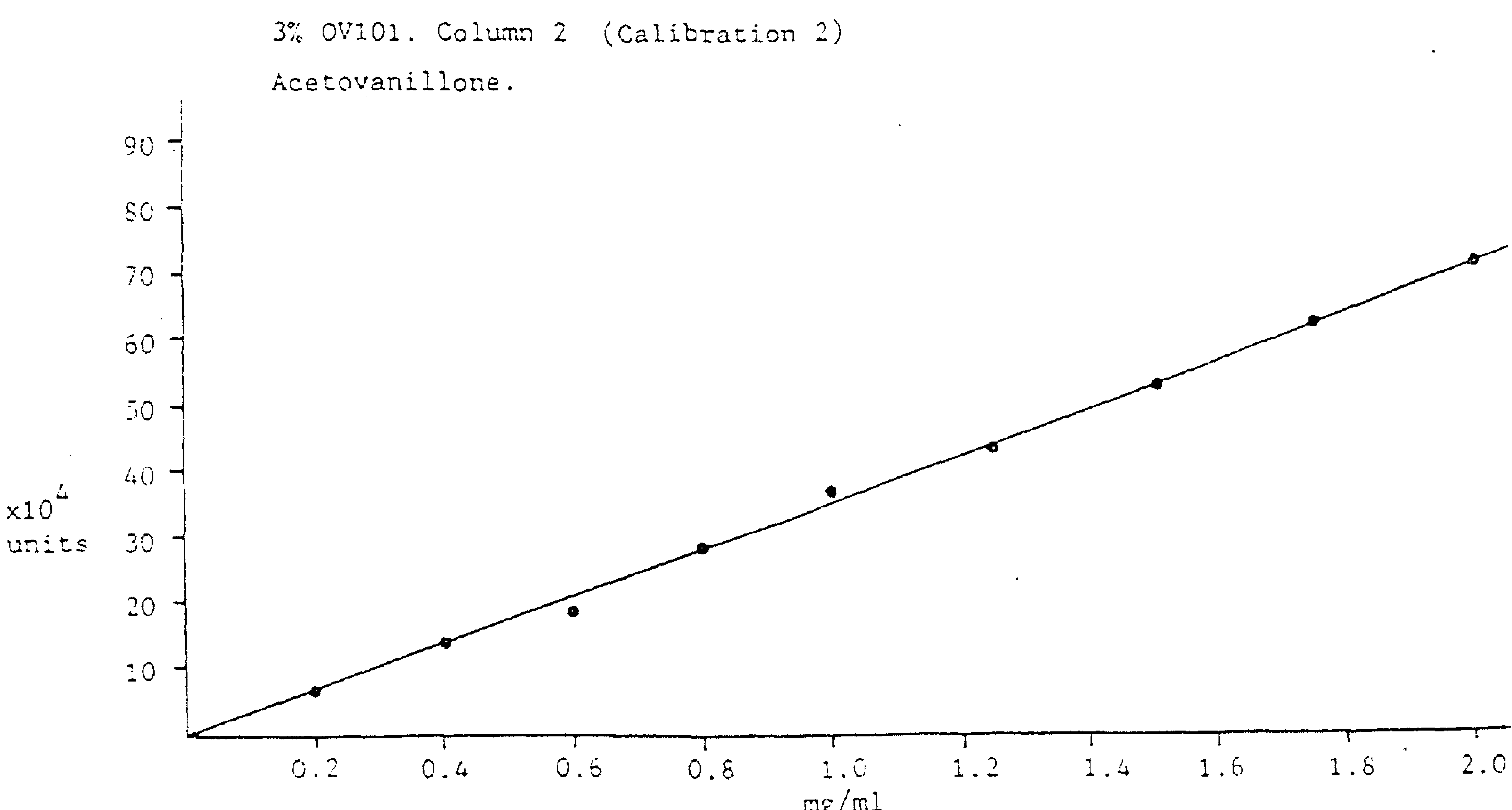


Fig.17

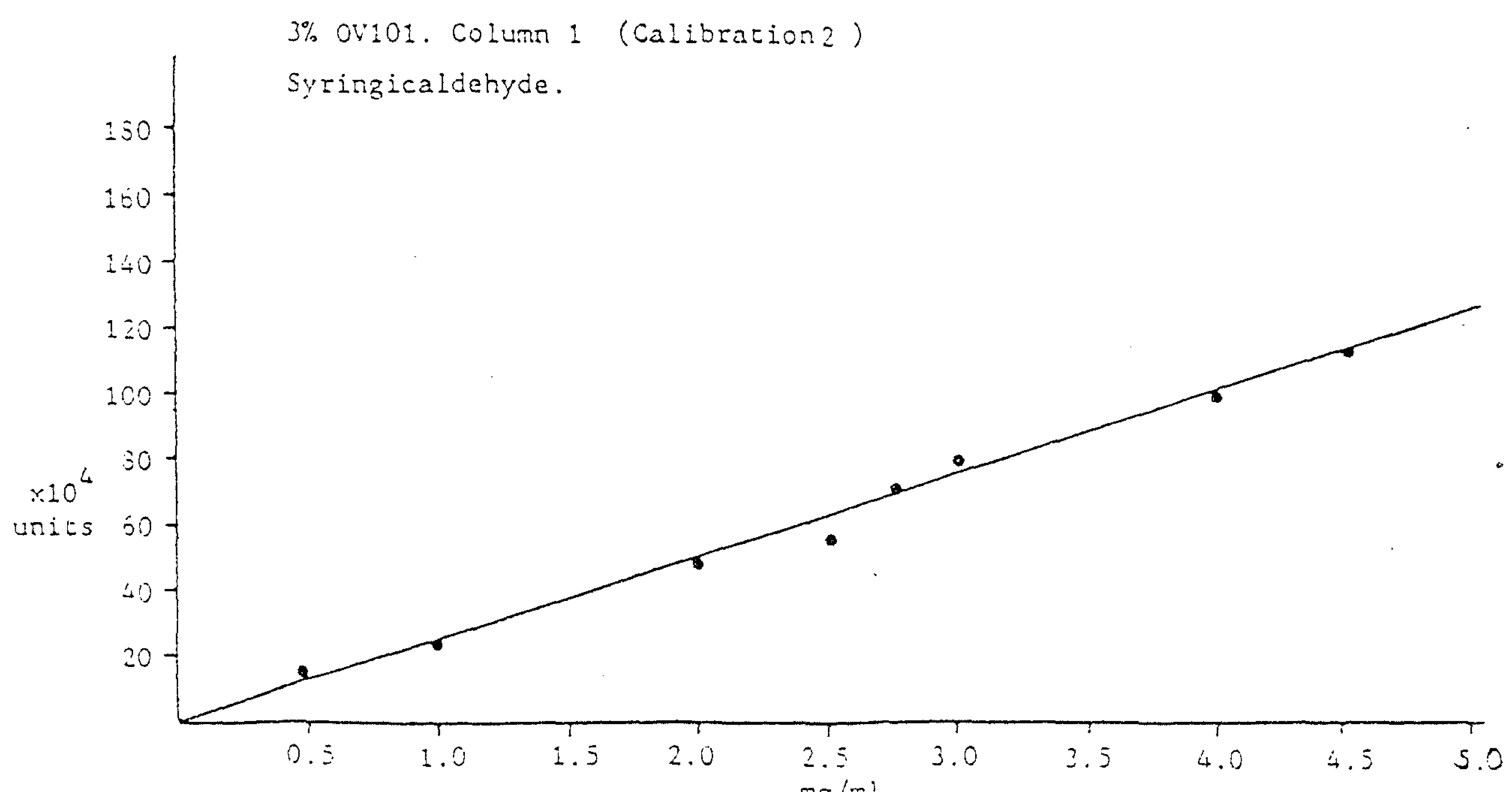


Fig.18

5% Pdegs. Column 1

p-hydroxybenzaldehyde.

Fig.19

5% Pdegs. Column 1

Vanillin.

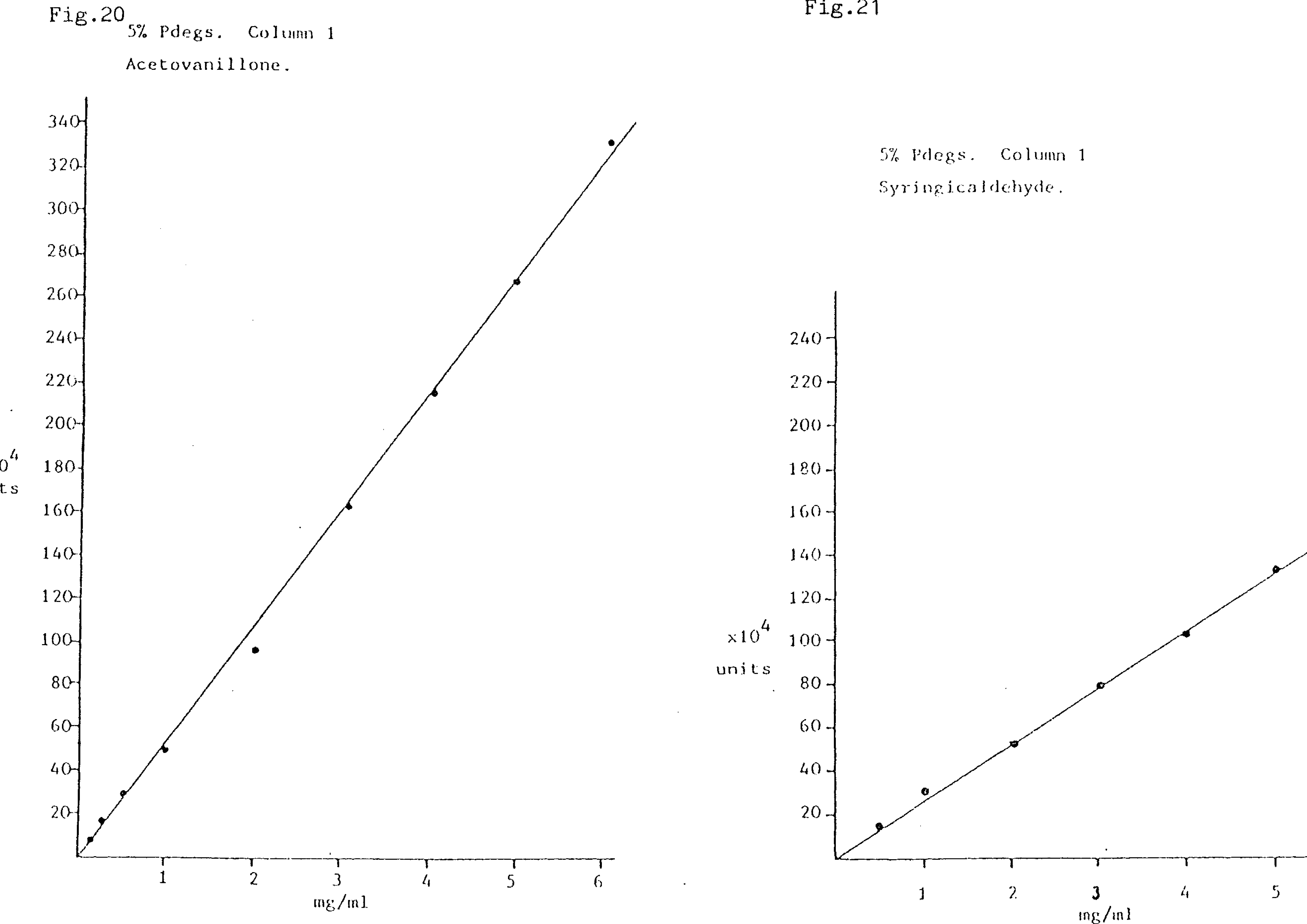


Fig.20 5% Pdegs. Column 1
Acetovanillone.

Fig.21 5% Pdegs. Column 1
Syringicaldehyde.

Fig.22

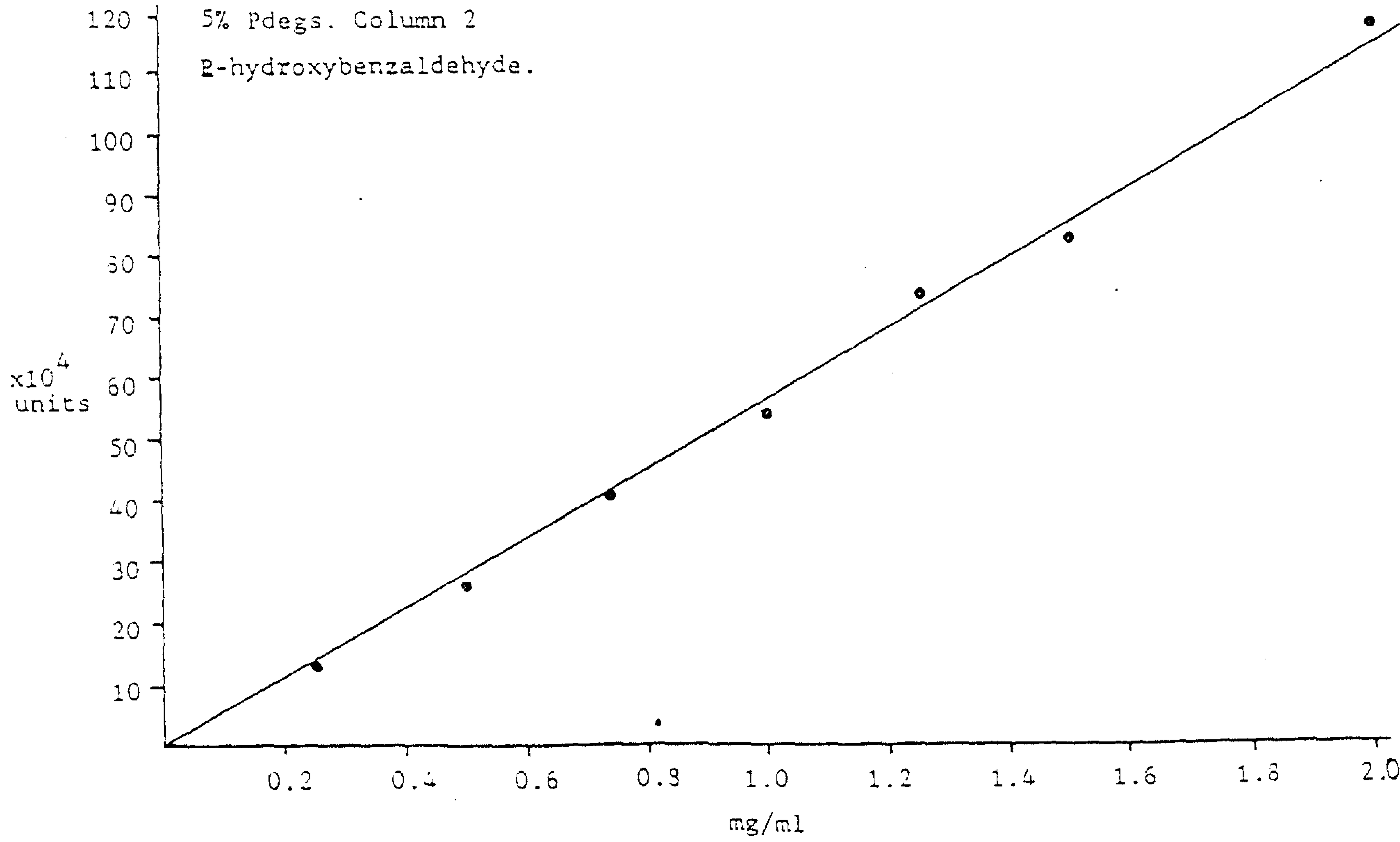


Fig.23

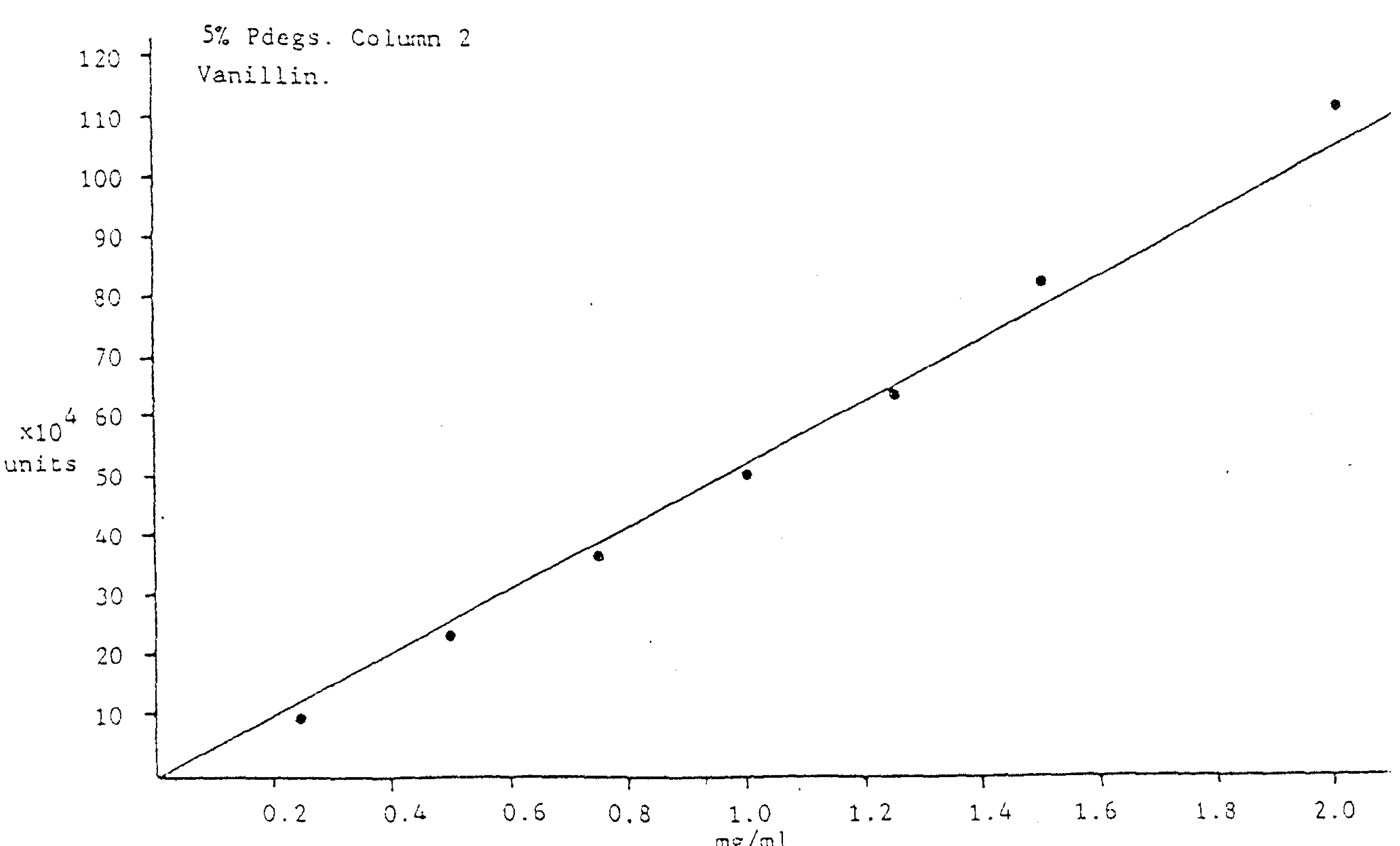


Fig. 24

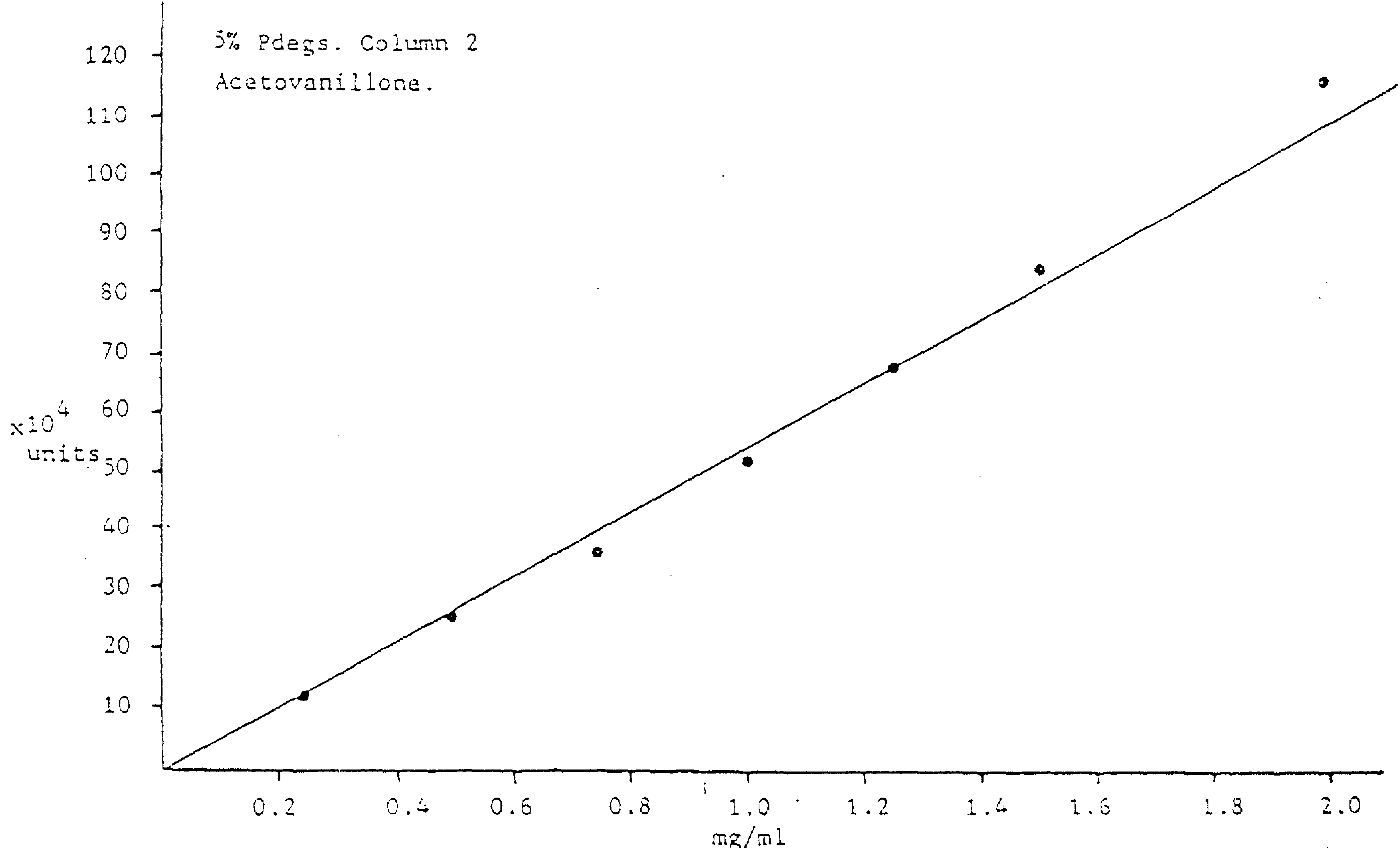


Fig.25

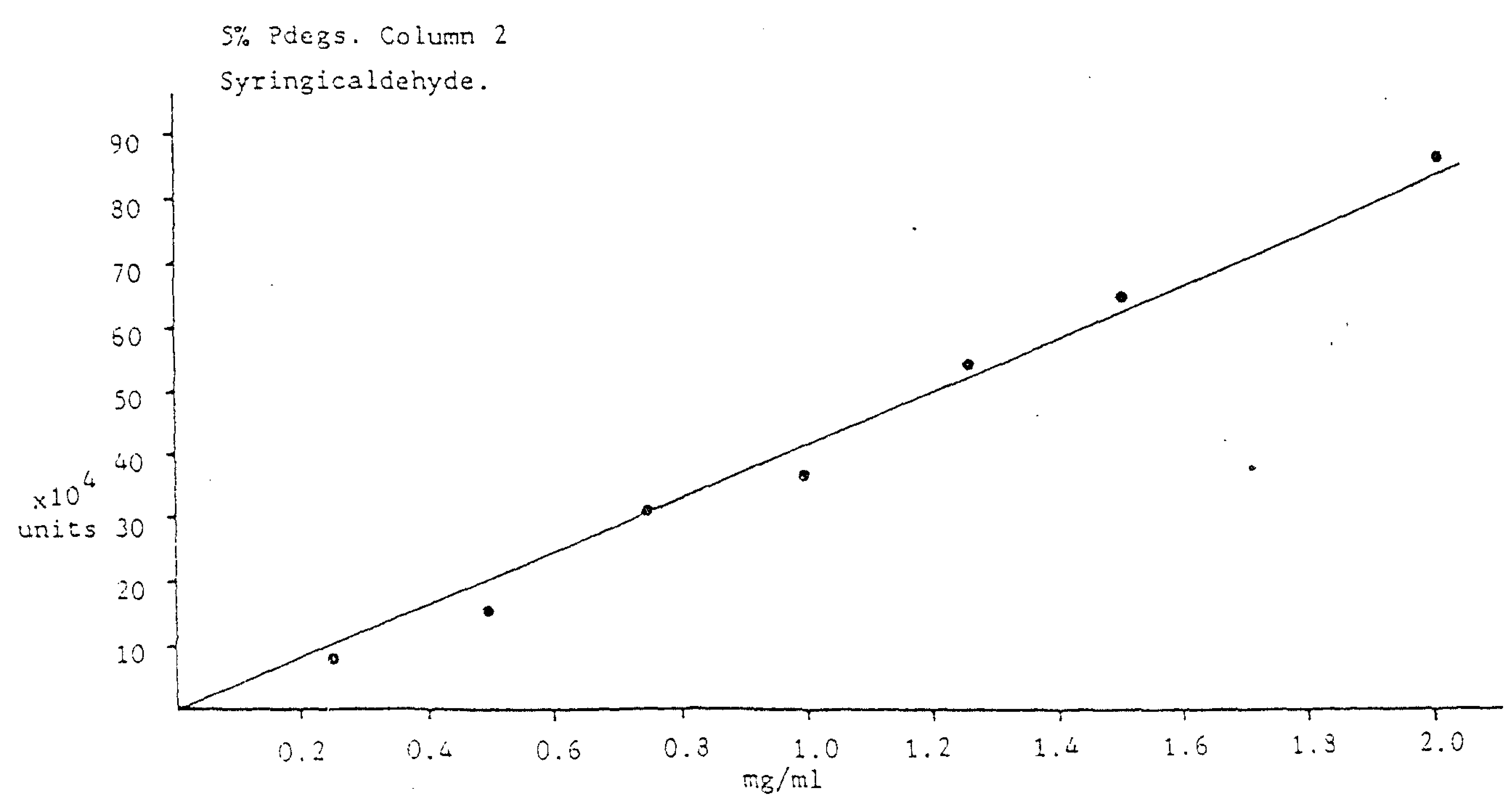


Fig. 26

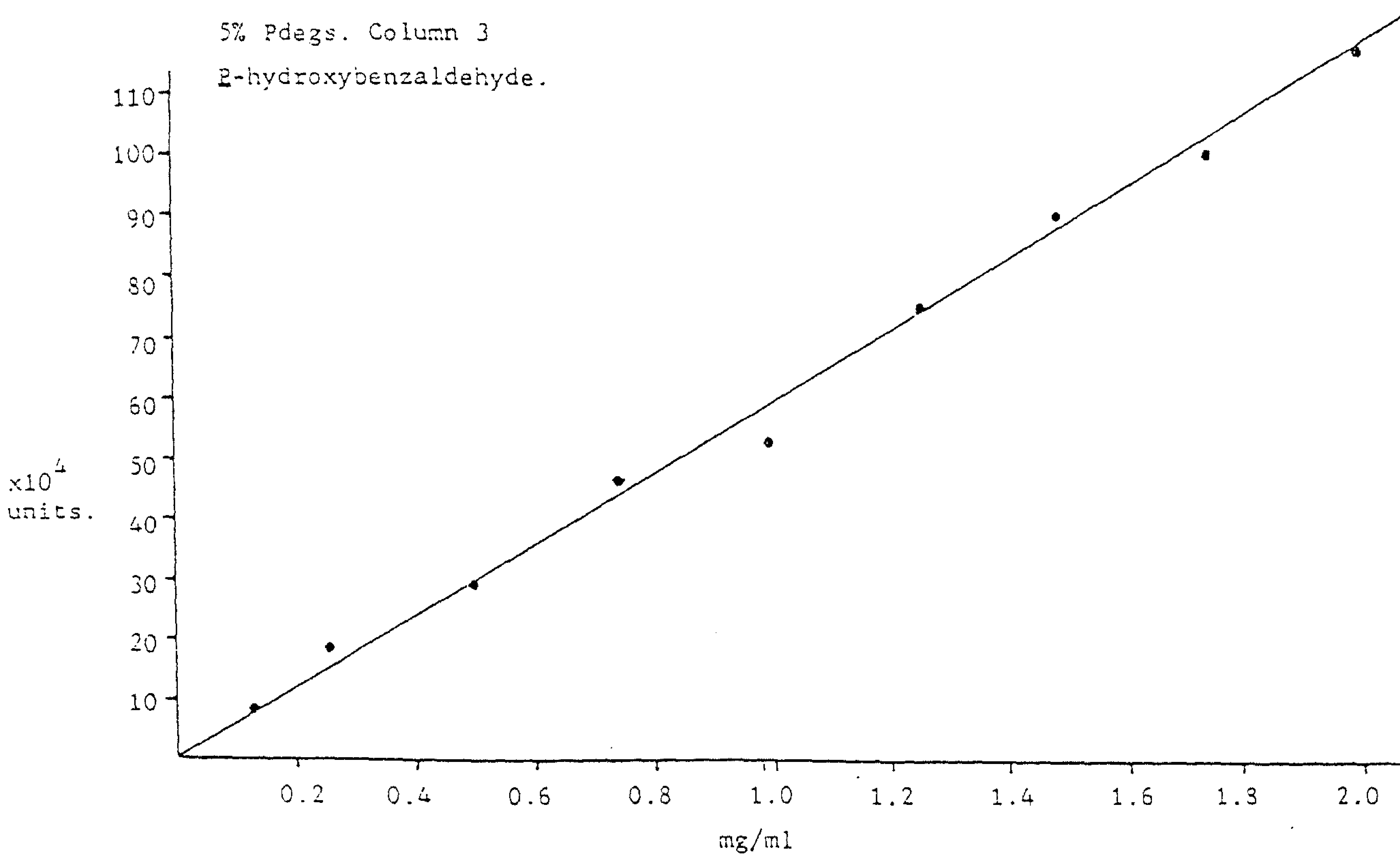


Fig.27

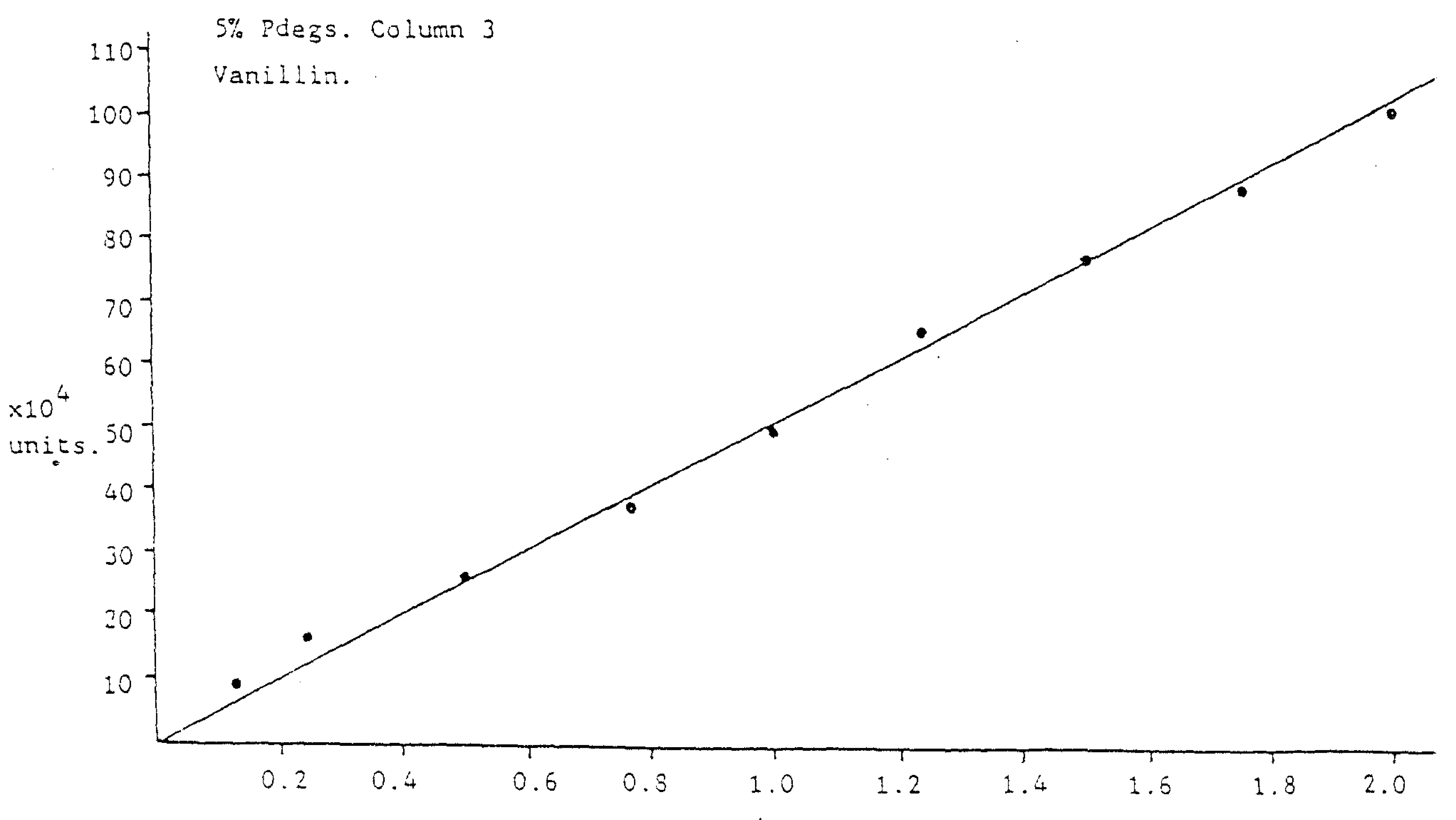


Fig. 28

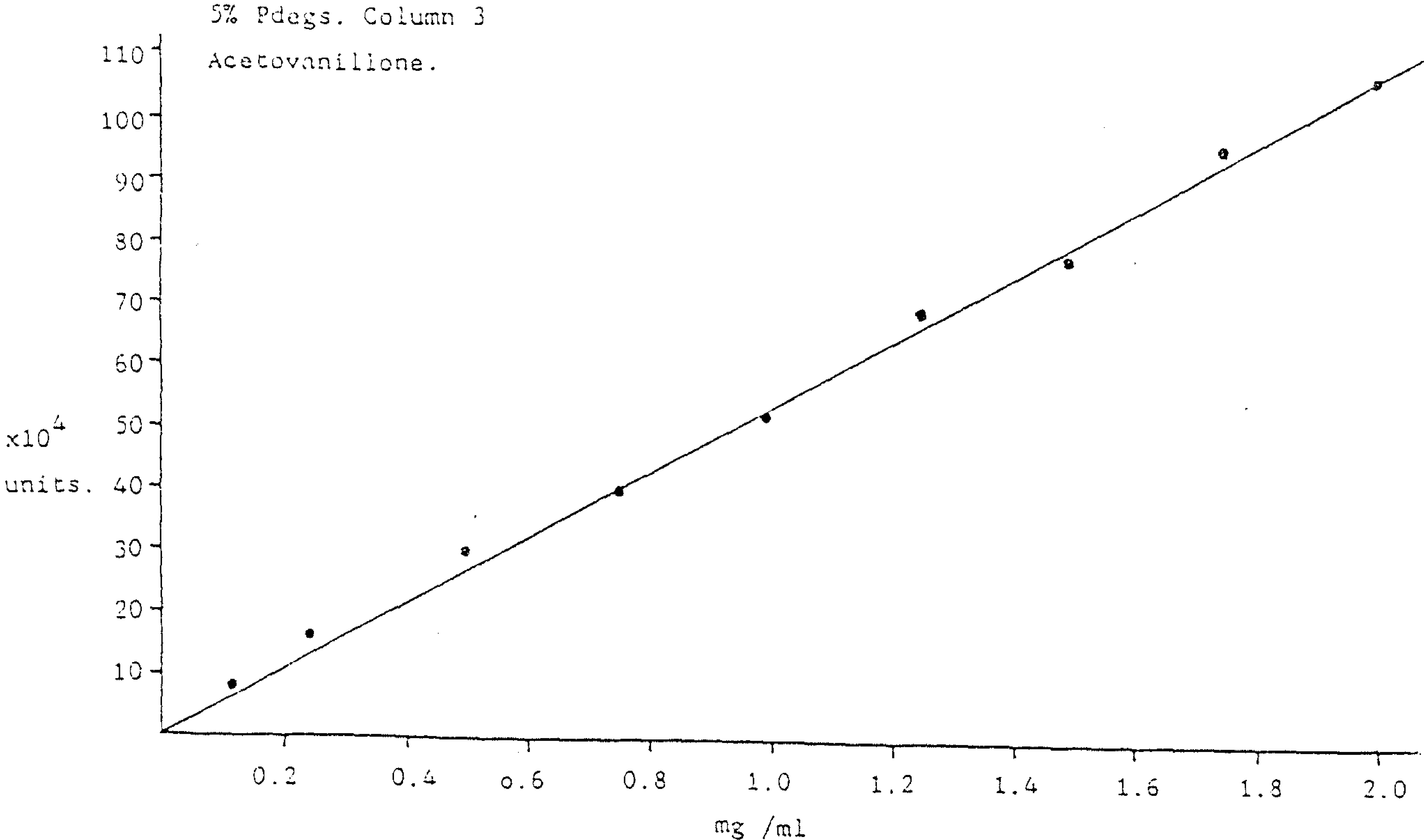
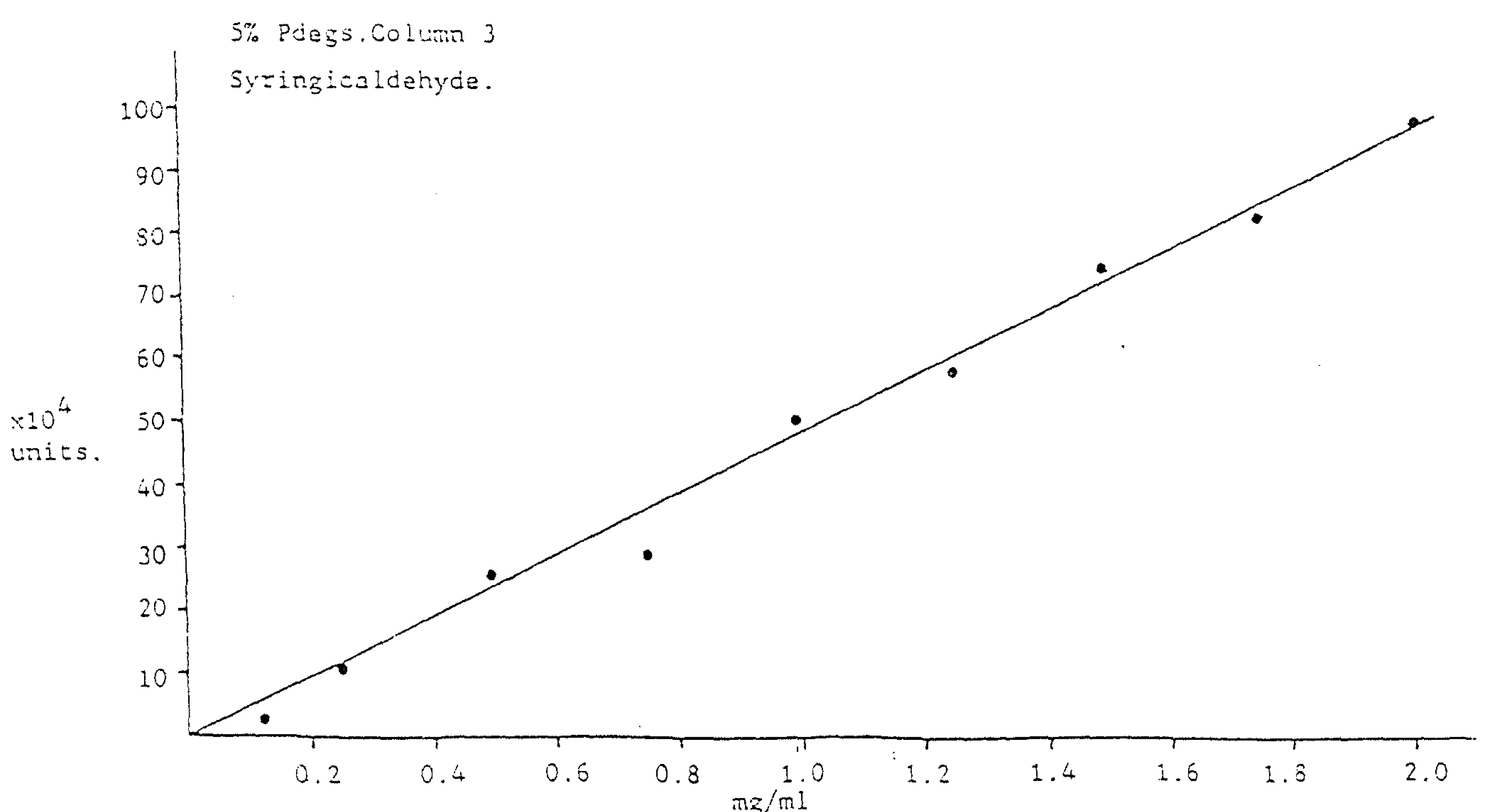


Fig.29



CHAPTER 3.

LIGNIN CHEMOTAXONOMY IN SPERMATOPHYTES.

Lignin Chemotaxonomy in Spermatophytes.

Chemotaxonomy.

Taxonomy is defined by Heywood (1976) as "that part of systematics which deals with the study of classification, including its bases, principles, procedures and rules". Since 1859 when Darwin published the "Origin of Species" and introduced the concept of evolution, phylogenetic relationships have added a new dimension to systematics.

Heywood (1976) defines a character as an attribute referring to the form, physiology or behaviour of what is being considered taxonomically and that in practise a character is any feature of an organism or group that can be measured or assessed. Two types of classification are recognised by botanists for higher plants. An artificial classification for identification based upon obvious characters such as flower and fruit morphology and a natural classification in which all the available criteria including cytological and biochemical characters are employed to indicate evolutionary relationships. For centuries anatomical and morphological characters have been used as well as chemical characteristics, notably the turpentines of pines. In the last twenty years a new surge of ideas and techniques in the field of systematics, that of biochemical systematics has occurred.

Biochemical systematics has come to occupy a major role providing a new class of data for use in constructing or modifying present classifications based on traditional characters. It also enables one to assess the probabilities of phylogenetic relationships particularly those referring to common ancestry and evolutionary sequences. The interest in biochemical systematics has been due to the development of rapid screening techniques such as gas chromatography and electrophoresis. These techniques identify a large number of organic compounds which can contribute to solutions of taxonomic problems. Secondary compounds of low molecular weight which are usually by-products of the major metabolic pathways such as flavonoids, terpenoids, alkaloids and

glycosides have been used to survey plant groups. Siekel, Hall, Feldman and Koeppen (1965) separated the heartwood from Pinus strobus and Pinus monticola on the basis of their flavanone content. Pinus strobus has a higher percentage of cryptostrobin and strobobanksin while Pinus monticola has a preponderance of pinocembrin. In 1966 Swan differentiated between several Canadian pines on the basis of the gas liquid chromatography of their terpenoid constituents.

Phytochemistry has made contributions to our understand-ing of relationships amongst living plants. An extension of this biochemical approach is now being applied to fossils although it is in a very early stage of development. Several workers have used lignin derivatives chemotaxonomically to assess phylogenetic relationships, particularly those referring to common ancestry evolutionary sequences. This present section of the thesis re-examines the lignin taxonomy of living plants and later sections will examine the possibilities of extending lignin taxonomy to fossil material.

The Maule Test.

The role of lignin taxonomy until the middle of the twentieth century was confined largely to lignin colour reactions. The test devised by Maule in 1901 has been one of the most valuable to botanists. Sections of woody stems are immersed in 0.5-1% potassium permanganate for 30 minutes, washed with water then treated with 20% hydrochloric acid for 15 minutes, washed and one or two drops of diluted ammonia are added. The sections are then viewed under the microscope. The wood of dicotyledons in general give a rose-red colour, that is a positive Maule reaction while those of conifers in general give a yellowish brown colour without a trace of red, that is a negative Maule reaction. On the basis of this test it was believed that there was a complete division between gymnosperms and angiosperms, although the cause of the differences was unknown.

In 1926 Sierch found that the Gnetales and some of the Cycads gave a positive Maule reaction. Crocker (1933) using a wide variety of botanical groups found that the three genera included in the Gnetales namely _Gnetum_, _Ephedra_ and _Welwitschia_ all gave positive Maule tests. Crocker also obtained one positive test with one of the _Podocarpus_ species namely _Podocarpus amarus_.

At about this time chemists were investigating the structure of lignin. In 1939 Cramer, Hunter and Hibbert showed that a marked difference existed between the degradation products of lignin isolated from angiosperms and from conifers. The lignins of the gymnosperms contain 14 to 16% methoxyl units whereas the angiosperms contain up to 20 to 22% methoxyl units. Further confirmation of differences between the lignin of angiosperms and gymnosperms has been found by Hibbert and co-workers from products obtained in the ethanolysis of wood, in particular Hibbert's ketones (Hunter, Cramer and Hibbert, 1939; Cramer, Hunter and Hibbert, 1939; Cooke, McCarthy and Hibbert, 1941 and Creighton, Gibbs and Hibbert, 1944). From spruce they obtained a large yield of vanillin whereas from hardwoods they obtained syringic aldehyde too.

That the red colouration of a positive Maule test is due to the presence of the syringyl radical was verified in the work of Creighton, Gibbs and Hibbert (1944). A wide selection of plants were subjected to the Maule test and more than fifty selected species were then subjected to the alkaline nitrobenzene oxidation technique by Freudenberg, Lautch and Engler (1940). The aldehydes were separated by fractional sublimation. In each case it was possible to isolate syringic aldehyde and vanillin from species giving a positive Maule reaction, while vanillin only was obtained from those giving a negative reaction. These studies were extended by Towers and Gibbs (1953) using a new micromethod and the correlation between a positive test and the detection of syringic aldehyde was confirmed.

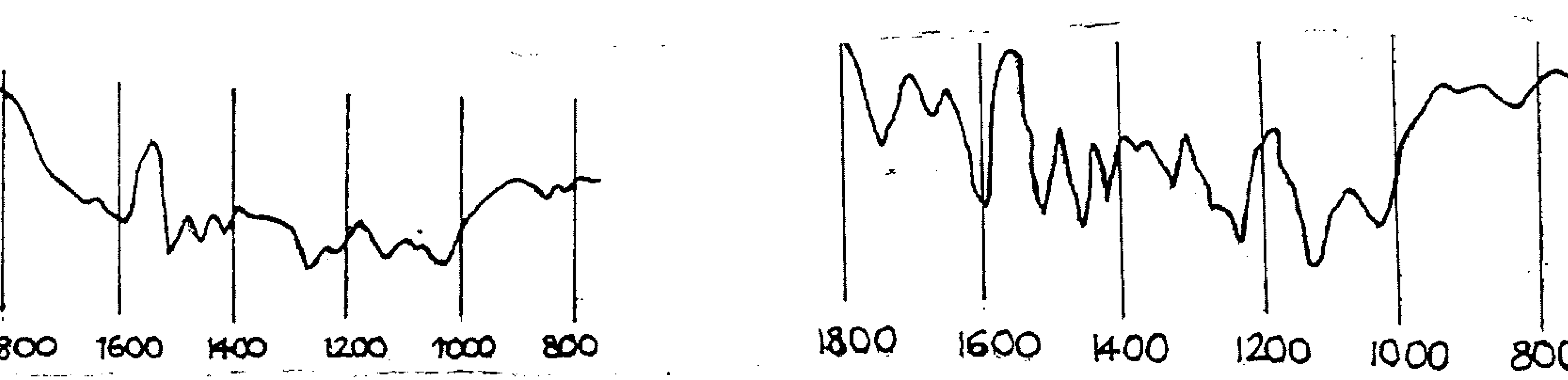
Lignin Chemotaxomony in Higher Plant Groups.

Initially a wide range of plants from differing plant groups, particularly those plant groups with secondary xylem development were used in lignin oxidation studies. The methods used to identify the lignin oxidation products included fractional sublimation and paper chromatography. As methods have become more precise and gas chromatography has been employed research has tended to concentrate on a smaller range of plant groups and on single plant groups.

Most botanists and wood chemists accept the broad division of the better known lignins into a) gymnosperm lignins b) angiosperm lignins and c) grass lignins. These may be distinguished on the basis of their nitrobenzene oxidation products. While gymnosperm lignins yield mainly vanillin both syringic aldehyde and vanillin are obtained from angiosperm and grass lignins. In addition grass lignins yield p-hydroxybenzaldehyde. This classification is unsatisfactory in leaving out herbaceous angiosperms and those of the Pteridophyta. Gibbs (1958) introduced the division of lignins into two major classes namely guaiacyl and guaiacyl-syringyl lignin. This division of the plant lignins has been accepted by Kawamura and Higuchi (1964). Guaiacyl lignins are called Type N lignins and guaiacyl-syringyl lignins are called Type L lignins based on different absorption points in their infra-red spectra of isolated milled wood lignins (as shown in the examples, figure(30). They divided both classes into sub-groups using minor differences of their infra-red spectra as criteria.

Figure (30). From Kawamura and Higuchi (1964).

Pinus thunbergii Type N   Fagus crenata Type L



wave number cm^{-1} wave number cm^{-1}

infra-red spectra of milled wood lignin

As more samples have been examined, and methods and techniques have improved, discrepances have occurred within the literature concerning the taxonomy of lignin degradation products. In 1940 Freudenberg, Lautsch and Engler employing the nitrobenzene oxidation of lignin found that spruce gave vanillin whereas maple gave a mixture of vanillin and syringic aldehyde. Creighton, Gibbs and Hibbert (1944) applied the nitrobenzene oxidation to over fifty species. Their results in general confirm the earlier work. The ratio of vanillin to syringic aldehyde in the isolated mixture of dicotyledons was shown to be in the ratio 1:3. The monocotyledons consisting of several grasses in this case, gave slightly lower yields of aldehydes and the ratio of vanillin to syringic aldehyde was approximately 1:1. The vanillin fraction isolated from these grasses were found to be contaminated with p-hydroxybenzaldehyde. Subsequently Creighton and Hibbert (1944) isolated free p-hydroxybenzaldehyde from the lignin of corn stalks, its presence serving as a distinguishing characteristic between monocotyledons and dicotyledons. Certain exceptions were found suggesting that differences in lignin could be used to aid the taxonomist in establishing true phylogenetic relationships of plants. Three species Podocarpus amarus, Podocarpus penduncculata and Tetraclinis articulata belonging to the Coniferales were found to give not only vanillin but yields of syringic aldehyde. The Gnetales were found to have yields of syringic aldehyde and their relationship to the

angiosperms was thus indicated rather than the gymnosperms. Belliolum haplopus and Zygogynum vieillardi both primitive angiosperms were found to have vanillin and syringic aldehyde in lower ratios of 1:1.1 and 1.2 respectively. These results are interesting because these two species belong to the Winteraceae which are characterized by the absence of vessels.

Using paper chromatography instead of the sublimation method of Creighton, Gibbs and Hibbert (1944), Leopold and Malmstrom (1952) identified small amounts of p-hydroxybenzaldehyde and syringic aldehyde. They detected very small amounts of p-hydroxybenzaldehyde in three dicotyledons as well as vanillin and syringic aldehyde. The wood from almost all the coniferous genera were found to give small and varying amounts of p-hydroxybenzaldehyde. The highest yields were given by members of the family Taxoidaceae, Pinaceae and some Cupressaceae. Syringic aldehyde was found to occur in some members of the Coniferales notably the Pinaceae and in one species Tetraclinis articulata the latter confirming earlier results.

In 1950 Bland, Ho and Cohen showed that lignins of at least six out of ten Eucalyptus species, dicotyledons, yielded p-hydroxybenzaldehyde although Towers and Gibbs (1953) indicated that this aldehyde is rather uncommon as a product of lignin oxidations in dicotyledons. Towers and Gibbs (1953) used a new micromethod developed by Stone and Blundell (1951) and used paper chromatography to separate and estimate aldehydes resulting from the alkaline nitrobenzene oxidation of wood. They found that p-hydroxybenzaldehyde was not restricted in occurrence to the grasses. They found it present in Psilotum, Equisetum, lycopsids and in all the monocotyledons examined but not in the dicotyledons that were examined. In contrast to the report by Leopold and Malmstrom (1952) syringic aldehyde was not detected in the gymnosperms apart from Podocarpus elatus.

Ibrahim, Towers and Gibbs (1962) determined whether there was any relationship between hydrolysed extracts of the leaves of plants and their lignin residues. No

syringic acid or sinapic acid was detected in the leaves of gymnosperms, apart from the expected exceptions which also had a positive Maule test. In the angiosperms one or both acids were found to be present.

In 1962 Pepper, Manolopoulo and Burton by using gas chromatography separated alkaline nitrobenzene oxidation products from aspen, spruce and wheat straw. The lignin fraction of those representatives of the three major divisions gave rise to all three types of nuclei. They concluded however that the relative abundance of each type still serves adequately as a form of taxonomic classification on the basis of these three species. However using gas chromatography and the cupric oxide oxidation of the lignin Pepper, Casselman and Karapally (1967) did not report any syringic nuclei from spruce wood.

Results.

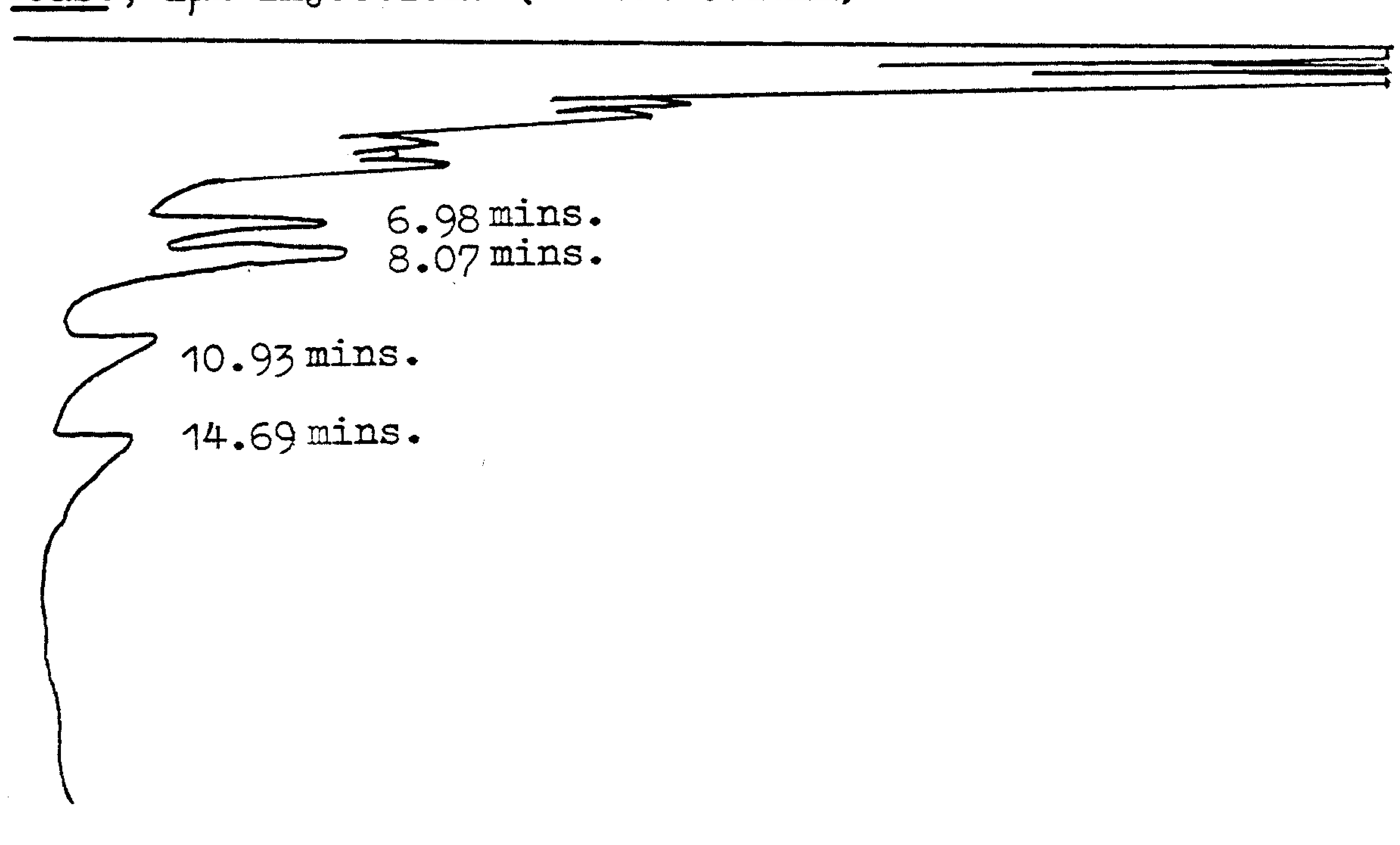
Abbreviations used in tables in this section are; p-OH = p-hydroxybenzaldehyde; VAN = vanillin; ACETO = aceto-vanillone and SYR = syringic aldehyde.

Control experiments.

1. The cupric oxidation method as already described was carried out in the usual way with the exception of omitting the plant material. The sample was made up to 3ml in a volumetric flask and injected in the usual way. Only the peak solvent was present on the chromatogram.

2. One gram of dried yeast was used to replace any plant wood and the oxidation was carried out in the normal way. Yeast was chosen to replace the usual plant material because it was thought highly unlikely to contain any lignin. The chromatogram is shown below. The components have not been identified but none of them correspond to the aromatic aldehydes found when plants containing lignin are oxidized.

Yeast, 2µl injection. (OV 101 column)



<u>The preferential loss of the aldehydes and acetovanillone.</u>

To see if any of the phenolic oxidation products are preferentially lost in the work up procedure 10 mg. each of p-hydroxybenzaldehyde, vanillin, acetovanillone and syringic aldehyde were dissolved in 10 ml. of warm 2N sodium hydroxide. Another 20 ml. of 2N sodium hydroxide was added, and the solution was then acidified to pH 3 with 2N sulphuric acid. The acidified mixture was continually extracted with 125 ml. of diethyl ether for 18 hours. The diethyl ether extract was washed with distilled water until neutral and dried over anhydrous magnesium sulphate and filtered into a clean flask. The magnesium sulphate residue was rinsed with 10 ml. of diethyl ether, and added to the filtrate. The filtrate was evaporated under reduced pressure and the residue was dissolved in diethyl ether and the final volume was adjusted to 3 ml. using a volumetric flask. This was repeated and the samples were then chromatographed quantitatively.

	mg.	
	I	II
p-hydroxybenzaldehyde	4.20	3.97
vanillin	4.50	4.57
acetovanillone	4.35	4.05
syringic aldehyde	2.85	3.00

As these results show, preferential loss does occur amongst the phenolic oxidation products, namely that of syringic aldehyde by an average of 13% more, than that lost by the other compounds.

Results with Gymnosperms.

Samples from living gymnosperms were oxidized using the cupric oxide method described previously and chromatographed quantitatively and qualitatively.

Figure (31).
A typical chromatogram of the lignin oxidation products of a member of the Cycadales.

Cycas revoluta petiole, 6µl injection. (5% Pdegs column).

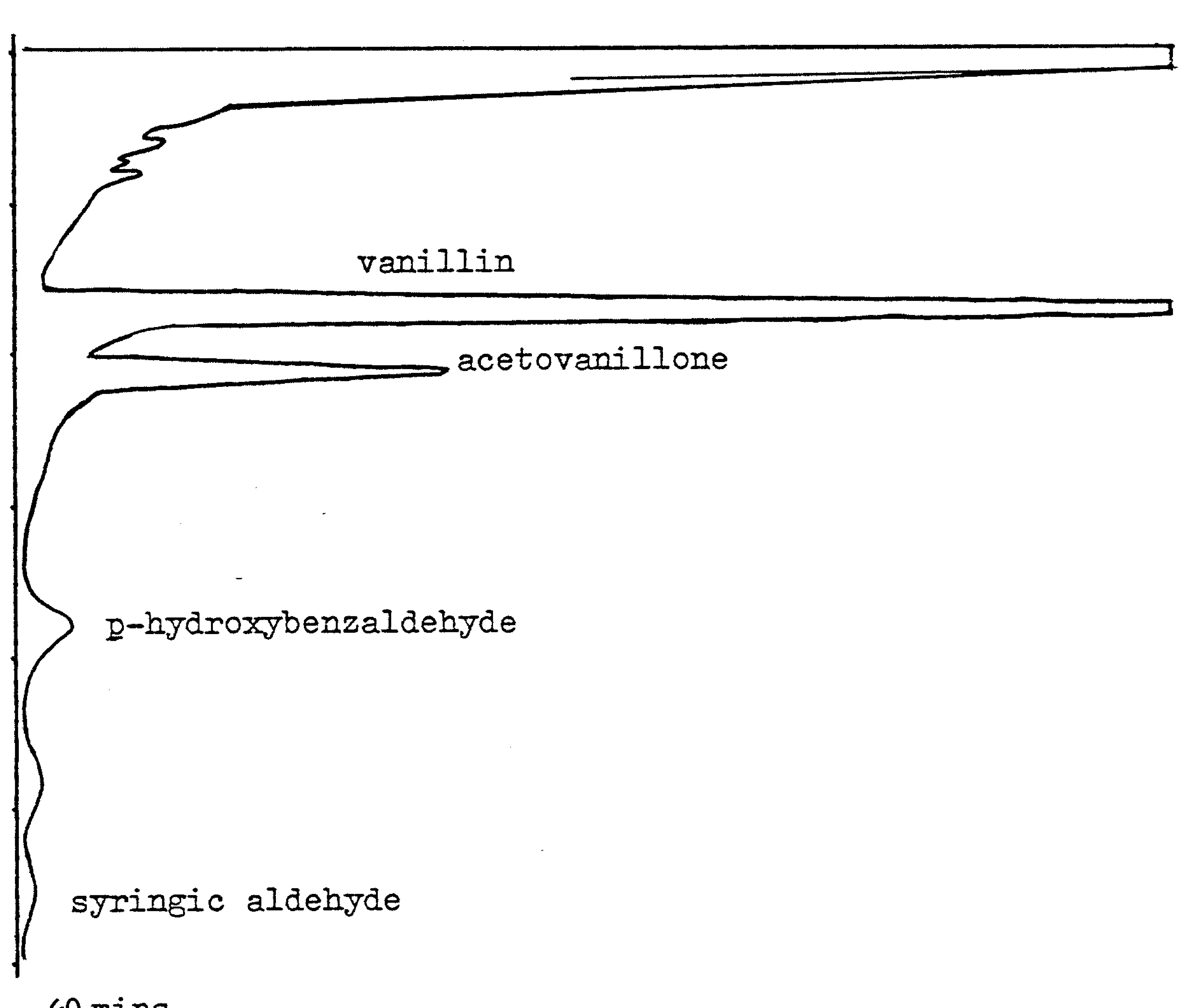


Table 2

The major lignin oxidation products from the Cycadales.

Unless otherwise stated 4µl injections were used.

	GYMNOSPERMS	Major oxidation products. mg/gm of woodmeal			
		p-OH	VAN	ACETO	SYR
	Cycadaceae				
1	Zamia furfuraceae - petiole	0.05	0.69	0.09	0.06
2		0.04	0.56	0.07	0.04
1	Zamia furfuraceae - leaves	0.13	1.35	2.81	0.41
1	Cycas revoluta - petiole	0.26	4.42	0.94	absent
2		0.43	1.84	0.71	absent
3		*0.26	3.50	1.30	0.06
1	Cycas revoluta - leaves	0.43	2.17	0.24	trace
1	Eucephalartos lebomboensis	0.22	1.85	0.25	trace
2	-petiole	0.21	1.61	0.16	trace

* 6µl injection

Figure (32)

A typical chromatogram of the lignin oxidation products of one of the Coniferales.

Juniperis communis woodmeal, 2µl injection. (OV 101 column).

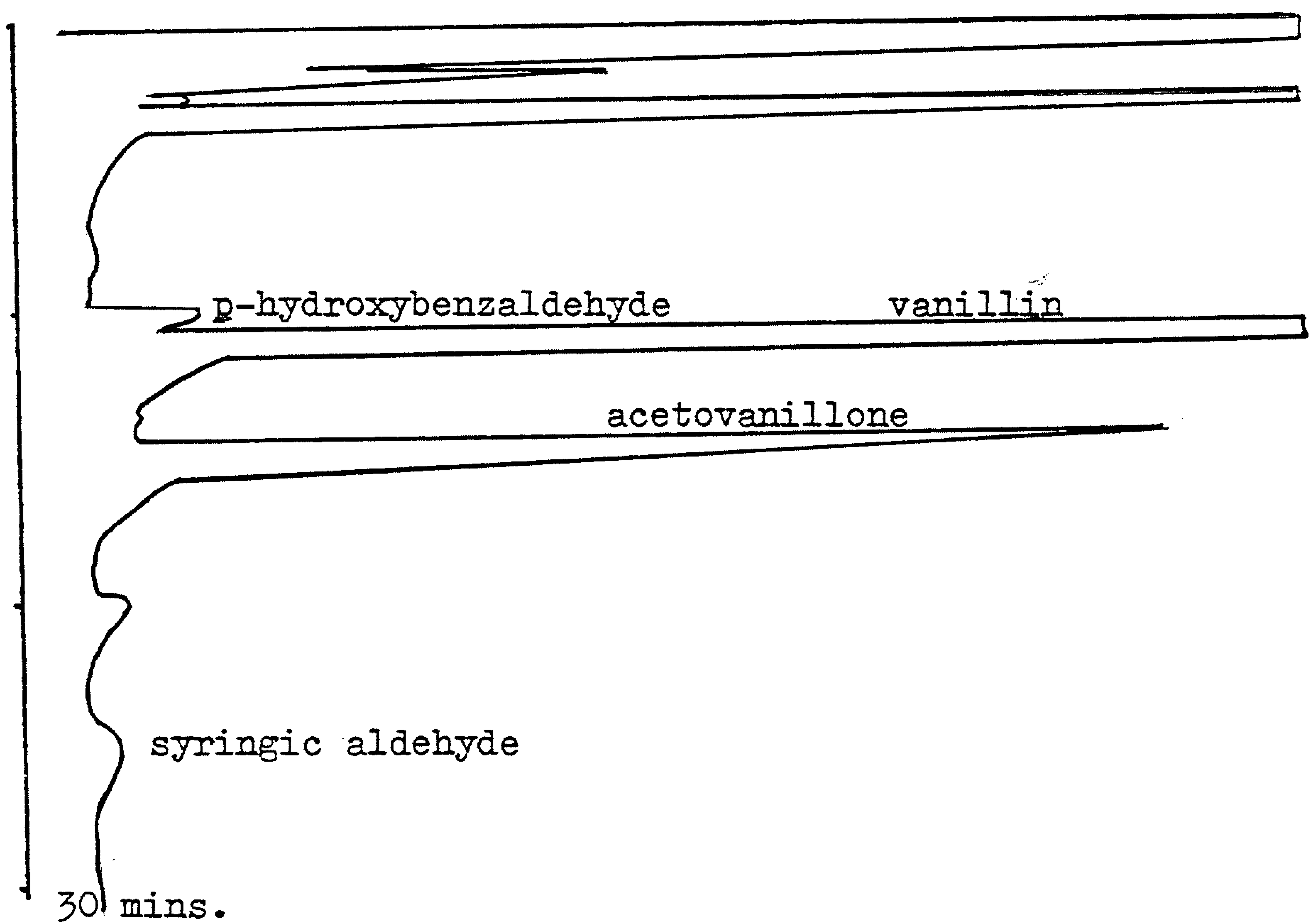


Table 3

The major lignin oxidation products from the sapwood of Gymnosperms.

Unless otherwise stated 1μl injections were used.

	GYMNOSPERMS	Major oxidation products. mg/gm of woodmeal			
		p-OH	VAN	ACETO	SYR
	Ginkgoaceae				
1	Ginkgo biloba	1.10	12.60	4.00	0.15
2		1.32	15.70	4.20	0.15
	Araucariaceae				
1	Araucaria araucana	0.60	15.80	3.00	0.30
2		*0.30	10.12	3.19	0.42
	Podocarpaceae				
1	Podocarpus andinus	0.07	14.70	3.60	0.15
2		0.22	14.70	4.20	0.45
	Cupressaceae				
1	Juniperis communis	0.60	9.30	2.25	absent
2		*0.47	13.80	3.30	0.15
3		*0.34	13.42	4.42	0.04
	Taxodiaceae				
1	Sequoia semperivirens	0.05	9.90	3.30	0.60
2		0.03	9.00	2.10	0.30
1	Sciadopitys verticillata	0.11	4.72	0.82	absent
2		1.20	7.50	0.90	absent
3		0.82	4.20	0.45	absent
4		*0.86	6.54	2.06	0.01
1	Taxodium distichum	0.76	6.62	1.92	absent
2		*0.85	7.83	2.34	0.45
1	Cryptomeria japonica	0.71	6.82	2.42	0.20
2		*0.94	8.65	2.42	0.35
	Pinaceae				
1	Abies alba	0.15	13.50	3.00	0.07
2		*0.15	11.55	3.60	0.03
1	Picea abies	0.60	6.22	1.50	absent
2		*0.60	8.47	3.04	0.03

continued

Table 3 continued.

	GYMNOSPERMS	Major oxidation products. mg/gm of woodmeal p-OH	VAN	ACETO	SYR
	Pinaceae				
1	*Larix decidua*	0.22	9.90	1.60	0.15
2		0.24	9.00	1.60	0.15
1	*Pinus sylvestris*	0.60	11.10	2.10	absent
2		0.22	7.87	1.87	absent
3		*0.41	5.32	2.02	0.02
1	*Pinus sylvestris*	'0.09	1.29	0.26	absent
2	-leaves	'0.07	1.06	0.26	absent
	Taxaceae				
1	*Taxus baccata*	0.04	3.00	0.52	absent
2		0.06	6.60	0.60	0.22
3		*0.41	8.55	0.23	0.22
	Gnetaceae				
1	*Gnetum scandens*	'0.51	2.41	0.42	1.43
2		'0.46	2.31	0.53	1.35

* 2µl injection
' 4µl injection

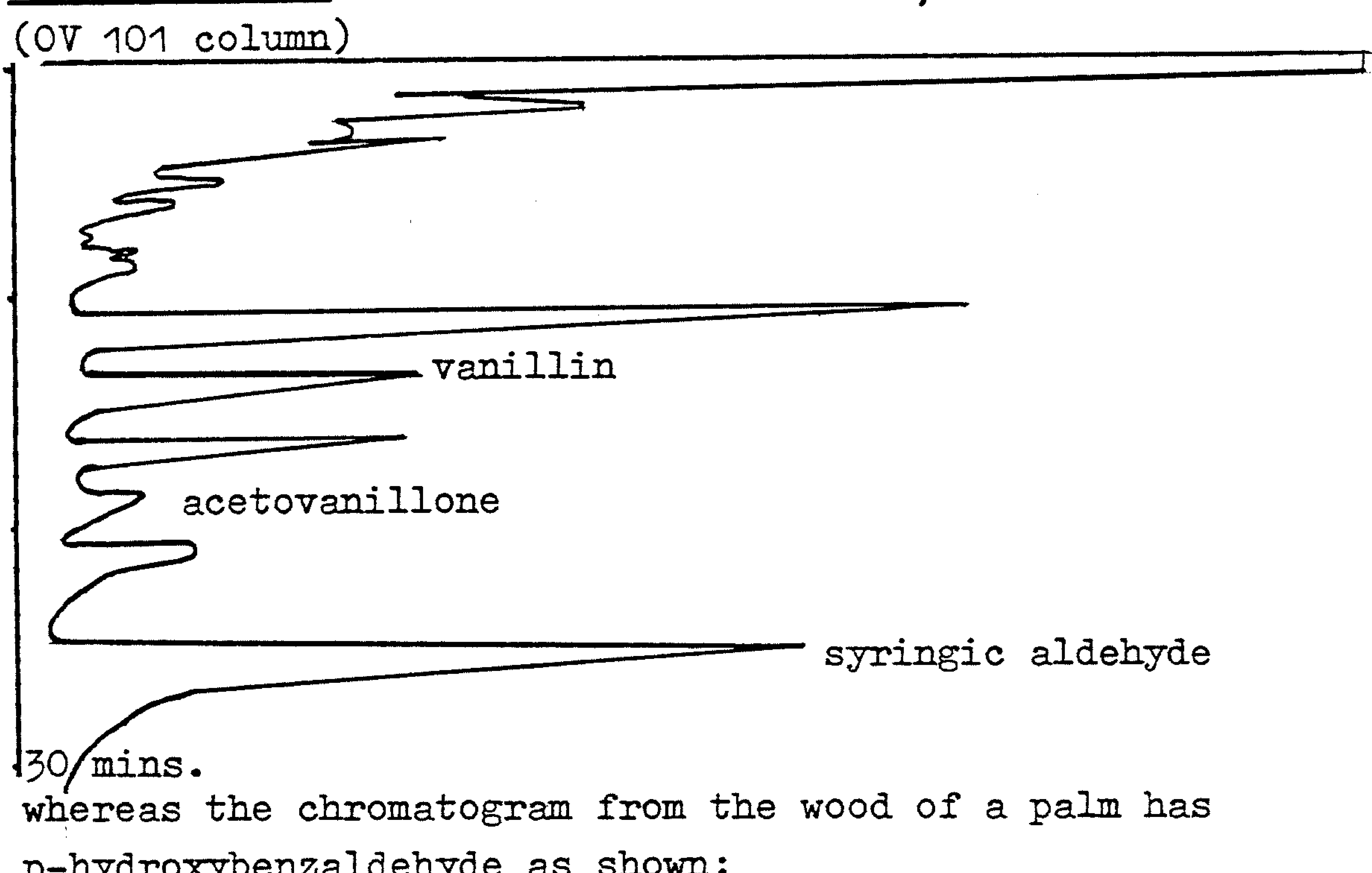
Results with Angiosperms.

Samples from living angiosperms were oxidized using the cupric oxide method described previously and chromatographed quantitatively and qualitatively.

Figures (33) and (34).

A typical chromatogram obtained from one of the monocotyledon fibres.

Agave sisalana fibres from the leaves, 2μl injection. (OV 101 column)



whereas the chromatogram from the wood of a palm has p-hydroxybenzaldehyde as shown:

Sabal palmetto woodmeal, 2μl injection. (5% Pdegs column)

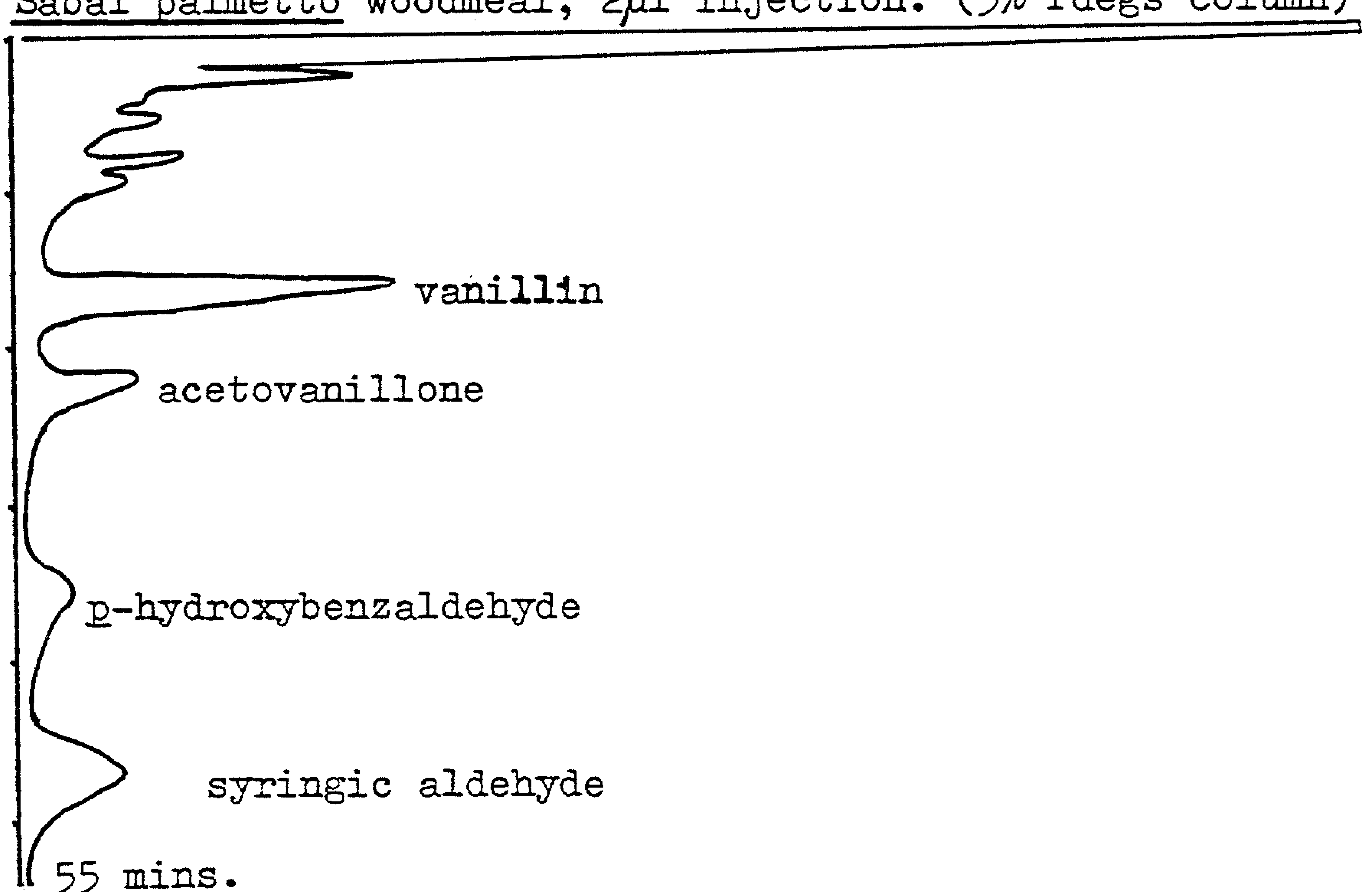


Table 4

The major lignin oxidation products from fibres and wood of Monocotyledons.

Unless otherwise stated 2µl injections were used.

	ANGIOSPERMS MONOCOTYLEDONS	Major oxidation products. mg/gm of woodmeal			
		p-OH	VAN	ACETO	SYR
	Agavaceae				
1	*Agave sisalana*	absent	1.12	0.37	5.96
2		absent	1.20	0.45	6.52
1	*Phormium tenax*	absent	1.05	0.45	4.05
2		absent	1.36	0.64	4.72
1	*Furcraea gigantea*	absent	2.70	0.67	10.50
2		absent	2.06	0.45	8.92
	Cocoideae				
1	*Cocos nucifera*	absent	3.92	0.75	5.40
2		absent	2.61	1.17	4.71
	Sabalineae				
1	*Sabal palmetto*	0.28	1.59	0.84	1.47
2		0.18	1.90	1.14	2.61
	Bambuseae				
1	*Bambusa* species	2.77	5.40	0.45	2.40
2		1.80	3.00	0.45	1.05
3		1.80	3.00	0.30	1.05

Larger injections of 10µls were made using *Agave sisalana* and *Phormium tenax* but no p-hydroxybenzaldehyde was detected.

Agave sisalana, *Phormium tenax* and *Furcraea gigantea* fibres were taken from the leaves whereas *Cocos nucifera* fibres were taken from the drupe. Woodmeal from the stalks of *Sabal palmetto* and the species of *Bambusa* were used.

Figure (35).

A chromatogram of an immature dicotyledon:

Magnolia grandiflora woodmeal, 2µl injection. (5% Pdegs column)

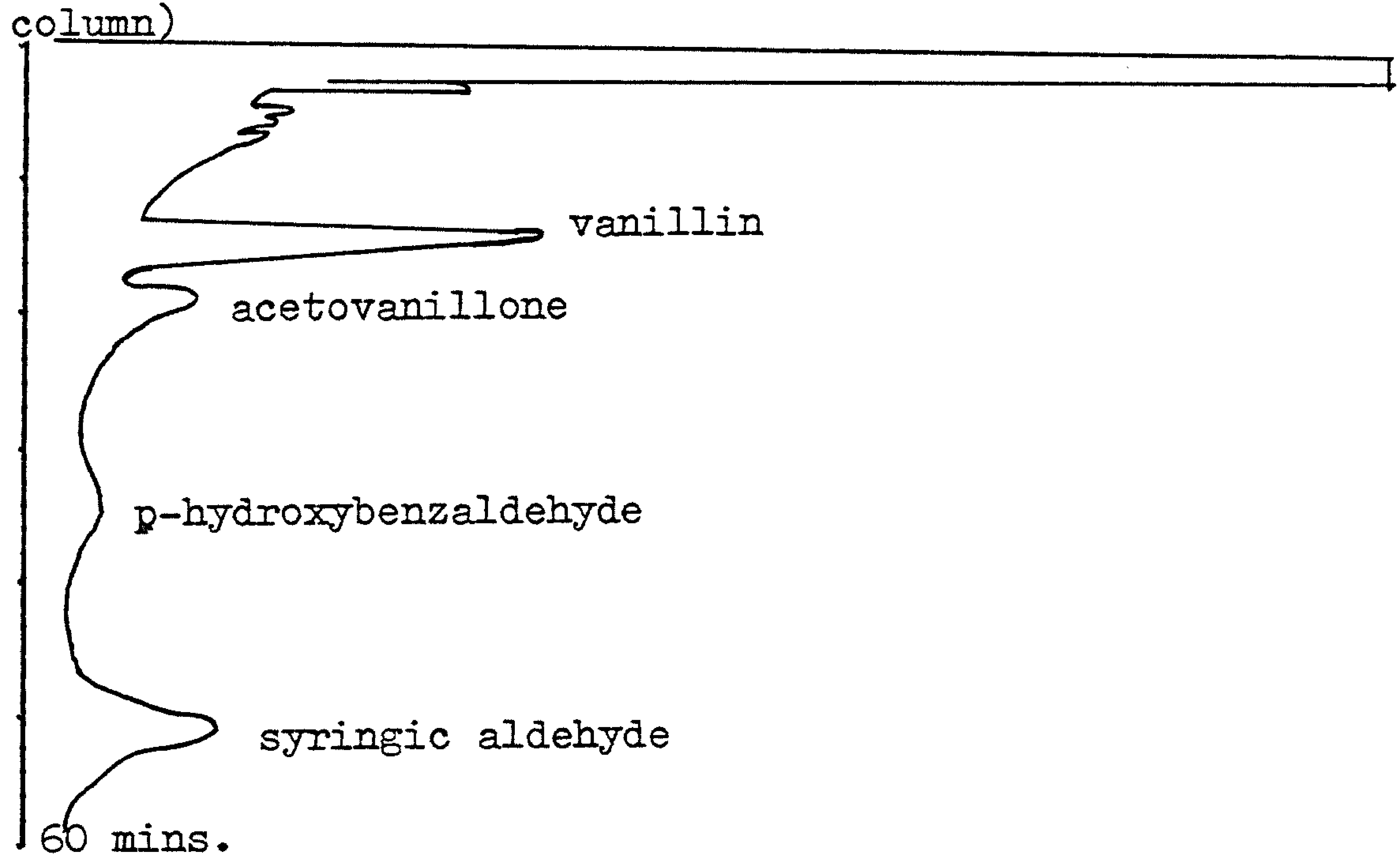


Figure (36).

A typical chromatogram of a mature dicotyledon:

Magnolia soulangeana woodmeal, 2µl injection. (5% Pdegs column)

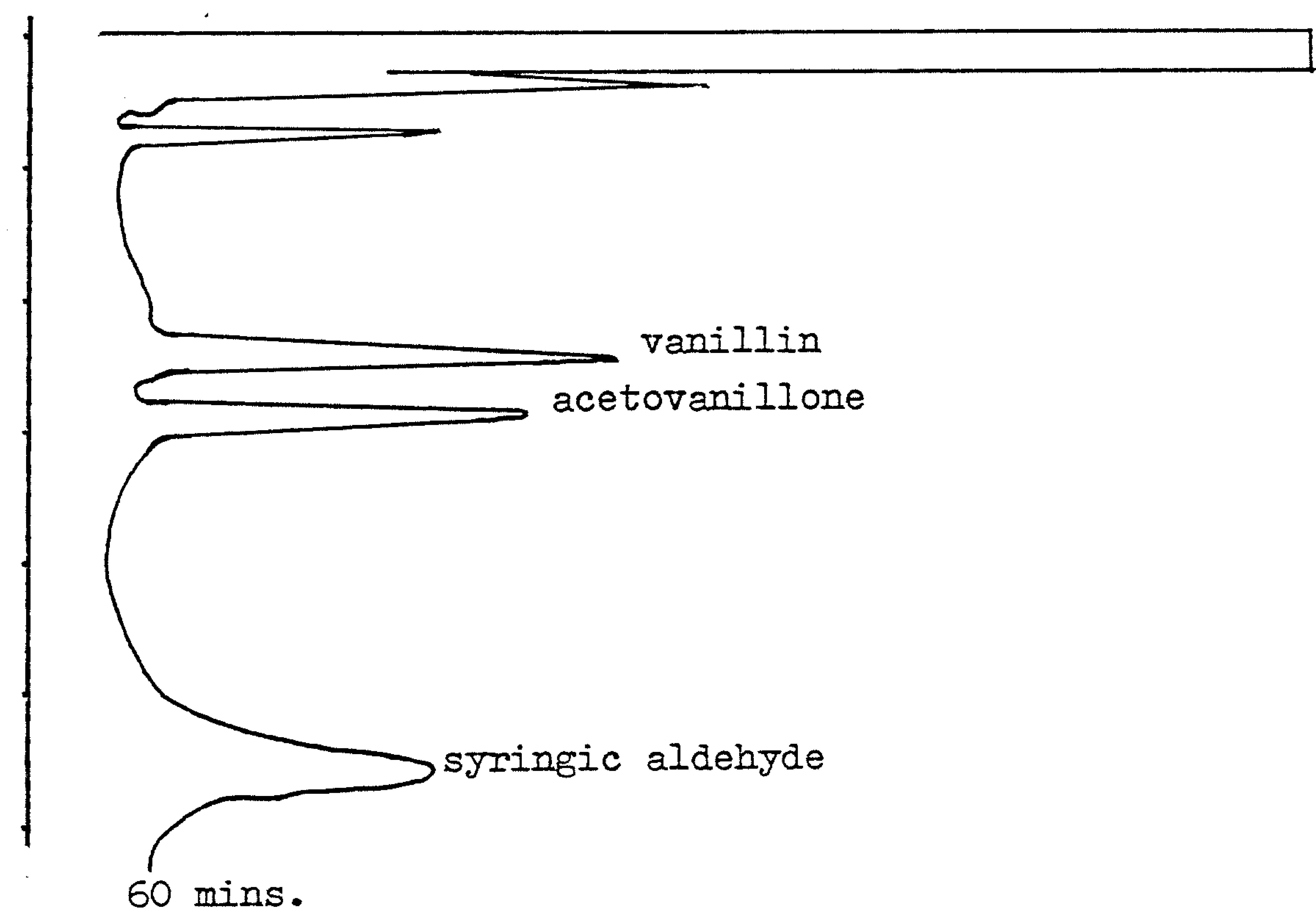


Table 5

The major lignin oxidation products from the sapwood of Dicotyledons.

Unless otherwise stated 2µl injections were used.

	ANGIOSPERMS	Major oxidation products. mg/gm of woodmeal			
	DICOTYLEDONS	p-OH	VAN	ACETO	SYR
	Magnoliaceae				
1	Liriodendron chinese	*trace	1.65	0.19	5.25
2		*trace	2.18	0.30	7.65
1	Liriodendron tulipiraflora	*0.04	1.65	0.19	5.25
2		*0.04	1.20	0.26	4.57
1	Magnolia kobus	*trace	2.10	0.30	6.67
2		*trace	2.70	0.37	8.55
1	Magnolia kobus-leaves	'0.18	1.00	0.44	1.09
1	Magnolia grandiflora	*0.06	1.27	0.19	2.77
2		*0.04	1.05	0.15	2.32
1	Magnolia soulangeana	absent	4.76	2.21	6.97
2		absent	5.51	1.69	9.37
	Tilaceae				
1	Tilia x europeae	*trace	2.10	0.22	6.45
2		*trace	1.92	0.32	5.32
	Ulmaceae				
1	Ulmus procera	absent	4.31	1.69	9.45
2		absent	5.07	2.85	7.44
1	Betula pendula	absent	2.80	0.50	5.00
2		absent	2.70	0.60	5.90
3		absent	3.00	0.30	6.60
	Fagaceae				
1	Quercus robur	absent	2.50	0.60	5.40
2		absent	3.00	0.60	8.40

* Twigs were used ranging from 2-4 years instead of mature wood.

' 4µl injection

No p-hydroxybenzaldehyde was found to be present in Quercus robur or Ulmus procera when 10µl injections were made.

Bark.

As the wood from trees was oxidized in order to obtain lignin oxidation products it was therefore interesting to find out what oxidation products would be obtained from the bark or cork layer. One coniferous bark and one angiospermous bark was chosen together with a laboratory cork. All procedures were the same as for the woods. 4µl injections were used.

Table 6. The lignin oxidation products from Barkmeal.

		Major oxidation products. mg/gm of barkmeal			
	BARK	p-OH	VAN	ACETO	SYR
	Gymnospermous				
1	Sciadopitys verticillata	0.28	1.25	1.06	0.16
2		0.25	1.21	1.14	0.19
	Angiospermous				
1	Betula pendula	0.57	2.28	0.49	1.75
2		0.39	2.25	0.55	2.11
1	laboratory cork	0.09	2.95	2.08	0.16
2		0.07	2.37	1.43	0.12

Discussion :Spermatophytes.

Acetovanillone together with the phenolic aldehydes p-hydroxybenzaldehyde, vanillin and syringic aldehyde were obtained when the lignin macromolecule was oxidized with alkaline cupric oxide using the method described by Pepper, Casselman and Karapally (1967). The phenolic aldehydes were identified and estimated by gas chromatography. Throughout the discussion the phenolic aldehydes are expressed as a percentage of the total aldehyde content. This follows the mode of presenting the data introduced by Creighton, Gibbs and Hibbert (1944). The total aldehyde content does not include the phenolic ketone acetovanillone.

Gymnosperms.

Tables 2 and 3 list the major oxidation products obtained from the lignin of a variety of gymnosperms. In the examples taken, the lignin was largely in the form of secondary xylem. Since the gymnosperms comprise such a large group of plants the sampling is incomplete: however the species were selected to represent a good cross-section of the group.

The phenolic aldehyde vanillin was the major constituent detected on oxidizing the lignin of the gymnosperms. In the Coniferales the average amount of vanillin detected was 94% of the total aldehyde content. The yield of vanillin from the gymnosperms found here is slightly lower than that found by previous authors (Freudenberg, Lautch and Engler, 1940; Creighton, Gibbs and Hibbert, 1944 and Towers and Gibbs, 1953). This is because acetovanillone is largely converted into vanillin when nitrobenzene is used as the oxidant whereas if cupric oxide oxidations are used this does not occur (Pepper, Casselman and Karapally, 1967). Acetovanillone was observed as an oxidation product for all the gymnosperms that were examined here. Indeed, throughout the present work, if vanillin was detected, so too was acetovanillone.

p-Hydroxybenzaldehyde was detected in small amounts for all the gymnosperms. Amounts ranging from 0.3% to 13% (average =5.4%) of the total aldehyde content were found. This confirms the earlier results (Leopold and Malmstrom,1952; Towers and Gibbs,1953; Pepper, Casselman and Karapally,1967 and Leo and Barghoorn,1970). Leopold and Malmstrom (1952) found that the highest yields of p-hydroxybenzaldehyde were given by members of the families Taxodiaceae and Pinaceae together with certain members of the family Cupressaceae. In the present work a higher percentage was detected in Sciadopitys verticillata, a member of the Taxodiaceae, with an average of 8.3% of the total aldehyde content. The species in the families Pinaceae and Cupressaceae which were examined here did not produce a high yield of p-hydroxybenzaldehyde. However Ginkgo biloba, regarded as a more 'primitive' member of the gymnosperms, had an above average proportion of the aldehyde p-hydroxybenzaldehyde (7.8%).

According to the literature, the occurrence of syringic aldehyde in the oxidation products is still uncertain. It is generally accepted that apart from one or two exceptions, (the Gnetales, Tetraclinis articulata and certain species of Podocarpus) the gymnosperms do not contain syringyl units (Creighton, Gibbs and Hibbert, 1944; Towers and Gibbs,1953; Harborne,1973 and Higuchi, 1980). Creighton, Gibbs and Hibbert (1944) and Leopold and Malmstrom (1952) reported the presence of syringic aldehyde in Tetraclinis articulata. The presence of syringic aldehyde, in the Gnetales was reported by Creighton, Gibbs and Hibbert (1944) confirming the earlier work of Crocker (1933). Creighton, Gibbs and Hibbert (1944) suggested a relationship to the angiosperms rather than to the gymnosperms was indicated. In the present work syringic aldehyde was obtained from Gnetum scandens as 32% of the total aldehyde content. Species of Podocarpus have been found to vary in the

amount of syringic aldehyde from 0% to 50% of the total aldehyde content (Creighton, Gibbs and Hibbert, 1944; Leopold and Malmstrom, 1952; Towers and Gibbs, 1953 and Kawamura and Higuchi, 1964). In the present work Podocarpus andinus was found to contain small amounts of syringic aldehyde, (1.2%).

Reports of small quantities of syringic aldehyde from lignin oxidations of other gymnosperms have been sporadic and contradictory. Leopold and Malmstrom (1952) reported the presence of syringic aldehyde in certain conifers notably the Pinaceae. Syringic aldehyde has also been reported in Pinus sylvestris by Ibrahim, Towers and Gibbs (1962) and evidence for syringyl units in this species has been obtained from chemical ionization mass spectroscopy by Metzer (1970). Leo and Barghoorn (1970) reported less than 0.01% of syringic aldehyde in Araucaria bidwillii and Cedrus deodora. In 1962 Pepper, Manolopoula and Burton detected the presence of syringic aldehyde in spruce although Morrison (1963) did not record it. In the present work syringic aldehyde was found to be present in small quantities in the oxidation products from all the gymnosperms which were examined as is shown in tables 2 and 3. In certain cases the standard 1 µl injections were not sufficient to detect the small amounts of syringic aldehyde present so a larger injection of 2 µl was used. The quantities of syringic aldehyde detected ranged from 0.2% to 5% of the total aldehyde content. During the extraction procedure it was found that syringic aldehyde was preferentially lost to the extent of 13%, (Chapter 3). Therefore the relative amount of syringyl units actually present from the lignin of gymnosperms is likely to be somewhat underestimated by this procedure. Various schematic structures, such as Freudenberg's (1965) model (Chapter 1) have been put forward for coniferous lignins.
The presence of syringyl units in the lignin of coniferous woods should therefore be taken into consideration when

attempting to establish the macromolecular structure of coniferous lignin.

Lignin oxidation products were examined from the leaves of *Pinus sylvestris*, *Zamia furfuraceae* and *Cycas revoluta*. Both *Z. furfuraceae* and *C. revoluta* leaves gave small amounts of syringic aldehyde as well as *p*-hydroxybenzaldehyde, vanillin and acetovanillone. With the leaves of *Pinus sylvestris*, syringic aldehyde was not detected. Obviously more information is needed concerning the oxidation products obtained from leaves. With *Zamia furfuraceae* the leaves were found to give a greater yield of oxidation products than from the petiole, presumably because the latter is less rich in lignin.

Ibrahim, Towers and Gibbs (1962) found a relationship between the phenolic acids in hydrolyzed extracts of the leaves in a range of plants as well as in their lignin residues. They found in general that lignins of gymnosperms which did not give syringic aldehyde did not have syringic or sinapic acid in their leaves. As our results show the presence of syringyl units in the wood of all the gymnosperms examined (and in certain leaves as well) it seems probable that syringic and sinapic acid will be present, in minute amounts. Clearly more investigations are necessary using more sensitive methods of analysis with respect to the correlation of lignin oxidations of wood and phenolic constituents of the leaves of gymnosperms.

Syringic aldehyde was detected in small amounts in the cycads examined. Larger injections (6 µl) were necessary because the cycads are less 'woody' than the other members of the gymnosperms. Kawamura and Higuchi (1964) had previously detected very small quantities of syringic aldehyde in *Cycas revoluta*.

Mass Spectral Interpretations.

The mass spectra of the authentics (*p*-hydroxybenzaldehyde, vanillin, acetovanillone and syringic

aldehyde, figures(37) to(40)are presented together with the mass spectra obtained from the major lignin oxidation products of the gymnosperm Sequoia semperivirens, table 7 and figures (41) to (43). The structural assignment of p-hydroxybenzaldehyde, vanillin, acetovanillone and syringic aldehyde based on the interpretation of fragmentation patterns is shown in Appendix 2.

Figure (37) p-hydroxybenzaldehyde.

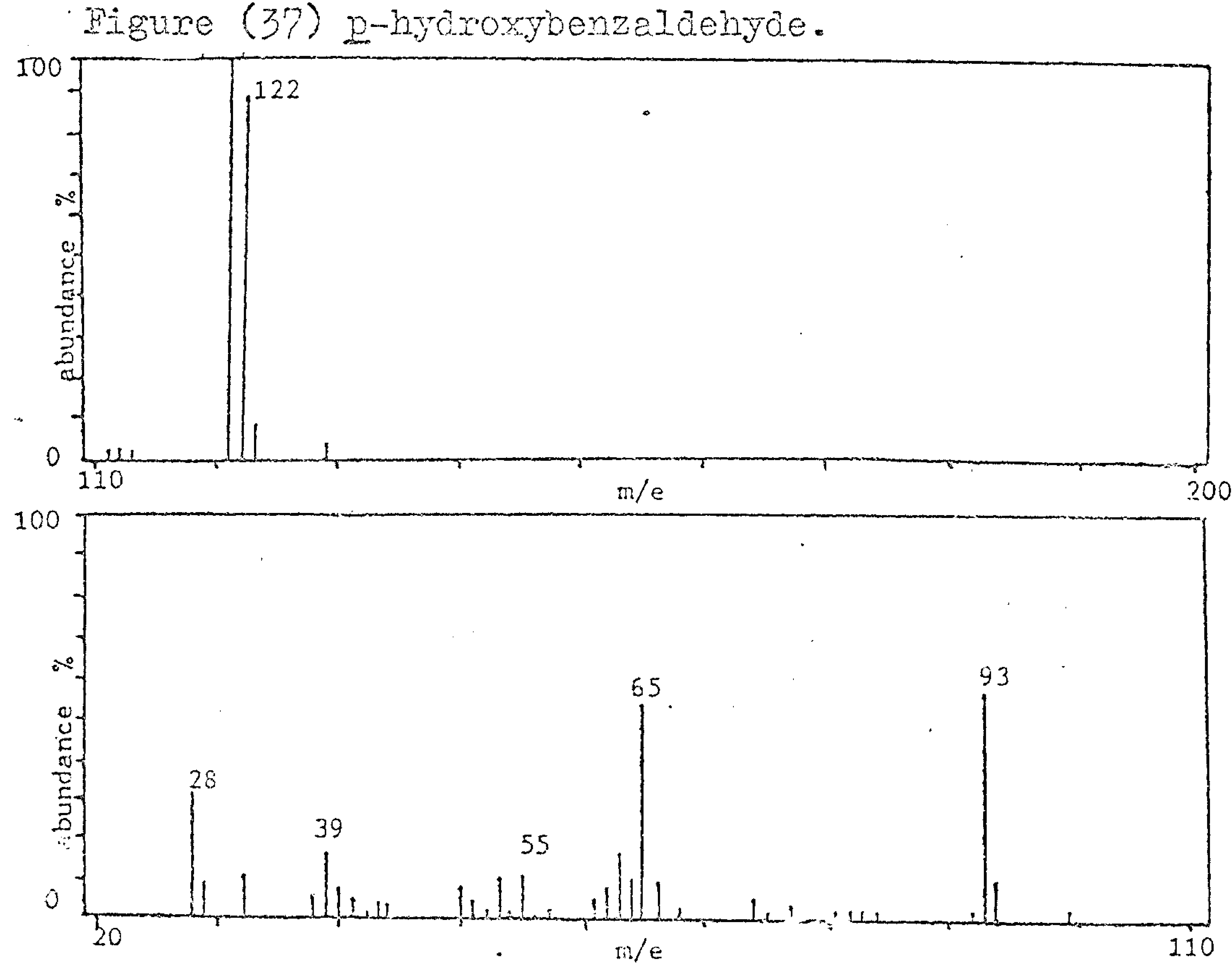


Figure (38) vanillin.

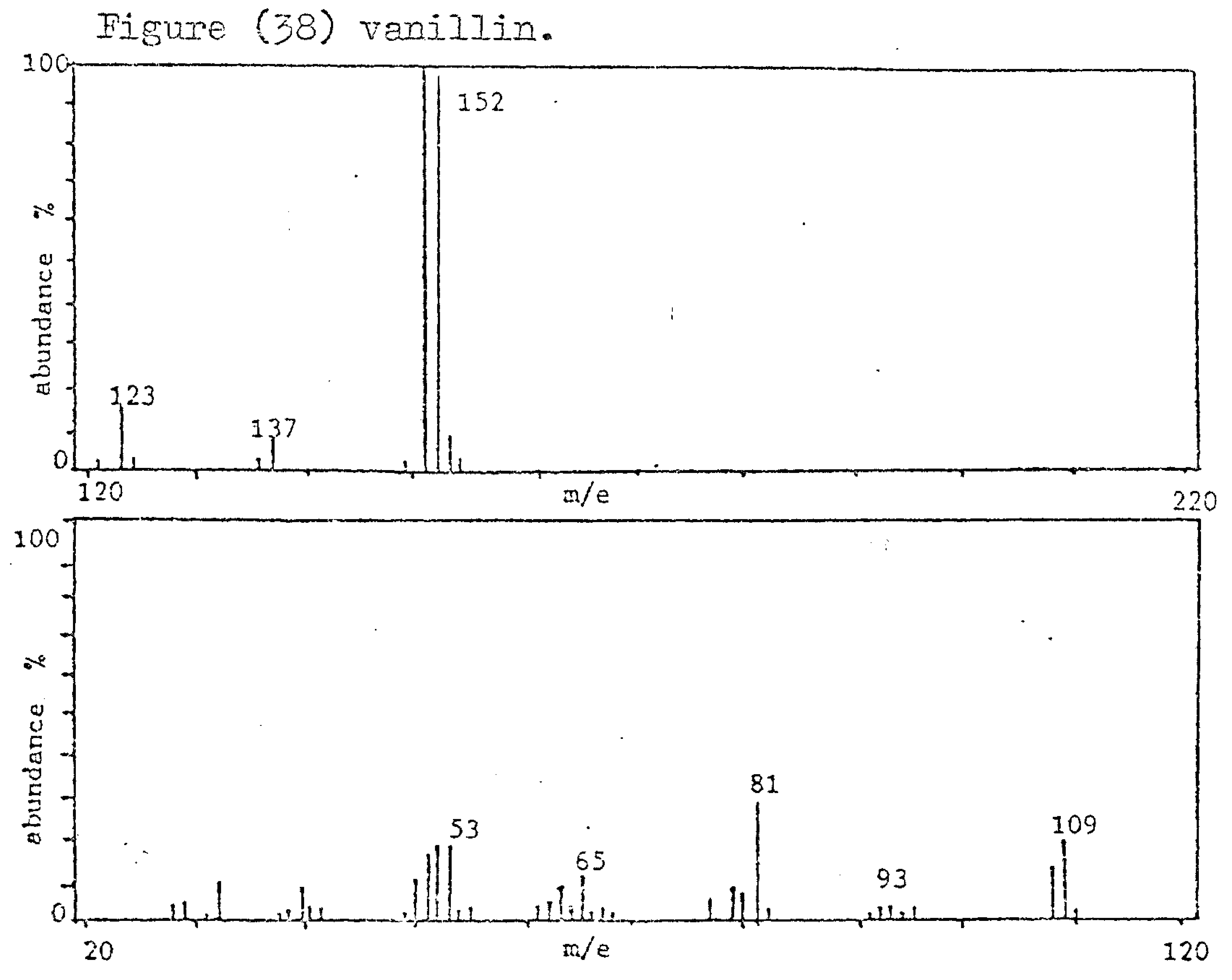


Figure (39) acetovanillone.

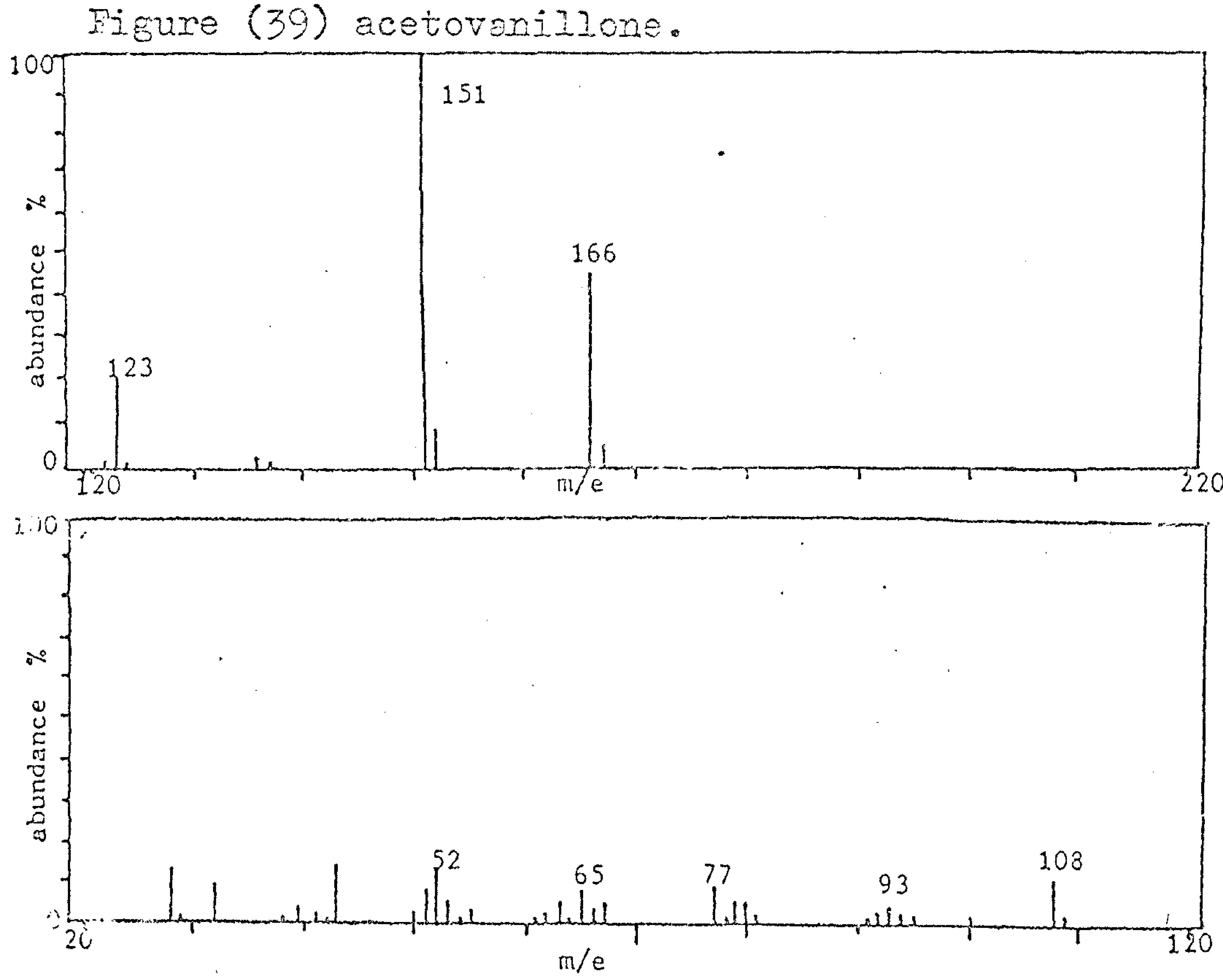


Figure (40) syringic aldehyde.

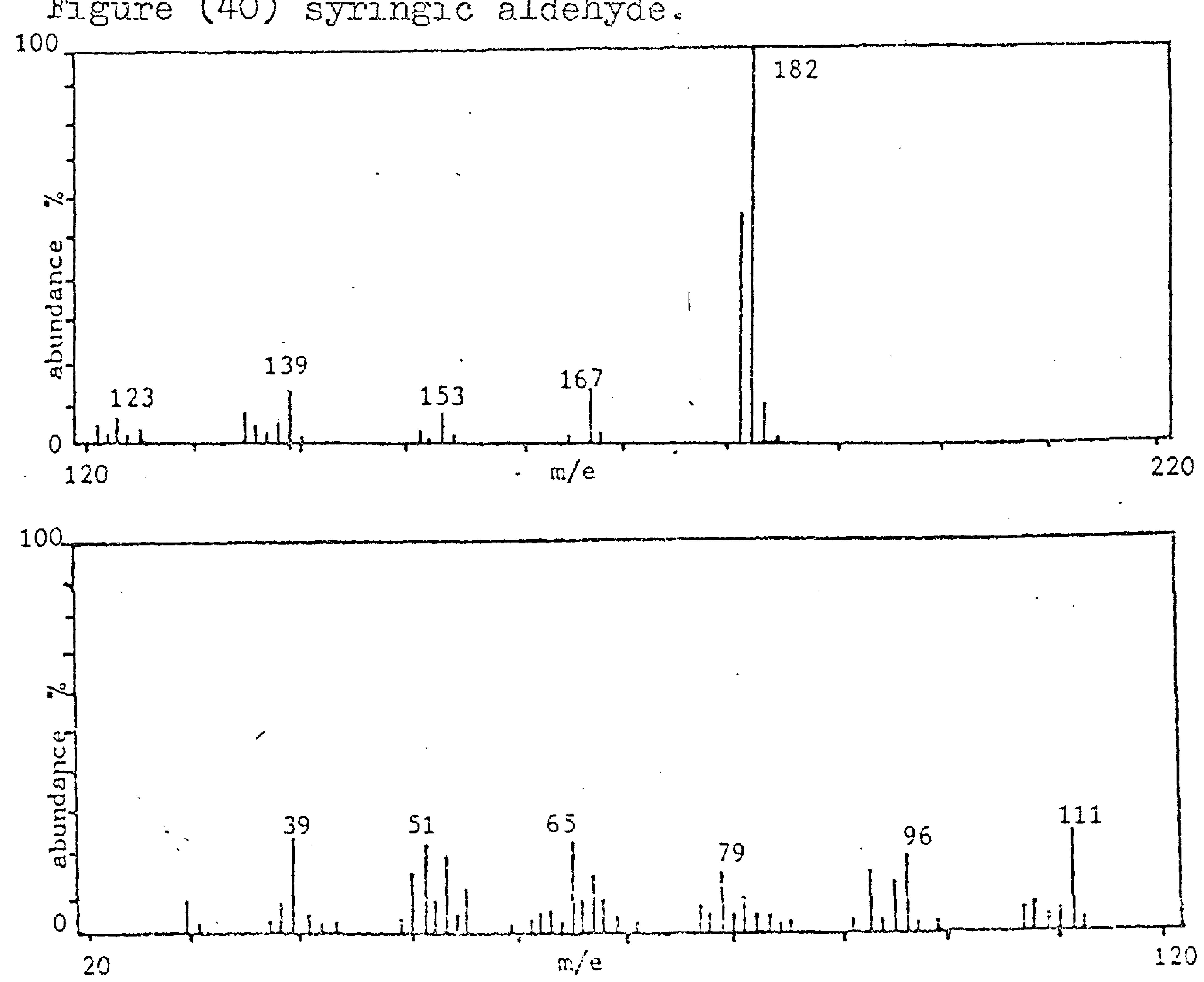


Table 7

G.C./M.S. of Oxidation Products from Lignin of S. semperivirens.

A.E.I. M.S. 30 double focusing mass spectrometer integrated with a Pye Unicam model 104 G.C. (3% OV 101 on Gas Chrom Q).

Retention Time(min.)	Mass * Spectrum m/e (relative abundance%)	M^+	Assigned molecular formulae.
26	83(66),70(39),57(34),56(36), 53(100),41(50).		
30	123(2),124(84),109(100),81(96), 55(30),28(34).	124	$C_7H_8O_2$ requires M=124 o or p methoxyl phenol. HO MeO ***
34	124(81),109(100),81(96),55(48), 53(30),32(65),28(100).		
35	98(54),57(100),55(32),41(43).		
39	111(78),82(100),68(64),55(31), 54(74),53(35),32(100),28(100).		
42	113(100),112(57),111(42),98(36), 84(70),82(59),68(41),57(34),56 (100),53(67),66(56),44(33),42 (73),41(42),39(38),32(100), 28(31).		
46	153(11),152(98),151(100),137 (9),123(25),109(32),108(10), 93(6),81(56),80(11),79(20), 77(13),67(9),65(21),63(20), 53(43),52(32),51(18),50(19), 40(12),39(16),32(100),28(100).	152	$C_8H_8O_3$ requires M=152 vanillin. HO CHO MeO **Figure 41

*For unidentified components only ions with a relative abundance greater than 30% are quoted for m/e less than 150.

**Structure assigned on basis of comparison with mass spectrum of authentic compound(figure38).

***Tentative structural assignment based on interpretation of fragmentation pattern (Appendix 2).

Table 7 cont.

Retention Time(min.)	Mass Spectrum m/e (relative abundance%)	M^+	Assigned molecular formulae.
72	167(3),166,(58),151(100),136(10), 123(35),108(13),93(5),77(22), 65(22),53(19),52(35),43(36), 32(100),28(6).	166	$C_9H_{10}O_3$ requires M=166 acetovanillone. CH_3 / C=O / ring / OH, OMe **Figure 42
129	183(4),182(31),167(2),153(2), 150(7),145(7),136(4),135(37), 125(29), 123(9),107(33),98(17), 94(57),93(63),91(31),85(12), 79(42),77(29),71(20),69(14), 67(31),57(100),55(17),45(16), 42(59).	182	$C_9H_{10}O_4$ requires M=182 syringic aldehyae MeO / HO ring CHO / MeO **Figure 43

**Structure assigned on basis of comparison with mass spectrum of authentic compound(figure 39 and 40).

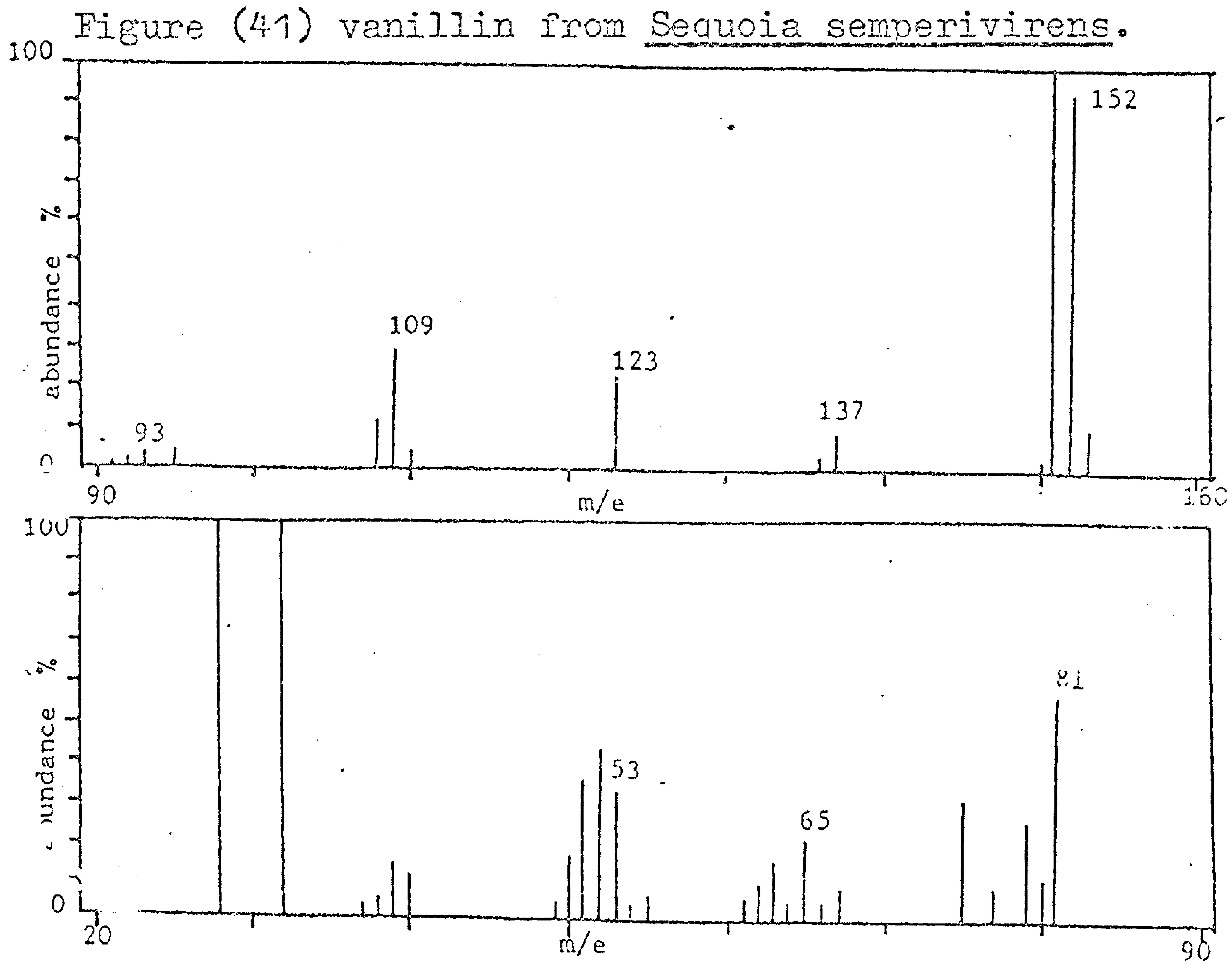


Figure (41) vanillin from *Sequoia semperivirens*.

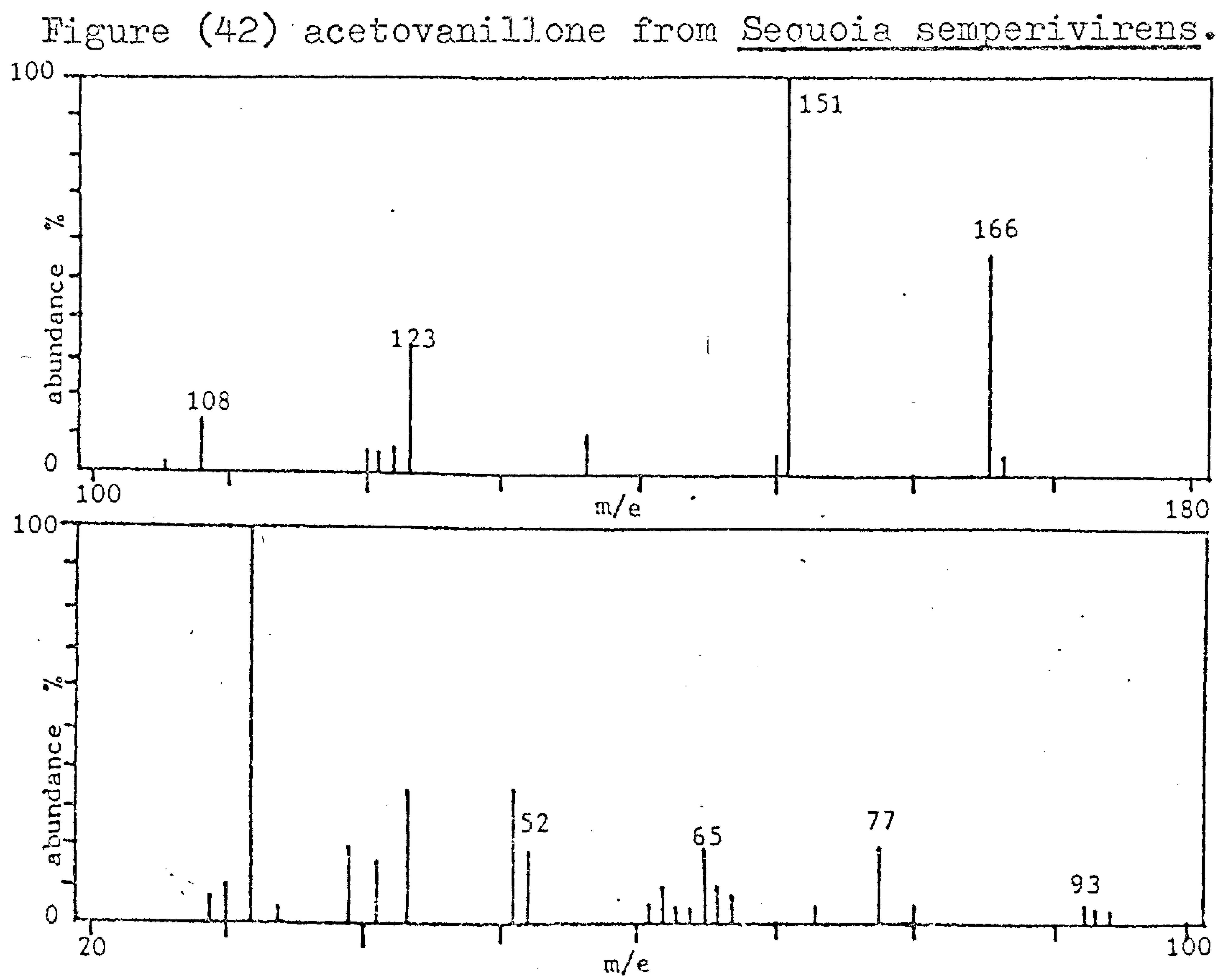
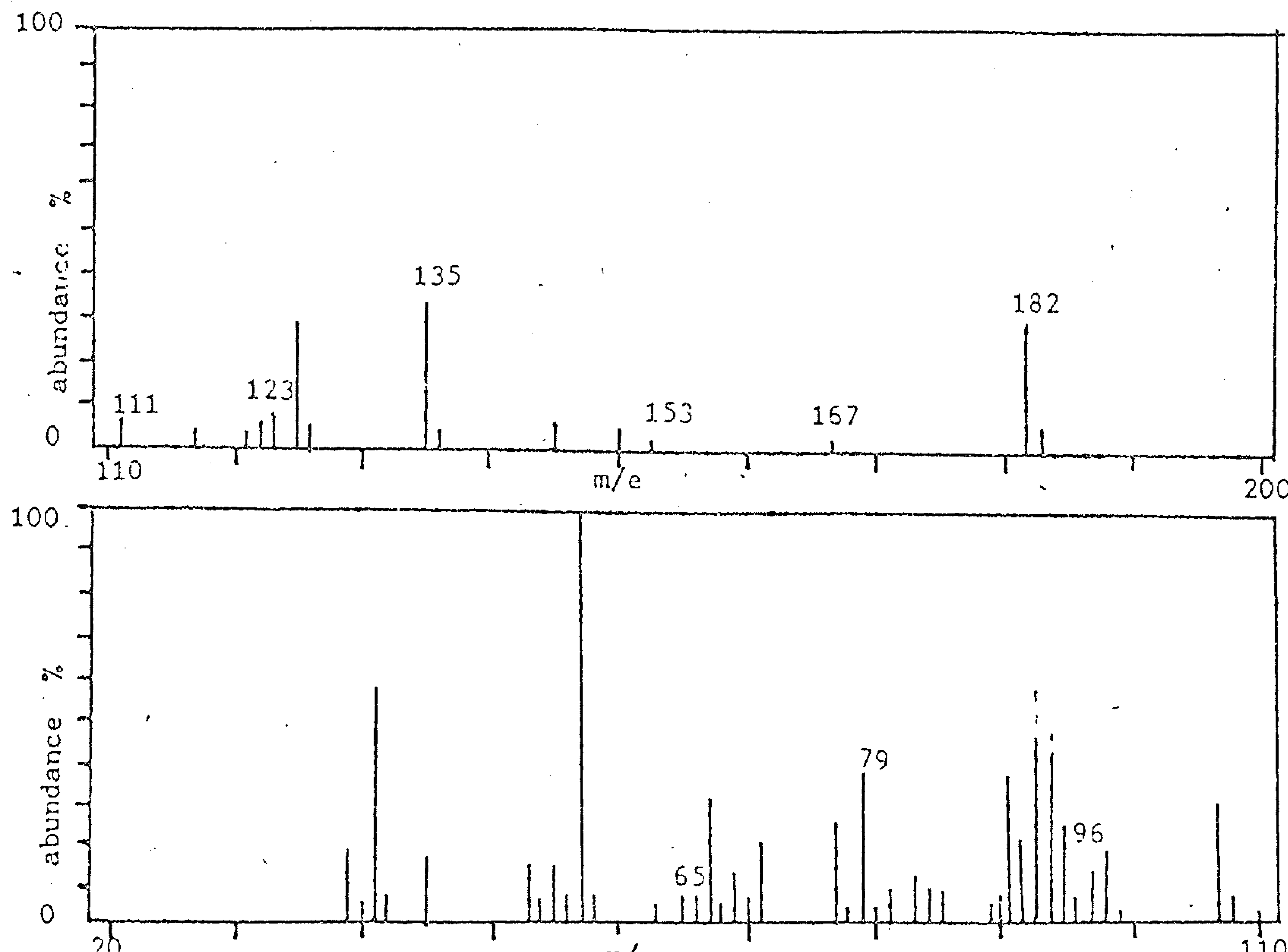


Figure (42) acetovanillone from *Sequoia semperivirens*.

Figure (43) syringic aldehyde from Sequoia semperivirens.



Angiosperms : Monocotyledons.

Vanillin together with a high proportion of syringic aldehyde and p-hydroxybenzaldehyde has been reported from monocotyledons belonging to the grass family (Creighton and Hibbert, 1944; Morrison, 1958; Gardner and Menzel, 1974 and Hedges and Parker 1976). Towers and Gibbs (1953) and Higuchi , Ho and Kawamura (1967) both observed that p-hydroxybenzaldehyde was given by other monocotyledons and was not restricted to the grass family. Table 4 lists the major oxidation prpducts from a range of monocotyledons and includes leaf and fruit fibres as well as woodmeal from the trunks of the palm Sabal palmetto and a Bambusa species. Vanillin was detected in all the species examined ranging from a higher proportion in the woodmeal of the Bambusa species (52% of the total aldehyde content) to lower proportions in the leaf fibres of the Agavaceae family, (20% of the total aldehyde content). The highest proportion of syringic aldehyde was found to be present in the fibres of the Agavaceae (80% of the total aldehyde content) whereas in the woodmeal from the Bambusa species only 20% of the total aldehyde content was syringic aldehyde.

The lignin oxidation products of monocotyledons are generally accepted as having a vanillin to syringic aldehyde ratio of 1:1 (Creighton and Hibbert, 1944; Sarkanen and Ludwig, 1971 and Harborne 1973). In the present work this ratio is approached in one species only, Sabal palmetto.

p-Hydroxybenzaldehyde was only found with the woodmeal of the Bambusa species and Sabal palmetto, but interestingly it was not detected in experiments with the fibres of the leaves of the other monocotyledons examined. Even larger injections (10μl) did not reveal p-hydroxybenzaldehyde for Agave sisalana and Phormium tenax. The p-hydroxybenzaldehyde content for the Bambusa species was 28% of the total aldehyde content, a rather high value. Bamboo is in many classification systems regarded as a member of the grass family. Members of the grass family have been found

to give substantial amounts of p-hydroxybenzaldehyde when their lignin has been oxidized (Creighton and Hibbert, 1944; Gardner and Menzel, 1974; and Hedges and Parker, 1976).

The fibres of the leaves and coconut drupe were taken from the strengthening tissues which are composed of highly lignified sclerenchymatous cells. The proportion of the phenolic aldehydes and ketones obtained from the oxidation of the lignin from the sclerenchyma tissue appears to be different to that observed from the lignified vascular tissues of the monocotyledons as shown in table 4.

The monocotyledonous fibres from the leaves of the Agavaceae family and the fibres from the drupe of Cocos nucifera gave the lignin oxidation products vanillin, acetovanillone and syringic aldehyde. In the fibres the syringic aldehyde content was higher than that of the vascular tissue of other monocotyledons examined, ranging from 77% to 84% of the total aldehyde content. This may be due to the presence of different types of units present in the lignin of fibres and xylem tissue. As all the leaf fibres in the present study have come from one family it will be necessary to examine other leaf fibres from different families within the monocotyledons. However from the results obtained, it does seem possible that a slightly different type of lignin is present in fibres of monocotyledons compared to that obtained from lignin found in the vascular tissues of monocotyledons.

Mass Spectral Interpretations.

The mass spectra obtained from the major lignin oxidation products of the monocotyledons Agave sisalana and Bambusa species are presented in table 8, figures (44) to (47) and table 9 respectively.

Table 8

G.C./M.S. of Oxidation Products from Lignin of *Agave sisalana*.

A.E.I. M.S. 30 double focusing mass spectrometer integrated with a Pye Unicam model 104 G.C. (3% OV 101 on Gas Chrom Q).

Retention Time(min.)	Mass* Spectrum m/e (relative abundance%)	M^+	Assigned molecular formulae.
37	282(1),208(5),198(7),181(6),167 (4),160(5),155(6),85(53),71(73), 57(100),55(66),29(100).		
44	153(12),152(100),151(96),137(6), 123(18),109(20),108(10),91(3), 81(25),79(8),65(10),63(8),51 (14),52(15),53(15),39(8),29(100).	152	$C_8H_8O_3$ requires M=152 vanillin HO-(ring)-CHO MeO **Figure 44
57	212(5),207(7),170(1),168(2), 155(14),85(44),71(80),57(100), 43(33),41(33),32(41),29(100).		
72	184(1),170(2),167(2),166(23), 165(6),156(1),154(12), 153(7), 154(58),151(100),139(12),123 (20),109(11),108(11),96(7), 93(9),81(9),79(13),77(10),71 (13),65(13),60(24),57(21),55 (15), 52(15),51(13),50(11),44 (11),43(48),42(11),40(16),32 (100),29(100).	166	$C_9H_{10}O_3$ requires M=166 CH_3 C=O (ring) OMe OH acetovanillone **Figure 45
78	281(2),267(2),226(7),183(1), 169(4),166(28),85(35),71(60), 57(100),43(79),41(30),40(31), 32(100),31(30),29(87).		

*For unidentified components only ions with a relative abundance greater than 30% are quoted for m/e less than 150.
**Structure assigned on basis of comparison with mass spectrum of authentic compound(figures 38 and 39).

Table 8 cont.

Retention Time(min.)	Mass* Spectrum m/e (relative abundance%)	M^+	Assigned molecular formulae.
84	183(2),181(100),180(78),167(24),153(8),29(88).		
90	241(4),208(5),197(1),187(1) 185(2),167(1),165(2),85(39),84(47),71(63),57(100),43(55),		
96	357(2),341(10),327(5),269(1),253(6),239(3),223(8),219(7),212(9),211(4),210(9),208(9),207(17),197(4),192(5),178(16),164(3),161(4),158(2),83(45),82(30),70(33),69(45),29(59).		
112	268(6),266(2),225(1),211(2),192(2),169(2),156(4),85(43),71(87),57(100).		
127	196(35),183(13),182(92),181(100),167(18),153(17),149(29),138(10),123(12),111(15),96(19),85(30),81(18),74(13),71(37),69(21),60(12),57(69),55(32),45(30),43(65),32(100),31(32),29(20).	182	$C_9H_{10}O_4$ requires M=182 syringic aldehyde. MeO HO CHO MeO **Figure 46.
147	197(13),196(100),181(100),15(47),138(6),110(5),85(15),71(15),57(27),43(47),32(21).	196	$C_{10}H_{12}O_4$ requires M=196 acetosyringone. MeO HO $COCH_3$ MeO ***

*For unidentified components only ions with a relative abundance greater than 30% are quoted for m/e less than 150.
**Structure assigned on basis of comparison with mass spectrum of authentic compound(figure 40).
***Tentative structural assignment based on interpretation of fragmentation pattern (Appendix 2).

Figure (44) vanillin from _Agave sisalana_.

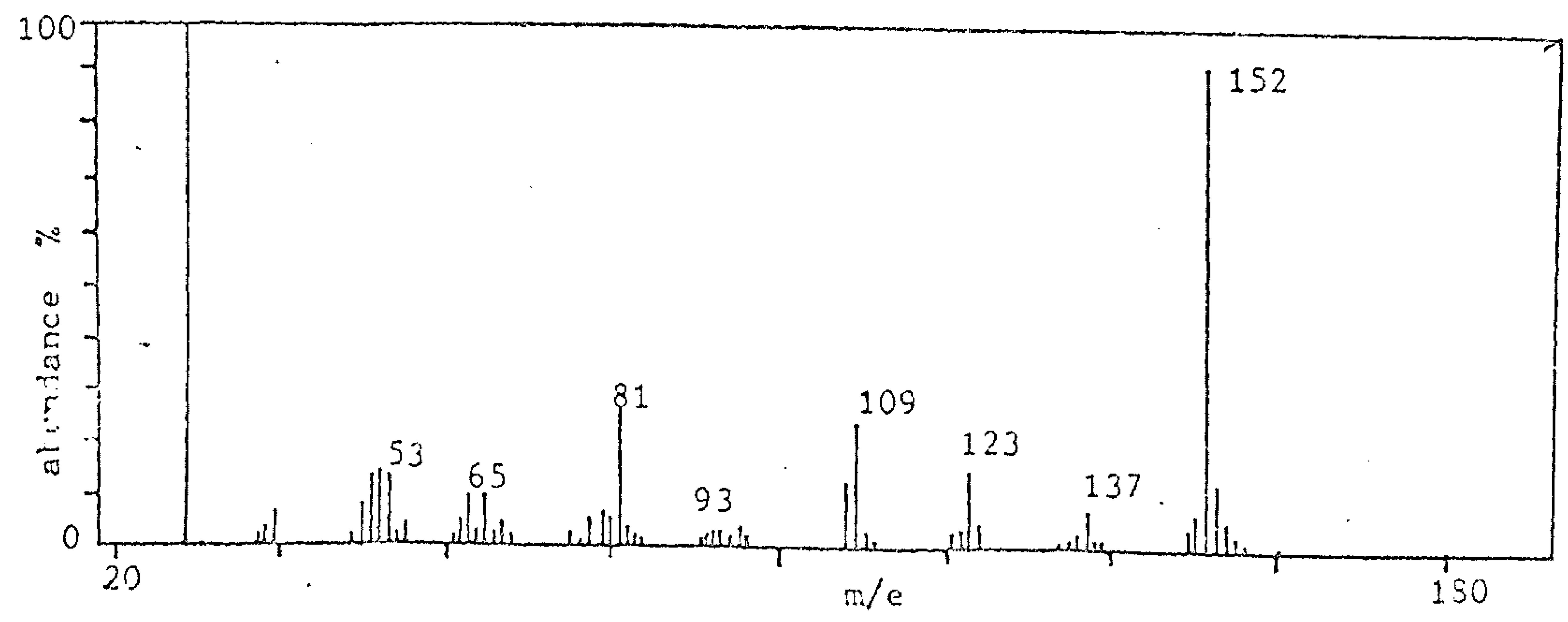


Figure (45) acetovanillone from _Agave sisalana_.

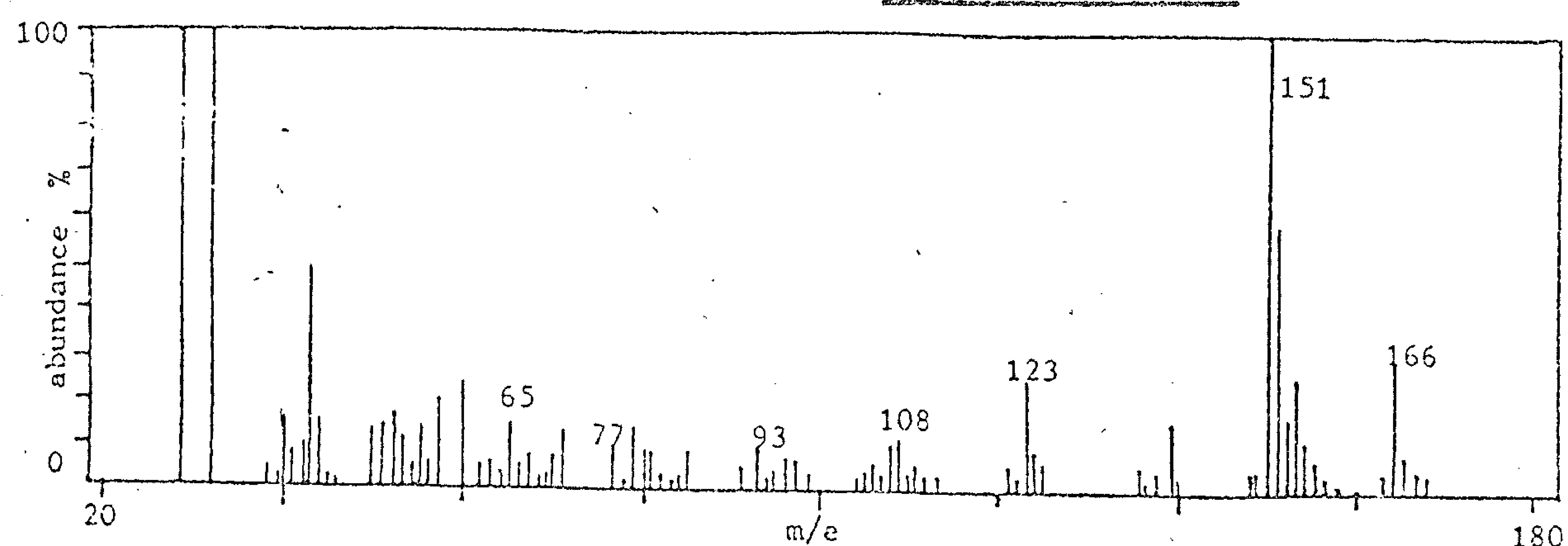


Figure (46) syringic aldehyde from _Agave sisalana_.

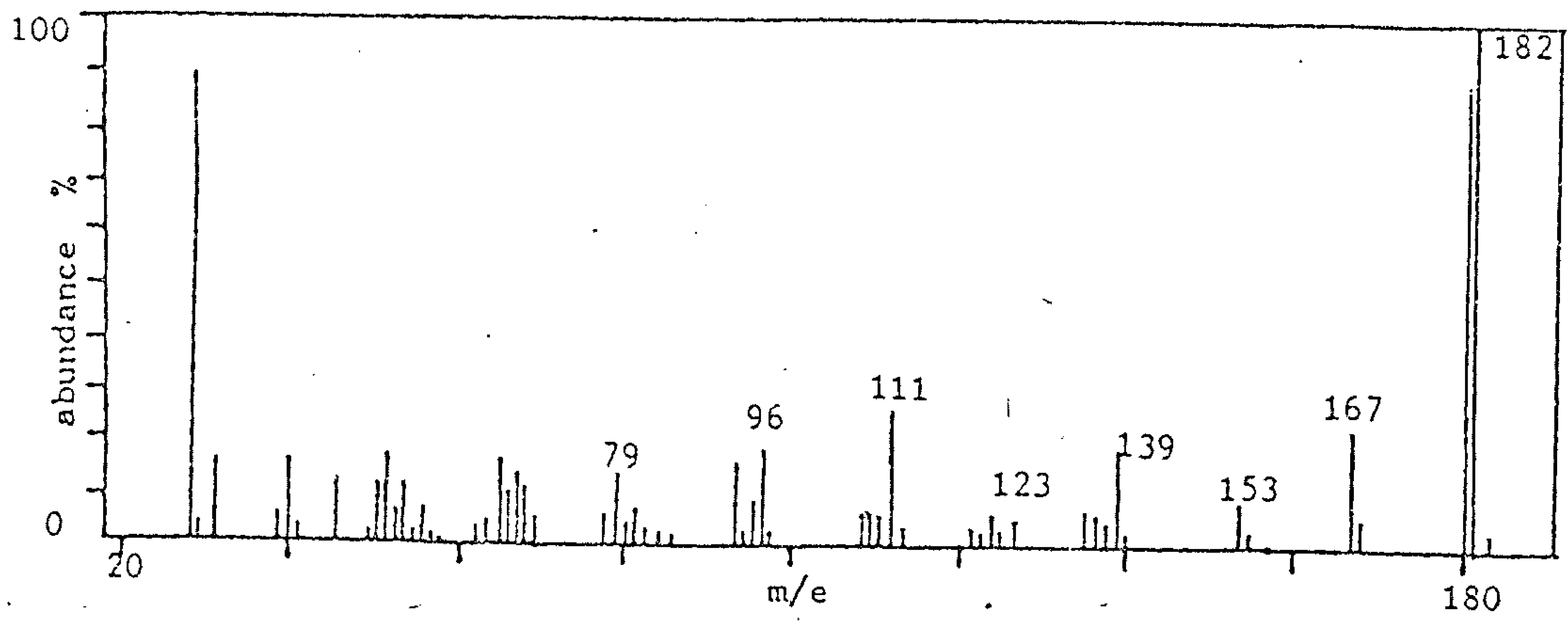


Figure (47) acetosyringone from _Agave sisalana_.

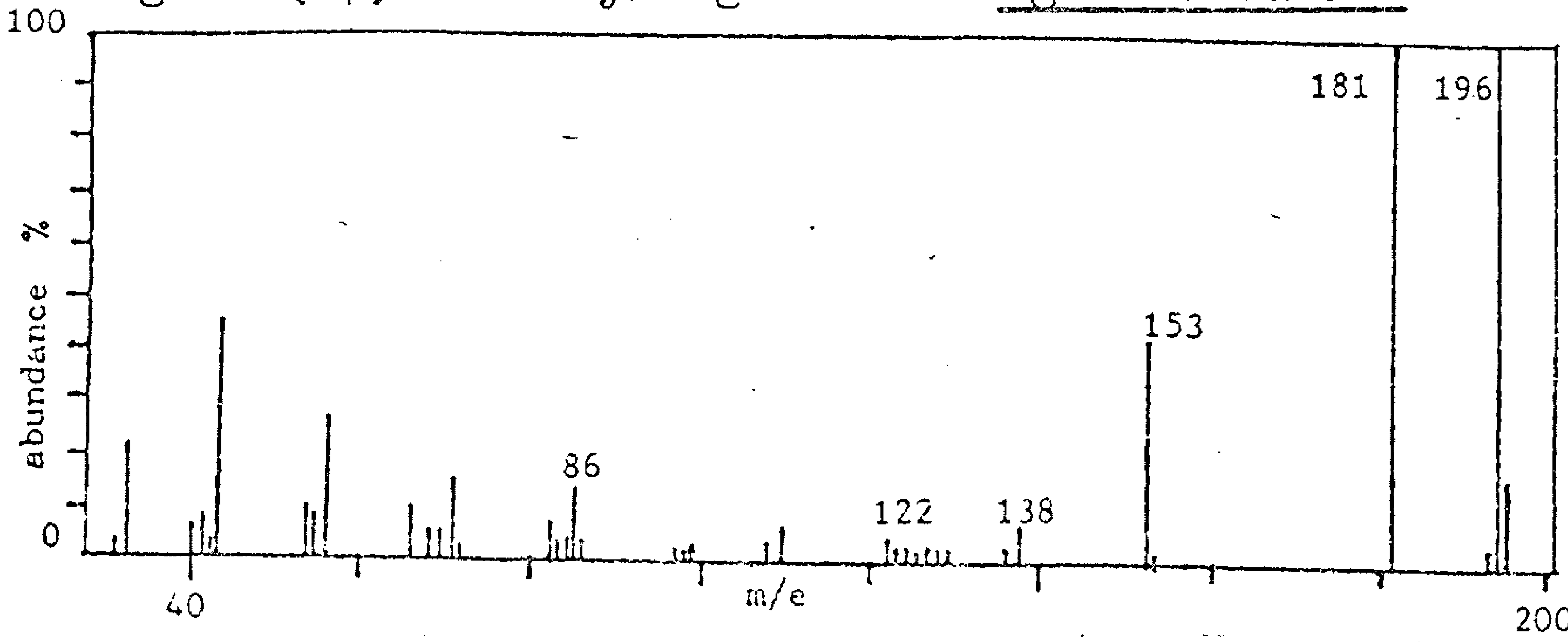


Table 9

G.C./M.S. of Oxidation Products from Lignin of *Bambusa* species.

A.E.I. M.S. 30 double focusing mass spectrometer integrated with a Pye Unicam model 104 G.C. (3% OV 101 on Gas Chrom Q).

Retention Time(min.)	Mass* Spectrum m/e (relative abundance%)	M^{+}	Assigned molecular formulae.
27	125(23),124(100),110(8),100(99),95(2),82(5),81(64),76(4),65(9),64(5),63(10),62(4),53(22),52(17),51(11),50(8),39(9),38(2).	124	$C_7H_8O_2$ requires M=124 o or p methoxyl phenol. HO MeO ***
36	138(4),136(4),126(5),125(16),124(94),123(8),122(35),121(8),120(2),110(10),109(100),107(68),93(3),91(5),82(6),81(97),77(7),53(25),52(21),50(2),32(49),28(100).	122	$C_7H_6O_2$ requires M=122 p-hydroxybenzaldehyde. HO CHO **
44	151(6),139(11),138(93),137(8),136(9),135(4),134(1),126(4),125(6),124(69),123(100),120(17),119(5),112(4),109(32),92(1),82(3),81(68),67(9),32(25),28(100).	138	$C_8H_{10}O_2$ requires M=138 4-ethyl resorcinol. OH OH C_2H_5 ***
79	154(100),139(62),111(39),96(37),93(37),65(30).		
91	154(19),153(19),152(100),151(100),139(9),137(8),136(2),124(3),123(22),122(10),121(12),111(6),110(2),109(31)108(11),95(6),93(16),81(50),80(10),79(22),77(12),65(28),63(20),53(31),52(35),51(32),50(17),39(25),32(18),29(6),28(45).	152	$C_8H_8O_3$ requires M=152 vanillin. HO CHO MeO **

*For unidentified components only ions with a relative abundance greater than 30% are quoted for m/e less than 150.
**Structure assigned on basis of comparison with mass spectrum of authentic compound (figures 37 and 38).
*** Tentative structural assignment based on interpretation of fragmentation pattern (Appendix 2).

Table 9 cont.

Retention Time(min.)	Mass* Spectrum m/e (relative abundance%)	M^+	Assigned molecular formulae.
125	121(100),93(31),65(35).		
138	167(7),166(56),151(100),136(18),123(33),121(33),118(4),108(14),95(2),94(5),93(12),92(5),91(2),80(6),79(7),77(16),67(8),66(7),65(22),63(8),55(6),52(19),51(8),50(6),43(34),41(4),40(4),32(30),28(38).	166	$C_9H_{10}O_3$ requires M=166 acetovanillone. CH_3–C=O on ring with OH and OMe **
318	183(10),182(100),[illegible](50),167(20),153(5),139(21),13[illegible](9),123(4),111(32),96(23),95(14),93(22),81(12),79(23),77(12),69(26),66(12),65(27),54(27),53(28),52(28),51(17),40(10),39(29),38(6),32(30),29(10),28(100).	182	$C_9H_{10}O_4$ requires M=182 syringic aldehyde MeO, HO, MeO – ring – CHO **
357	197(9),196(58),181(100),153(22),152(3),138(8),125(5),123(6),122(5),110(7),108(8),85(9),83(9),78(9),77(9),75(9),67(17),66(6),65(7),53(6),50(4),43(50),28(100).	196	$C_{10}H_{12}O_4$ requires M=196 acetosyringone. MeO, HO, MeO – ring – $COCH_3$ ***
433	223(2),230(3),205(2),196(19),183(2),182(46),181(74),167(11),153(9),150(10),149(100),116(30),101(30),75(77),43(36),40(33),39(35),32(100),28(100).		

* For unidentified components only ions with a relative abundance greater than 30% are quoted for m/e less than 150.
**Structure assigned on basis of comparison with mass spectrum of authentic compound (figures 39 and 40).
***Tentative structural assignment based on interpretations of fragmentation pattern (Appendix 2).

Angiosperms : Dicotyledons.

Table 5 lists the major oxidation products obtained from a range of woody dicotyledons. Vanillin, acetovanillone, and syringic aldehyde were detected in all the species examined using 2µl injections. Many authors including Creighton, Gibbs and Hibbert (1944), Towers and Gibbs (1953) and Sarkanen and Ludwig (1971) have shown that dicotyledons contain vanillin and syringic aldehyde in the ratio 1:3. In this work the ratio of vanillin to syringic aldehyde was found to vary between 1:2 and 1:3. The lower ratio is attributed to the presence of acetosyringone, which is believed to oxidize to syringic aldehyde under the nitrobenzene oxidation conditions. The ratio of vanillin to syringic aldehyde in one of the 'primitive' groups of dicotyledons, the Winteraceae, which lack vessels, was found to have lower ratios than those of the more advanced dicotyledons examined, (Towers and Gibbs, 1953). Liriodendron tulipiraflora was examined by Creighton, Gibbs and Hibbert (1944) and the ratio of vanillin to syringic aldehyde was 1:3.2 whereas within the same family, the Magnoliales, also regarded as a 'primitive' group, Towers (1951) found that for Magnolia grandiflora and Magnolia acuminata this ratio was 1:1.5 and 1:1.2 respectively. The five species of Magnoliaceae examined here, all of which possessed vessels, had vanillin to syringic aldehyde ratios of 1:2, similar to other dicotyledons that were examined in this work.

For the oxidation products from dicotyledons there is some confusion over the presence or absence of small amounts of p-hydroxybenzaldehyde. As early as 1950 Bland, Ho and Cohen found p-hydroxybenzaldehyde in the Eucalyptus family. Subsequently it has been obtained by De Stevens and Nord (1952) from enzymatically liberated lignin of birch , and from various dicotyledons (Leopold

and Malmstrom, 1952; Pepper, Manolopoula and Burton, 1962; Pepper, Cassalman and Karapally, 1967, and Leo and Barghoorn, 1970). However Towers and Gibbs (1953) found that p-hydroxybenzaldehyde was generally absent from dicotyledons other than Tetracentron sinense and Ibrahim, Towers and Gibbs (1962) found that it was uncommon in dicotyledons. In the present work branch wood ranging from 2 to 4 years in age gave trace amounts of p-hydroxybenzaldehyde as did the leaves of Magnolia kobus. No p-hydroxybenzaldehyde was detected from the mature wood even when larger injections (10 µl) were used. Hedges and Parker (1976) detected p-hydroxybenzaldehyde from the leaves of Ilex opaca.

Mass Spectral Interpretations.

The mass spectra obtained from the major lignin oxidation products of the dicotyledon Magnolia soulangeana are presented in table 10 and figures (48) to (51).

Table 10

G.C./M.S. of Oxidation Products from Lignin of *Magnolia soulangeana* .

A.E.I. M.S. 30 double focusing mass spectrometer integrated with a Pye Unicam model 104 G.C. (3% OV 101 on Gas Chrom Q).

Retention Time(min.)	Mass* Spectrum m/e (relative abundance%)	M^+	Assigned molecular formulae.
26	184(11),182(2),155(3),151(4),150(30),135(47),85(35),54(71),57(100),43(86),41(30).		
39	209(1),191(4),181(2),179(3),162(2),160(10),158(3),125(37),111(86),74(50),57(30),43(48),31(100),29(57),28(100).		
42	166(14),165(13),164(100),139(50),111(65),74(30),51(47),45(40),31(34).		
51	153(9),152(100),137(5),123(17),109(15),108(4),81(24),64(1),63(5),62(3),61(2),53(9),52(11),51(6),50(5),32(4).	152	$C_8H_8O_3$ requires M=152 vanillin. HO–⟨ring⟩–CHO, MeO **Figure 48
55	267(40),241(1),209(6),207(8),194(9),182(11),179(6),175(6),174(55),167(9),166(11),164(12),159(22),156(2),151(100),139(40),132(100),104(45).		
62	167(6),166(56),151(100),136(4),135(1),123(23),122(2),121(2),108(8),94(2),85(4),77(9),71(8),67(6),65(9),57(8),55(7),52(9),51(8),43(19).	166	$C_9H_{10}O_3$ requires M=166 acetovanillone. CH_3–C=O on ring, OMe, OH **Figure 49

*For unidentified components only ions with a relative abundance greater than 30% are quoted for m/e less than 150.

**Structure assigned on basis of comparison with mass spectrum of authentic compound(figures 38 and 39).

Table 10 cont.

Retention Time(min.)	Mass* Spectrum m/e (relative abundance%)	M^+	Assigned molecular formulae.
98	267(7),226(5),208(5),207(4),187 (2),185(3),182(2),168(8),167(7), 158(7),157(8),85(44),69(30),57 (100),45(30),42(32)28(100).		
135	189(2),184(8),183(1),182(28), 181(62),169(4),167(18),165(4), 153(18),151(5),149(10),141(8), 139(28),137(23),135(12),125(4), 123(10),113(20),110(17),99(12), 97(12),95(17),85(32),83(4),72 (4),71(73),59(22),57(18),57 (100),56(12),53(12),50(15),43 (67),41(32),38(4),34(4).	182	$C_9H_{10}O_4$ requires M=182 syringic aldehyde. MeO HO⟨⟩ CHO MeO **Figure 50
147	197(11),196(100),181(53),153 (14),138(7),108(3),85(8),75 (4),71(5),67(13),28(43),32(21), 28(12).	196	$C_{10}H_{12}O_4$ requires M=196 acetosyringone. MeO HO⟨⟩ $COCH_3$ MeO ***
160	283(2),281(2),270(3),268(4), 260(2),242(1),211(5),193(21), 192(99),191(23),180(31),177 (95),175(8),162(8),155(7),145 (50),85(36),71(81),67(100), 32(51).		

*For unidentified components only ions with a relative abundance greater than 30% are quoted for m/e less than 150.
**Structure assigned on basis of comparison with mass spectrum of authentic compound(figure 40).
***Tentative structural assignment based on interpretation of fragmentation pattern (Appendix 2).

Figure (48) vanillin from <u>Magnolia soulangeana</u>.

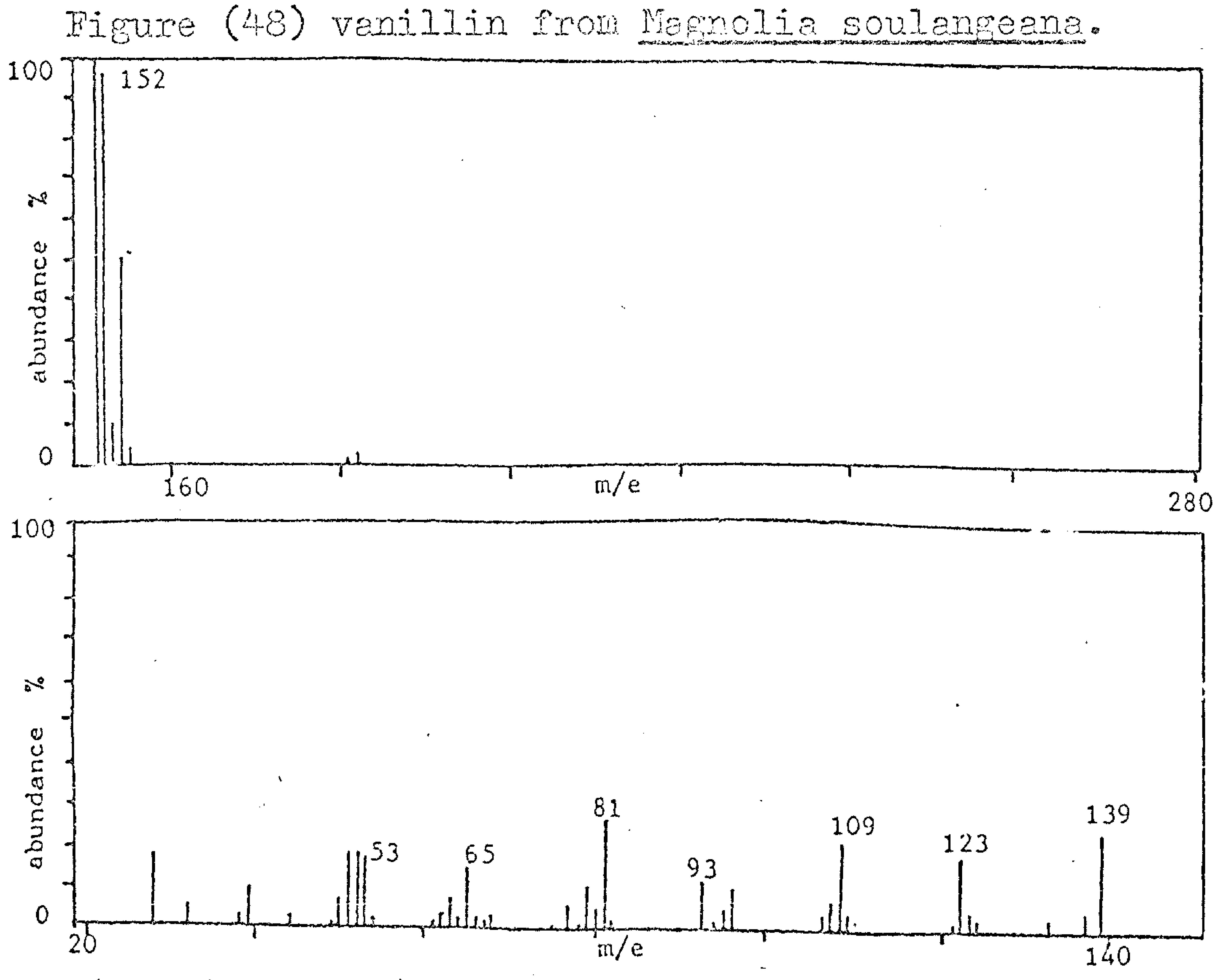


Figure (49) acetovanillone from <u>Magnolia soulangeana</u>.

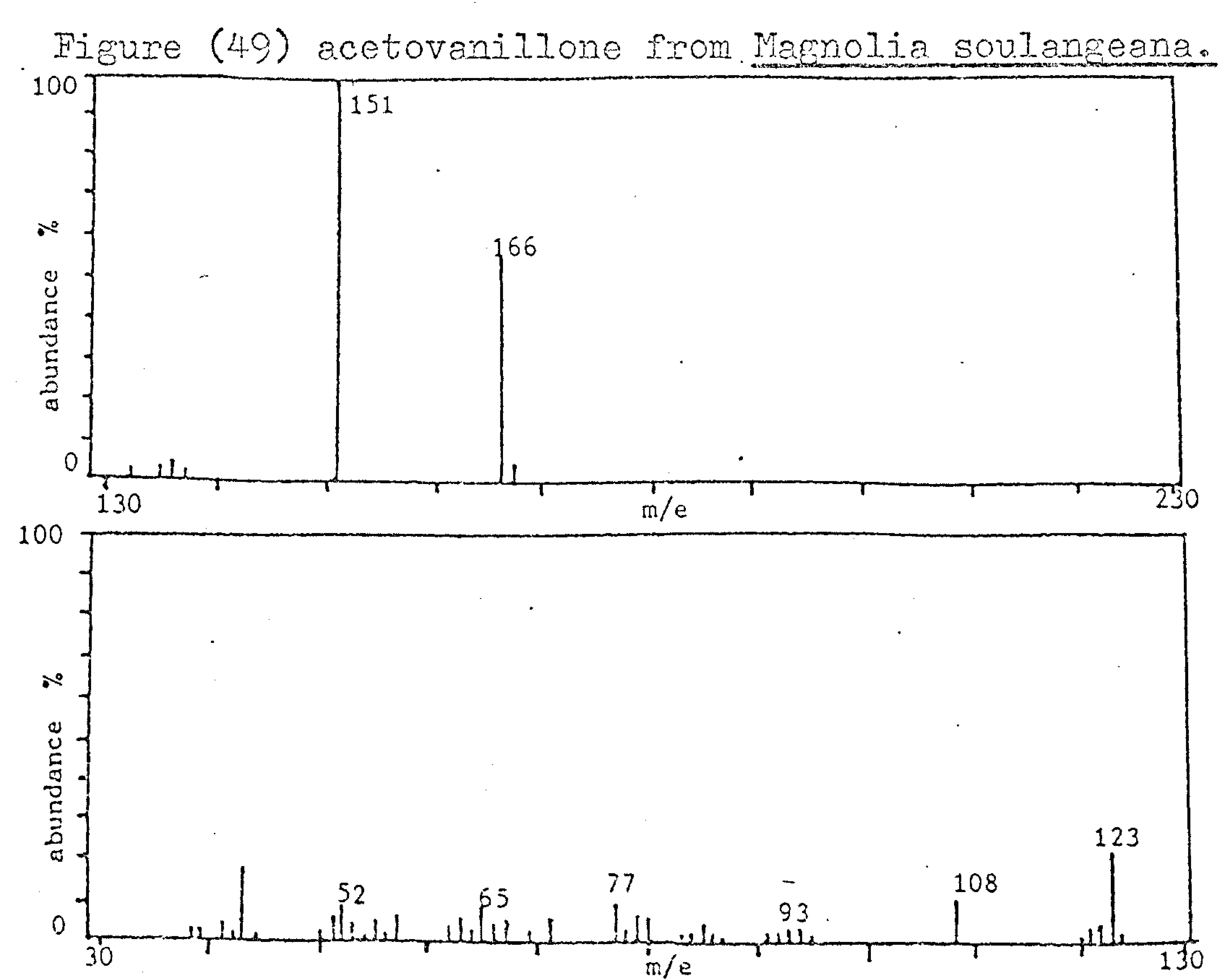


Figure (50) syringic aldehyde from *Magnolia soulangeana*.

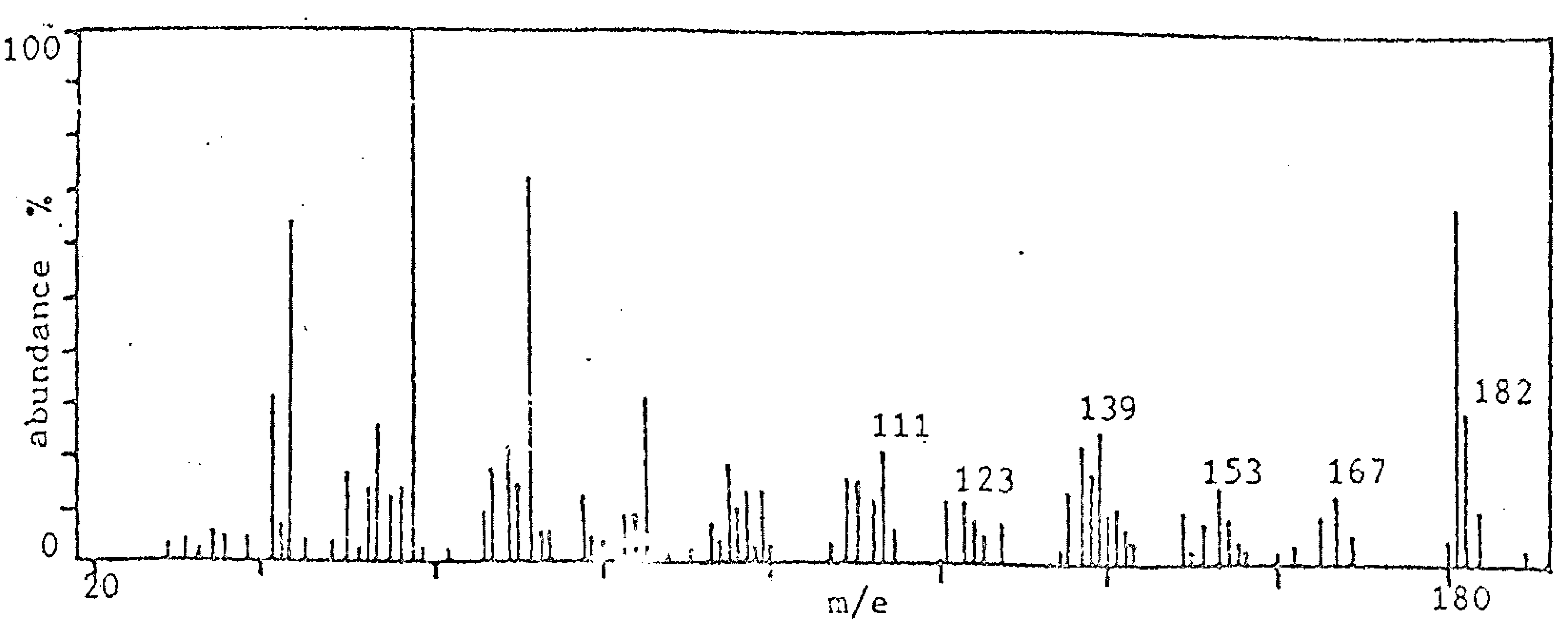
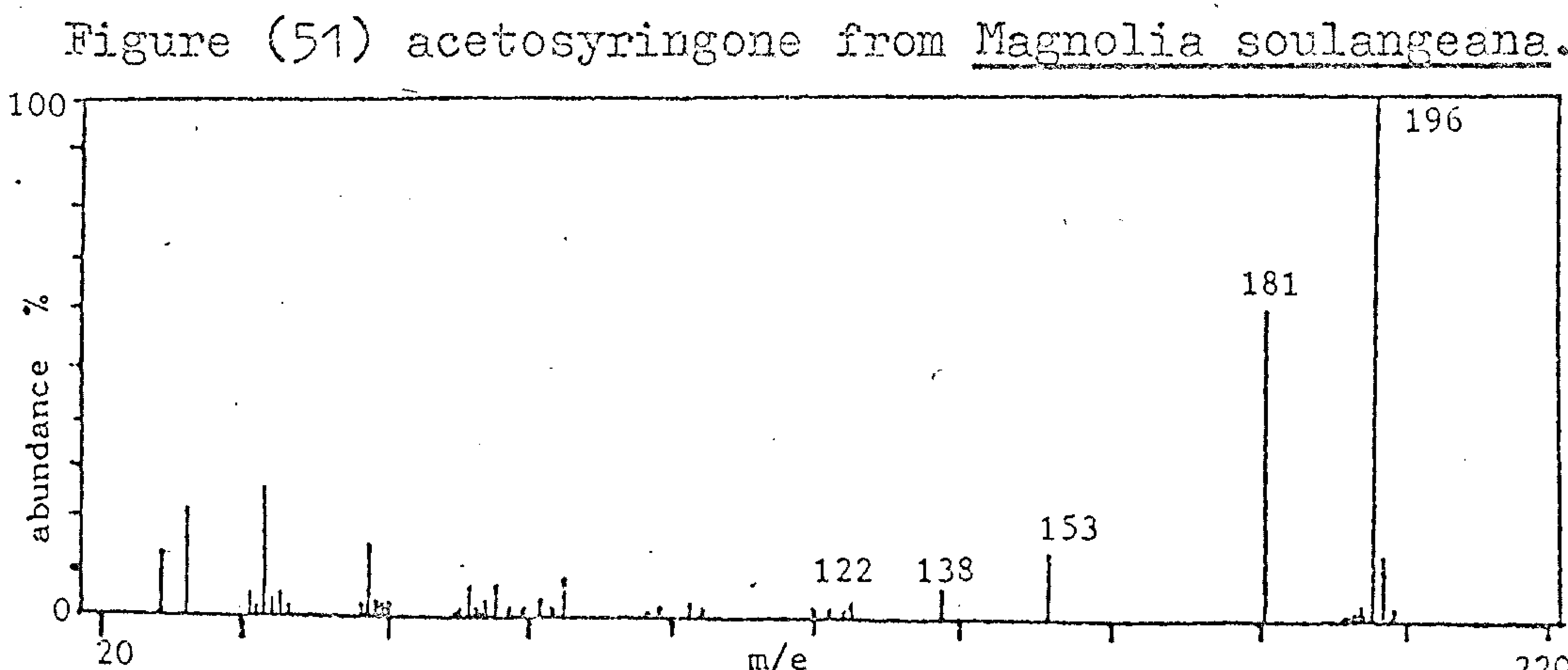


Figure (51) acetosyringone from *Magnolia soulangeana*.



Bark.

Table 6 lists the major lignin oxidation products obtained from the bark of an angiosperm, Betula pendula and one gymnosperm, Sciadopitys verticillata together with a laboratory cork. Most of the secondary walls of the bark cells are suberized: however the cellulose primary wall often remains unsuberized, and can become lignified (Esau, 1965). Sclerified phelloids (a type of sclerified fibre) can occur in bark. These cells are unsuberized and may become lignified (Bamber, 1962).

As shown in table 6, the analysis of the oxidation products provides evidence for the precursors coumaryl, guaiacyl, and syringyl units in the lignins of the three bark samples studied. Sarkanen and Hergert (1971) found that the general elemental composition and functional groups of bark lignin were very similar to those of the lignin isolated in the same manner from the wood of the corresponding species. To some extent this is supported by the results obtained in this work but some surprising differences do occur. Vanillin was found to be the major oxidation product obtained from Sciadopitys verticillata bark, being 73% of the total aldehyde content. This is similar tothe major oxidation product vanillin obtained from the wood of the same species, which was 86% of the total aldehyde content. The abundance of p-hydroxybenz-aldehyde was found to be similar for both the bark and the wood of Sciadopitys verticillata, (15.5% and 13.8 % of the total aldehyde respectively). Surprisingly, however, the proportion of the syringic aldehyde in the coniferous bark was substantially higher than that obtained from the wood or indeed from any of the gymnospermous wood examined. In bark, 10.5% of the total aldehyde was syringic aldehyde: the corresponding figure for wood was 0.13%.

In Betula pendula bark, vanillin and syringic aldehyde were found to be the major oxidation products (48% and 42% of the total aldehyde content, respectively). In wood of Betula pendula similar results were obtained (33% and 67% of vanillin and syringic aldehyde). However

p-hydroxybenzaldehyde was present as 10% of the total aldehyde content for the bark of Betula pendula whereas for the mature wood no p-hydroxybenzaldehyde was detected.

The laboratory cork has a higher proportion of vanillin (92%) and a lower proportion of syringic aldehyde (5% of the total aldehyde content). Therefore it seems more likely that this cork was taken from a gymnosperm although more samples of lignin analyses for bark are necessary.

Summary.

Vanillin remains the major lignin oxidation product obtained from the gymnosperms and syringic aldehyde remains the major oxidation product obtained from the dicotyledons examined. However small amounts of other phenolic aldehydes and ketones have been detected for the gymnosperms and dicotyledons. Not all monocotyledons on oxidation of their lignin, gave vanillin and syringic aldehyde in equal amounts as previously thought. All three phenolic aldehydes p-hydroxybenzaldehyde, vanillin and syringic aldehyde have been detected in the barks that were examined. p-Hydroxybenzaldehyde was not detected for mature dicotyledons or for the fibres of the monocotyledons that were examined in this work. Small amounts of these phenolic aldehydes have been detected in this work because a very sensitive system of analytical gas liquid chromatography with a column that separated the phenolic aldehydes and ketones as such, rather than as derivatives, was used, as described in Chapter 2. This was important as regards the small amounts of syringic aldehyde detected from the lignin of the gymnosperms. The types of lignin units found in the plant materials that we have examined differ to some extent from those reported by other authors. This is because many authors who have used gas chromatography for this analysis have restricted themselves to analysing only a few species and not including a wide enough range.

There has been a correlation drawn between plants that possess vessels in their xylem and the presence of a large proportion of syringyl units obtained from the lignin of these plants (Sarkanen and Ludwig, 1971 and Lewis, 1980). This is reinforced by the observations that certain angiosperms belonging to the Magnoliales such as _Drimys_ and _Tetracentron_ which lack vessels have low amounts of syringyl units (Sarkanen and Hergert, 1971). Members of

the Gnetales have numerous small solitary vessels present in their wood together with tracheids. The Gnetales have been shown in the present work and by Creighton, Gibbs and Hibbert (1944) to have a substantial proportion of syringyl units in their lignin.

The apparent association of syringic aldehyde with the presence of vessels in the wood does not explain why small amounts of syringic aldehyde were detected in all the gymnosperms that were examined in this work. Nor does it correlate with the observation that some of the highest amounts of syringic aldehyde were obtained from monocotyledon fibres. (No vascular tissue is present in the fibres). It does not explain why syringic aldehyde was reported in considerable quantities from certain species of Podocarpus (Creighton, Gibbs and Hibbert, 1944; Leopold and Malmstrom, 1952; Towers and Gibbs, 1953 and Kawamura and Higuchi, 1964) and Tetraclinis articulata (Creighton, Gibbs and Hibbert, 1944 and Towers and Gibbs, 1953) both of which are composed of tracheids with associated fibres and not vessels.

As the fibres from the leaves of the Agavaceae and the fibres from the drupe of Cocos nucifera examined in this work gave higher proportions of syringic aldehyde than did the wood from the monocotyledons examined, it could be argued that it is the fibres that play a part in determining whether the lignin is of a guaiacyl type or syringyl type. Fergus and Goring's work (1976) on one species, Betula papyrifera, indirectly supports this proposal. Using ultra-violet light microscopy they found that the lignin of the vessel secondary walls and the middle lamella was composed of guaiacylpropane units while the lignin deposited in the secondary walls of the ray parenchyma was composed mostly of syringylpropane units. The middle lamella around the fibres and ray cells which were also lignified contained guaiacyl and syringylpropane units.

This implies that lignin precursors can originate within the differentiating cell and be incorporated into the cell.

There are two main types of fibres found in plants, libriform fibres and fibre-tracheids. Libriform fibres are generally found in the angiosperms and are long pointed elements in which the pits are simple and slit like. They are commonly thick walled. There are stages of gradation between libriform fibres and tracheids with bordered pits, the fibre-tracheids. They are most commonly found in gymnosperms although they are present in angiosperms. They have small bordered pits with slit like apertures often extending beyond the pit borders. Their definition is difficult and often intermediate stages are encountered. Brazier and Franklin (1961) define fibre tracheids as 'fibres' with distinctly bordered pits. We can speculate further here and suggest that fibre-tracheids may contribute to the very small amounts of syringic aldehyde detected, such as in the gymnosperms ; and that tissue high in fibre content, such as the angiosperms give high amounts of syringic aldehyde. Futhermore we can suggest that the syringic aldehyde content in bark may be due in part at least to the sclerified phelloid cells which are fibre type cells.

Lignin is a polymer of compounds with a phenylpropane skeleton. Although information on some of the stages of lignin formation is available as outlined in Chapter 1, the total integrated series of biochemical reactions remains obscure.

Dicotyledonous twigs were found to give small quantities of p-hydroxybenzaldehyde but it was not detected in the experiments with mature wood. p-Hydroxybenzaldehyde was also found to be present in high amounts from bark and the lower plants. The enzyme tyrase converts tyrosine to trans-p-coumaric acid and has been shown to exist in the

grass family (Brown, 1961). The possibility of an enzyme such as tyrase or an excess in these plants could account for the presence of p-hydroxybenzaldehyde.

The following reactions lead to the formation of syringyl lignin, (as shown in Chapter 1) :

Caffeic Acid ⟶ Ferulic Acid ⟶ 5-Hydroxyferulic Acid ⟶

Sinapic Acid ⟶ Sinapyl Alcohol ⟶ Syringyl Lignin

The angiosperms have been found to give high amounts of syringic aldehyde. Conversions to syringyl lignin are thought to be carried out by the o-methyltransferases which are widely distributed in plants. It has been suggested by Shimada, Kuroda and Higuchi (1973) and Kuroda, Shimada and Higuchi (1975) that one or more enzymes mediating the reactions are not present in gymnospermous plants. However syringic aldehyde has been detected in all the gymnosperms examined in this work. Therefore gymnosperms must possess small amounts of the enzymes that convert caffeic acid to syringyl lignin.

CHAPTER 4.

LIGNIN CHEMOTAXONOMY IN CRYPTOGAMS.

Lignin Chemotaxonomy in Cryptogams.

Cryptogams or lower plants encompass those plants which reproduce by spores rather than seeds. Considering the extensive investigations concerning different aspects of lignin from higher plants, surprisingly little attention has been paid to lignin in lower plants. In 1958 Manskaya stated that the methoxyl content of 'primitive' plants is generally lower than that of higher plants. In Kawamura and Higuchi's lignin classification of 1964 ,(see page 78) based on infra-red spectra the lignin of the Pteridophyta is classed as Type Np, a subdivision of the gymnospermous or Type N lignin. This subdivision arises because there is virtually no absorption at 2850 cm^{-1} in the lignin of the pteridophytes. This absorption arises mainly from the methoxyl groups, and this result confirms Manskaya's earlier statement.

The tree ferns show an interesting distribution of lignin. These ferns possess no vascular cambium and consequently have no secondary xylem. Vanillin and _p_-hydroxybenzaldehyde have been reported by Sarkanen and Latif (in Sarkanen and Ludwig, 1971) in _Dicksonia_, _Alsophila_ and _Cyathea_.

Both Towers and Gibbs (1953) and Ibrahim, Towers and Gibbs (1962) have reported the presence of vanillin and _p_-hydroxybenzaldehyde on nitrobenzene oxidation of _Equisetum hyemale_.

Considerable investigations have been carried out in the Lycopsida. They are a group of plants whose history extends back to at least the Devonian and whose ancestors were both herbaceous and arborescent types. The arborescent types made a large contribution to the Upper Carboniferous swamp forests. Towers and Gibbs (1953) first reported vanillin and _p_-hydroxybenzaldehyde as products of alkaline nitrobenzene oxidation of lignin from _Lycopodium clavatum_, _Isoetes muricata_ and three species of _Selaginella_. Both _Isoetes muricata_ and the _Selaginella_ species also had syringic aldehyde. Towers and Maass (1965) confirmed this

using both nitrobenzene and cupric oxide oxidations as well as ethanolysis. In addition they found that the genera Lycopodium and Diphasium had syringic acid in addition to having guaiacyl compounds. They suggested therefore that this lignin is different from that of either gymnospermous or angiospermous lignins. Phylloglossum drummondii was found to have a gymnospermous type of lignin similar to that of the Lycopodium species by White, Tse and Towers (1967).

The location of guaiacyl syringyl lignins in cryptogams other than Selaginella species have not been proven up to now. Any phylogenetic significance of this 'advanced' biochemical feature is still to be elucidated.

The presence of lignin in the Bryophyta is a much debated topic at present and will be dealt with later. Sphagnum has been investigated more thoroughly than other mosses because of its importance in peat formation. Lindberg and Theander (1952) investigated lignin in Sphagnum balticum and Sphagnum fuscum. Both species were found to give about 5% Klason lignin containing about 1% of methoxyl. Nitrobenzene oxidations revealed the major constituent of the aldehyde fraction to be p-hydroxybenzaldehyde. Traces of vanillin, syringic aldehyde and formyl vanillin were also identified. However in 1951 Kratzl and Eibl could not detect any vanillin on oxidation of sphagnum material. Morrison (1958) reported the presence only of p-hydroxybenzaldehyde as an oxidation product from Sphagnum as did Farmer and Morrison (1964) with Sphagnum cupidatum. In 1967 Nilsson and Tottmar's studies of several species of sphagnum cell wall pigment polymers they found p-hydroxybenzaldehyde and vanillin by cupric oxide oxidation. Bland, Logan, Menshun and Sternhell (1968) isolated lignin from Sphagnum by milling followed by acetone extraction avoiding acids or alkalis to see if this would give results supporting its classification as a lignin rather than as cell wall pigments. Nitrobenzene oxidation gave p-hydroxybenzaldehyde, vanillin and syringic aldehyde.

Lignin was found to be absent in a Marchantia species and Funaria hygrometrica by Towers and Gibbs (1953). In

1969 Siegel established the presence of lignin in the New Zealand gametophytes of Dawsonia and Dendroligotrichum by ultraviolet spectra, lignin colour reactions and nitrobenzene oxidations. This was in contrast to the results observed with north temperate species such as Polytrichum and Funaria. Erickson and Mische (1974) investigated six species of Hepaticae for the presence of lignin by an oxidative degradation method and found them all devoid of lignin, although they contained other types of phenolic cell wall material.

In this section the tree ferns have been examined together with a range of other lower plants.

Results with Cryptogams.

Results with tree ferns.

Samples from living tree ferns were oxidized using the cupric oxide method described previously and chromatographed quantitatively and qualitatively.

Figure (52)
A chromatogram of the lignin oxidation products of one of the tree ferns.

Dennstaedtia bipinnata meal from the stem, 2µl injection. (OV 101 column)

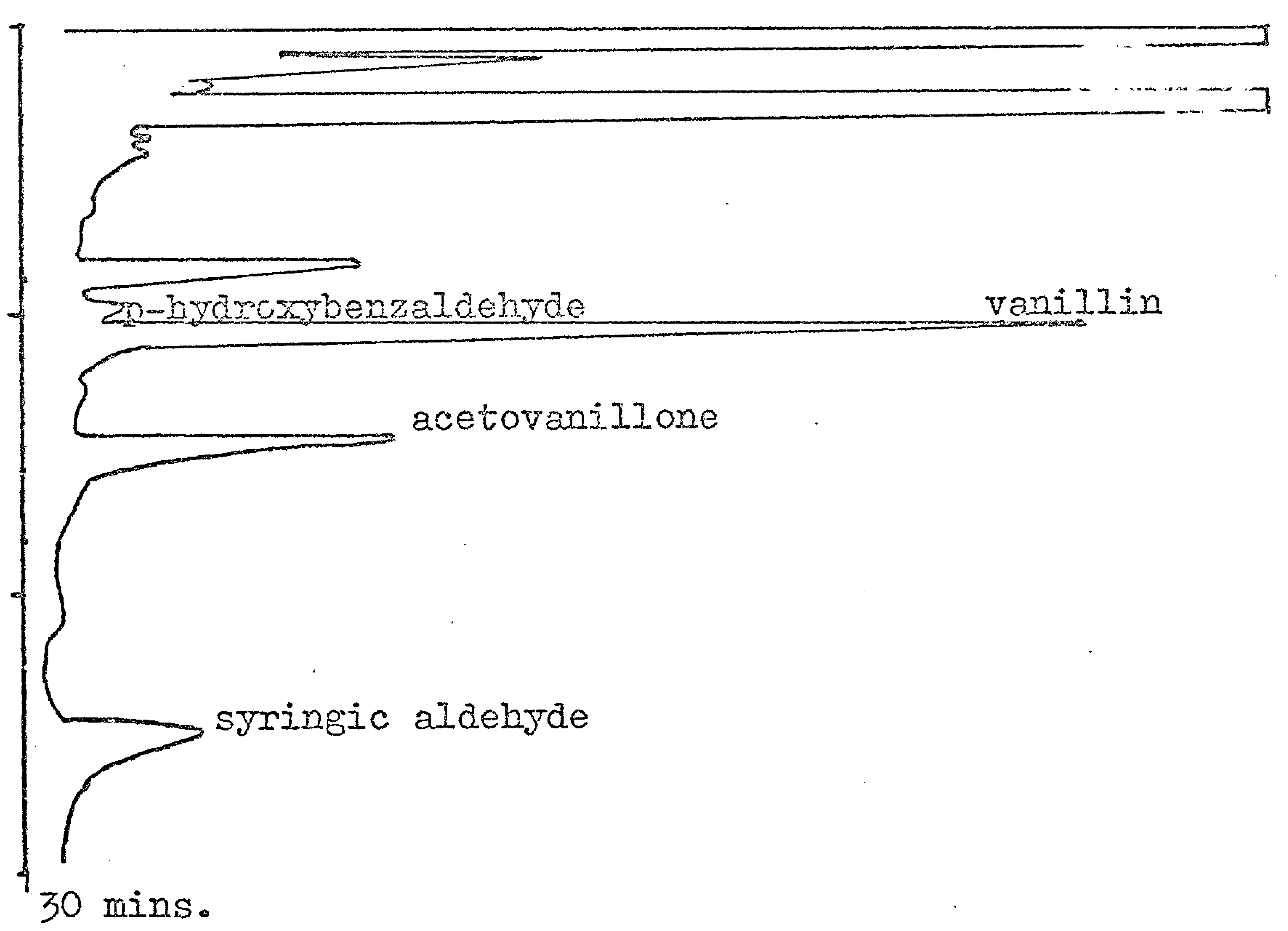


Table 11

The major lignin oxidation products from stems of tree ferns.

Unless otherwise stated 2µl injections were used.

		Major oxidation products. mg/gm of meal.			
	PTEROPSIDA	p-OH	VAN	ACETO	SYR
	Cyatheaceae				
1	Dicksonia squarrosa	0.05	5.25	1.61	absent
2		0.19	6.45	1.95	absent
1	Cibotium barometz	0.26	6.11	2.55	absent
2		0.06	6.49	2.63	absent
3		*0.11	5.55	1.56	0.02
1	Cyathea arborrea	0.12	9.00	3.04	absent
2		0.03	8.63	2.74	absent
3		*0.30	7.25	2.05	0.04
	Dennstaedtiaceae				
1	Dennstaedtia bipinnata	0.02	3.04	1.31	0.98
2		0.04	3.19	1.39	0.98
3		0.05	3.26	1.42	1.12
	Adiantaceae				
1	Pteris podophylla	0.03	3.34	1.54	absent
2		0.09	3.33	1.83	absent
3		*0.06	3.57	1.98	0.02

* 4µl injection

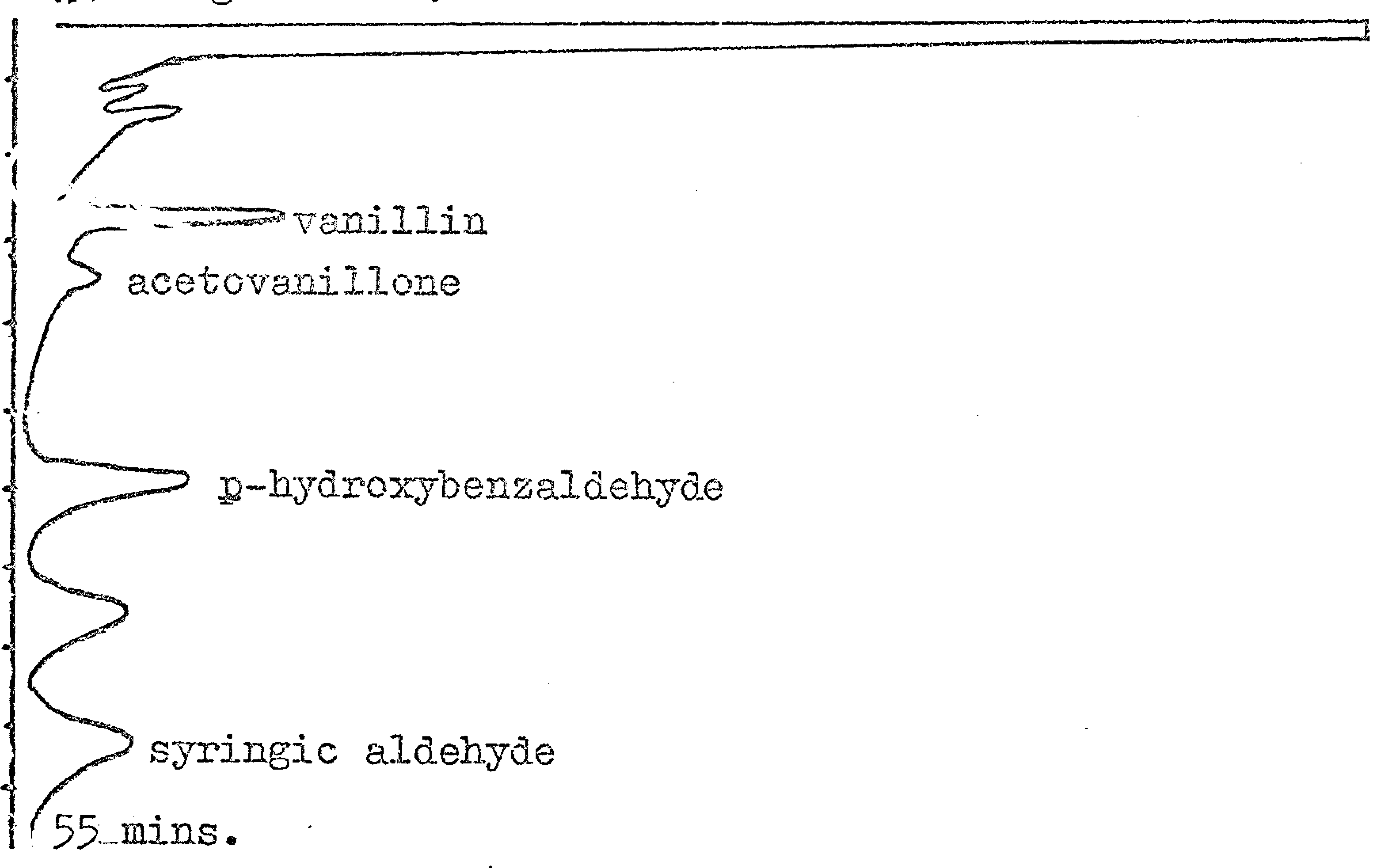
Results with Sphenopsids and Lycopsids.

One species of Equisetum and a range of living Lycopsids were oxidized using the cupric oxide method described previously and chromatographed quantitatively and qualitatively.

Figures (53) and (54)

A chromatogram of the lignin oxidation products of one of the Selaginellales.

Selaginella wildenovii meal from stem, 2µl injection.
(5% Pdegs column)



whereas the chromatogram from a Lycopodium species is as follows:

Lycopodiella cernua meal from stem, 2µl injection.
(5% Pdegs column)

vanillin

acetovanillone

p-hydroxybenzaldehyde

55 mins.

Table 12

The major lignin oxidation products from shoots of the Sphenopsids and Lycopsids.

Unless otherwise stated 2µl injections were used.

		Major oxidation products. mg/gm of meal.			
	SPHENOPSIDA	p-OH	VAN	ACETO	SYR
	Equisetaceae				
1	Equisetum fluviatile	0.30	0.30	0.15	0.15
2		0.21	0.40	0.10	0.15
	LYCOPSIDA				
	Selaginellaceae				
1	Selaginella species	0.34	0.52	0.15	4.[illegible]
2	(214 70 01983)	0.37	0.64	0.15	4.83
1	Selaginella wildenovii	2.60	1.70	0.20	5.00
2		3.00	2.00	0.40	7.00
3		4.05	1.50	0.75	6.15
1	Selaginella myosurus	0.67	2.56	0.48	1.00
2		0.43	2.67	0.67	0.67
	Lycopodiaceae				
1	Lycopodiella cernua	0.07	5.21	1.95	absent
2		0.22	6.37	1.95	absent
1	Lycopodium squarrosum	* 0.04	0.20	0.16	absent
2		* 0.03	0.25	0.25	absent

* 4µl injection

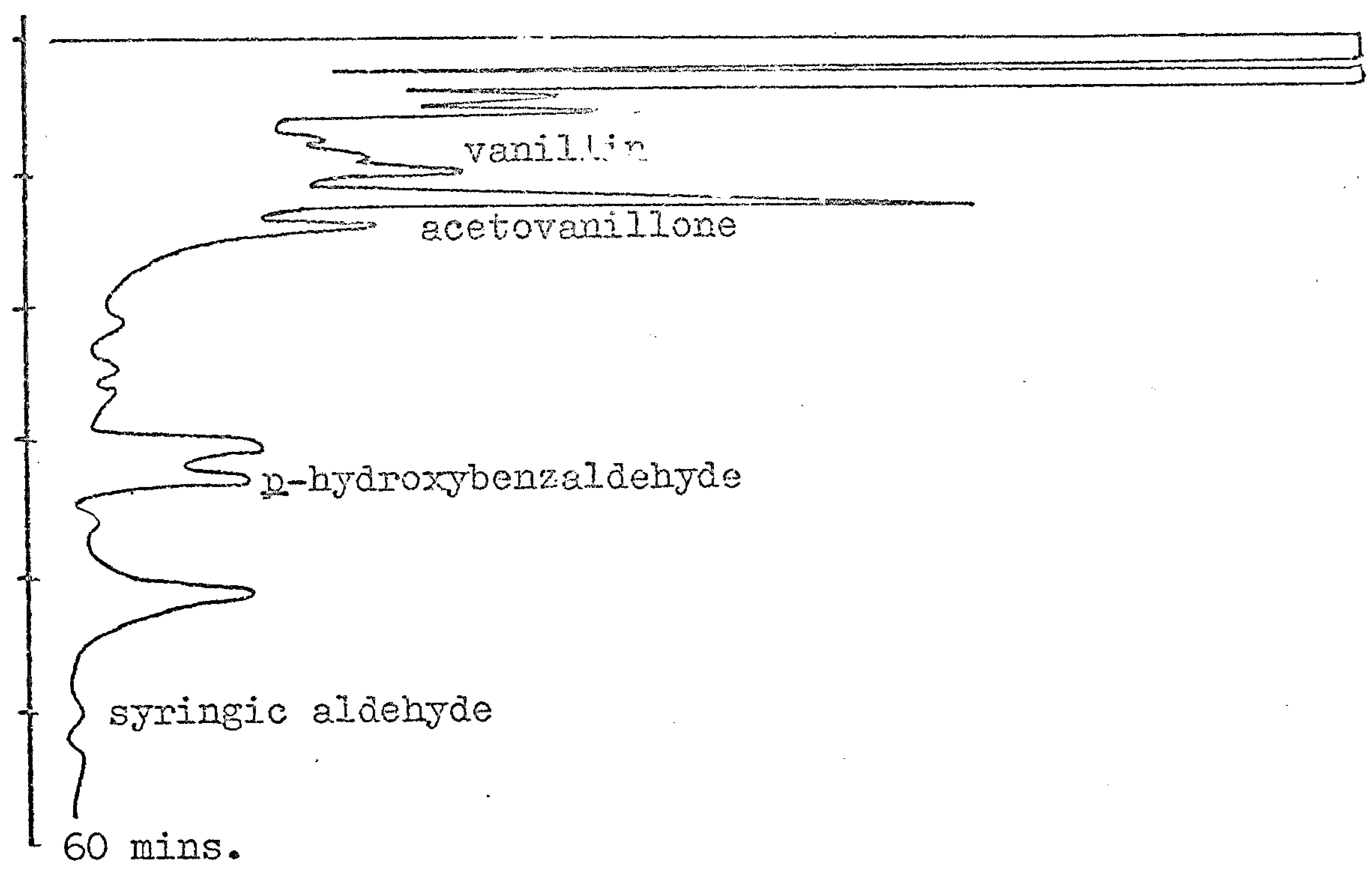
Results with Bryophytes.

Two species from the Hepaticae and one species from the Musci were oxidized using the cupric oxide method described previously and chromatographed quantitatively and qualitatively.

Figure (55)

A chromatogram of the lignin oxidation products of one of the mosses.

Polytrichum commune gametophyte, 4µl injection. (5% Pdegs column)



Mixed chromatography using the authentics, p-hydroxybenzaldehyde, vanillin, acetovanillone and syringic aldehyde were added to Polytrichum commune to confirm the presence of small amounts of the lignin oxidation products as shown in figure (56).

Figure (56)

A chromatogram of the lignin oxidation products of *Polytrichum commune* with the added authentics.

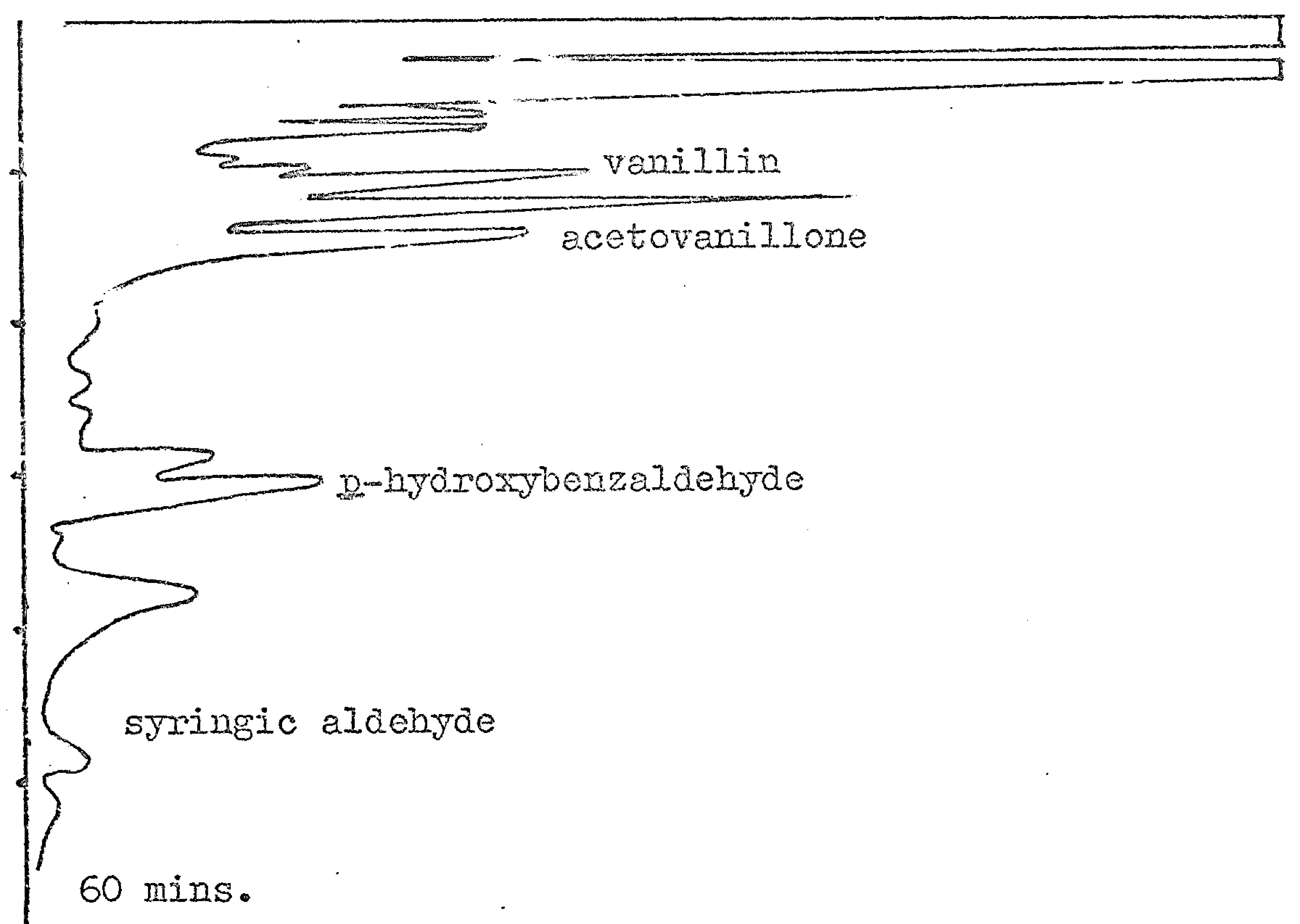


Table 13

The major lignin oxidation products from the Musci and the Hepaticae.

Unless otherwise stated 4µl injections were used.

		Major oxidation products. µg/gm of meal			
	BRYOPHYTA	p-OH	VAN	ACETO	SYR
	Musci				
	Polytrichaceae				
1	Polytrichum commune	6.25	0.68	15.62	trace
	(sporophyte)				
1	Polytrichum commune	46.25	15.00	30.00	trace
2	(gametophyte)	57.00	23.75	45.00	trace
	Hepaticae				
	Plagiochilaceae				
1	Plagiochila aspleniodes	14.40	22.50	35.00	trace
2	(gametophyte)	21.90	45.62	41.24	trace
	Marchantiaceae				
1	Conocephalum conicum	145.00	128.12	162.50	200.00
2	(gametophyte)	126.25	104.37	103.75	290.00
3		132.36	121.42	107.25	245.36

Discussion : Cryptogams.

Tree ferns.

The major lignin oxidation products obtained from a range of woodmeals from the tree ferns using the alkaline cupric oxide method are summarized in table 10. As before gas chromatography was used to separate the constituents and a typical result is shown in figure (52). p-Hydroxybenzaldehyde, vanillin and acetovanillone were detected in all the species examined using a 2 µl injection.

An average vanillin content of 97.9% of the total aldehyde content was present in all the species examined, indicating a guaiacyl type lignin, apart from Dennstaedtia bipinnata. Dennstaedtia bipinnata was found to have a lower vanillin content,(74.8% of the total aldehyde content)and a surprisingly substantial proportion of syringic aldehyde (24.2% of the total aldehyde content). The presence of syringic aldehyde in this species is not yet understood. The classification of ferns is based largely upon reproductive structures. Within the Dennstaedtiaceae, Holtum (1949) grouped a number of subfamilies some of which, he believed, had affinities with the Dicksoniaceae. Sporne (1975) believes that many evolutionary processes have taken place and that the group is difficult to define and so it seems that the subfamilies warrant elevation to family status. Dennstaedtiaceae is regarded as the most 'primitive' subfamily because some species still retain the gradate arrangement of sporangia in the sorus and the sorus has a superficial origin. However the presence of syringyl units is regarded by many as being an'advanced' characteristic. It would therefore be of interest to see what lignin residues occur in a range of species from the subfamilies of the Dennstaedtiaceae.

As syringic aldehyde was detected in Dennstaedtia

bipinnata larger injections (4 µl) were made of genera of this family (Cibotium barometz, Cyatheae arborrea and Pteris podophylla) to see if any syringic aldehyde could be detected. Small amounts of syringic aldehyde were found to be present. Previously only p-hydroxybenzaldehyde and vanillin have been reported from the tree ferns (Sarkanen and Ludwig, 1971). Kawamura and Higuchi (1964) using the infra-red spectra from milled wood lignin of Alsophila dnertensina and Cyathea boninsimer concluded that they had guaiacyl lignin. The tree ferns of course do not possess any secondary thickening of the vascular system. The xylem in these species is composed of tracheids (Bower, 1963 and Foster and Gifford, 1974) and not vessels, yet syringic aldehyde has been observed, notably in Dennstaedtia bipinnata. Lignification can occur in the sclerotic sheaths in the stele (Bower, 1963; Bierhorst, 1971 and Foster and Gifford, 1974) of the tree ferns and in the axes of the tree ferns (Bierhorst, 1971 and Foster and Gifford, 1974). Lignification may also occur in the endodermis of the lower vascular plants. Van Fleet (1961) found that endodermal cells may become modified by a deposition of suberin over the entire inner surface of the wall. Later a layer of cellulose which may become lignified can cover the suberin lamella and the cellulose wall may become incrusted with oxidized products of phenolics and other substances. Again,, a tentative association with the presence of syringic aldehyde and sclenchyma cells can be made.

Small amounts of p-hydroxybenzaldehyde were detected in all the species of tree ferns examined ranging from 0.85% of the total aldehyde content in Dennstaedtia bipinnata to 2.9% of the total aldehyde content in Cyathea arborrea.

Mass Spectral Interpretations.

The mass spectra obtained from the major lignin oxidation products of the tree fern Dennstaedtia bipinnata are presented in table 14 and figures (57) to (60).

Table 14

G.C./M.S. of Oxidation Products from Lignin of *Dennstaedtia bipinnata*.

A.E.I. M.S. 30 double focusing mass spectrometer integrated with a Pye Unicam model 104 G.C. (3% OV 101 on Gas Chrom Q).

Retention Time(min.)	Mass Spectrum m/e (relative abundance%)	M^+	Assigned molecular formulae.
14	125(9),124(100),123(3),122(4),121(4),110(10),109(97),108(5),107(3),95(2),96(4),82(4),81(66),80(4),79(2),77(5),65(5),64(3),63(6),62(2),61(1),53(13),52(11),51(9),50(8),39(6),38(3),37(2),29(91).	124	$C_7H_8O_2$ requires M=124 o or p methoxyl phenol. HO MeO ***
41	153(9),152(46),151(15),150(23),149(12),137(30),136(10),124(12),123(60),122(8),121(9),109(72),108(31),100(6),95(12),94(2),93(10),91(6),81(100),80(17),79(26),77(18),74(10),69(9),67(11),65(24),63(24),55(11),53(37),52(38),51(38),50(21),40(8),39(14),38(7),32(15),29(19),28(19).	152	$C_8H_8O_3$ requires M=152 vanillin. HO CHO MeO **Figure 57
65	167(10),166(100),152(2),151(100),147(4),136(8),124(1),123(40),122(4),108(18),101(9),93(5),80(8),79(8),77(10),67(8),66(4),65(9),52(9),51(8),43(13),41(3),32(5),28(8).	166	$C_9H_{10}O_3$ requires M=166 acetovanillone. CH_3 C=O OMe OH **Figure 58

**Structure assigned on basis of comparison with mass spectrum of authentic compound(figures 38 and 39).
***Tentative structural assignment based on interpretation of fragmentation pattern (Appendix 2).

Table 14 cont.

Retention Time(min.)	Mass Spectrum* m/e (relative abundance%)	M^+	Assigned molecular formulae.
73	291(10),281(22),269(19),231(44),225(14),219(17),205(26)201(13),196(12),191(10),183(12),173(15),170(23),165(23),154(36),163(19),156(18),131(42),121(50),98(45),93(37), 83(95),82(92),76(69)49(52),47(60),45(74),43(100),40(96),32(91),29(50),28(100).		
86	196(6),194(9),191(5),183(4),182(28),181(21), 177(14),167(6),164(8), 155(11),154(28),153(31),141(27),125(13),124(9),123(32),122(62), 121(26),113(23),111(10),107(17),105(19),97(23),91(35),89(8),85(23),71(39),70(30),57(54),55(55),54(53),43(59),41(33),40(100),39(27),42(100),30(32),28(100).	182	$C_9H_{10}O_4$ requires M=182 syringic aldehyde. MeO HO ⟨benzene ring⟩ $COCH_3$ MeO ** Figure 59
92	282(2),281(7),269(3),256(2),243(3),236(6),226(19),207(5),184(10),181(11),169(13),168(4),155(22),154(29),85(82),71(81),57(100),55(62),43(73),41(63),32(100),28(100),.		

*For unidentified components only ions with a relative abundance greater than 30% are quoted for m/e less than 150.
**Structure assigned on basis of comparison with mass spectrum of authentic compound(figure 40).

Figure (57) vanillin from Dennstaedtia bipinnata.

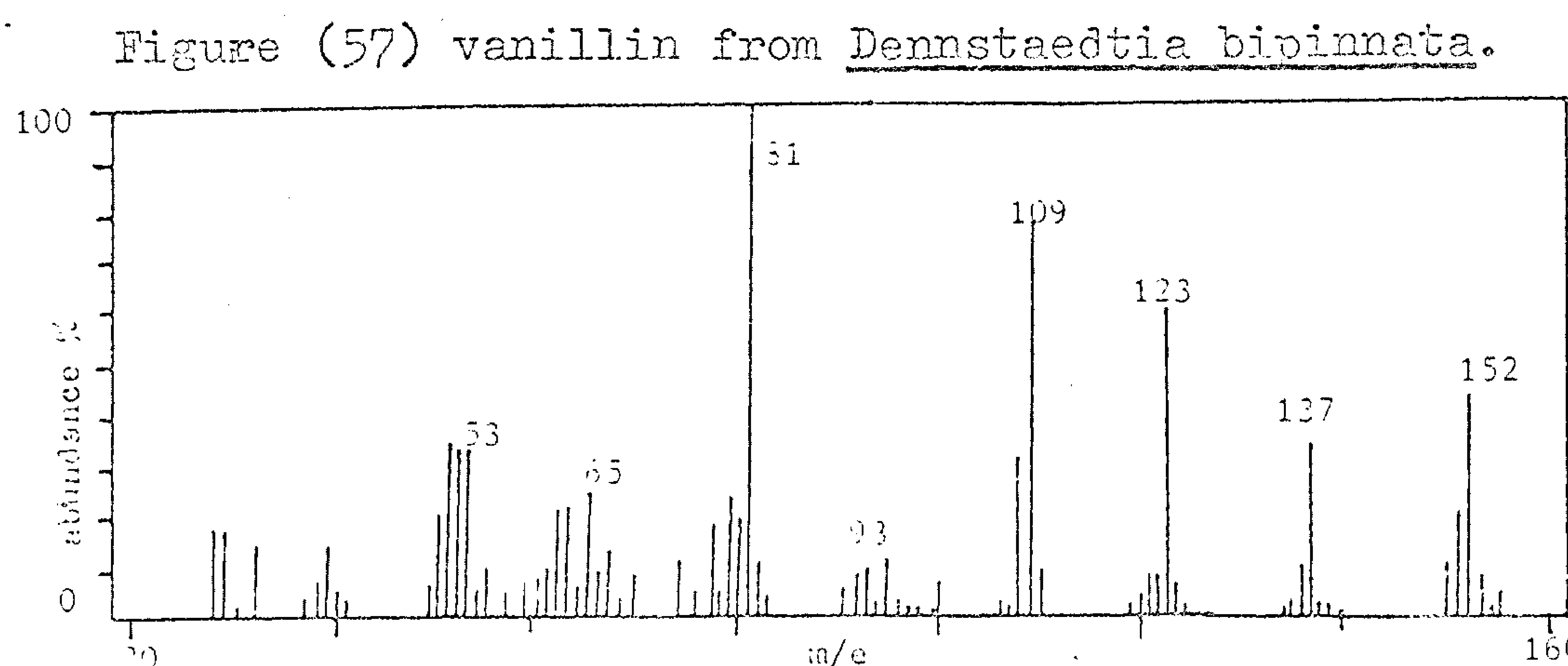


Figure (58) acetovanillone from Dennstaedtia bipinnata.

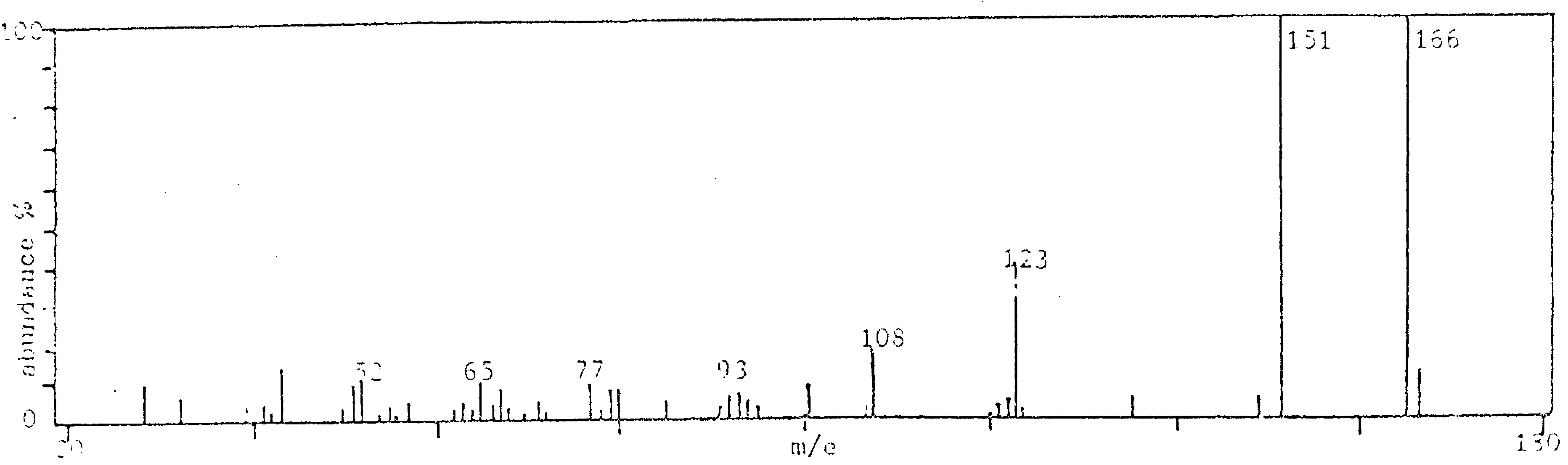


Figure (59) syringic aldehyde from Dennstaedtia bipinnata.

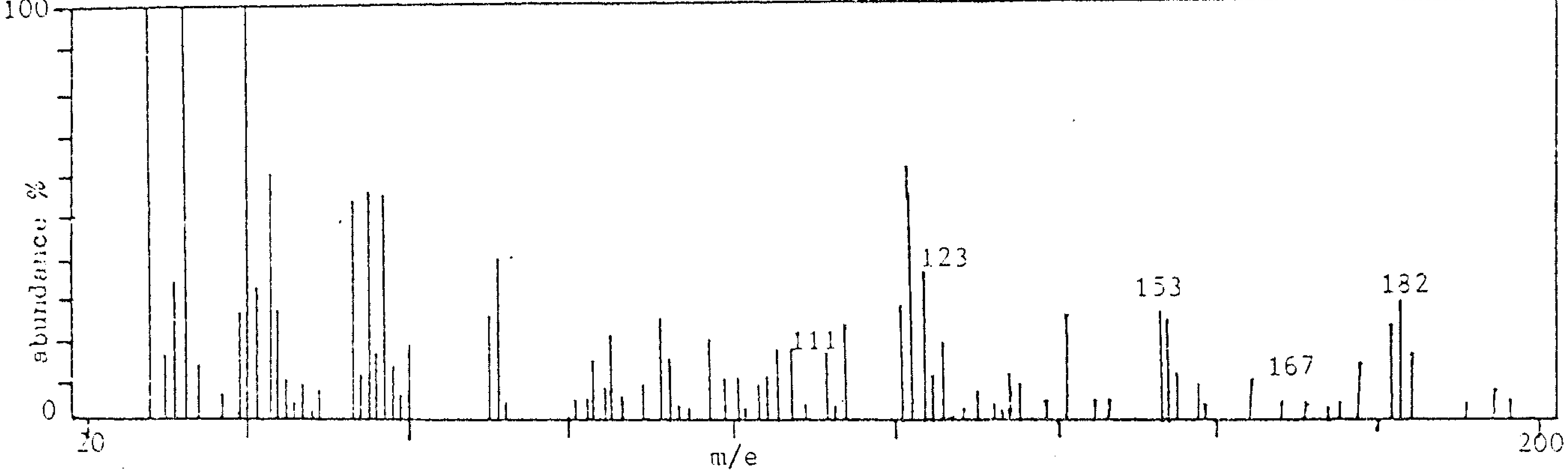
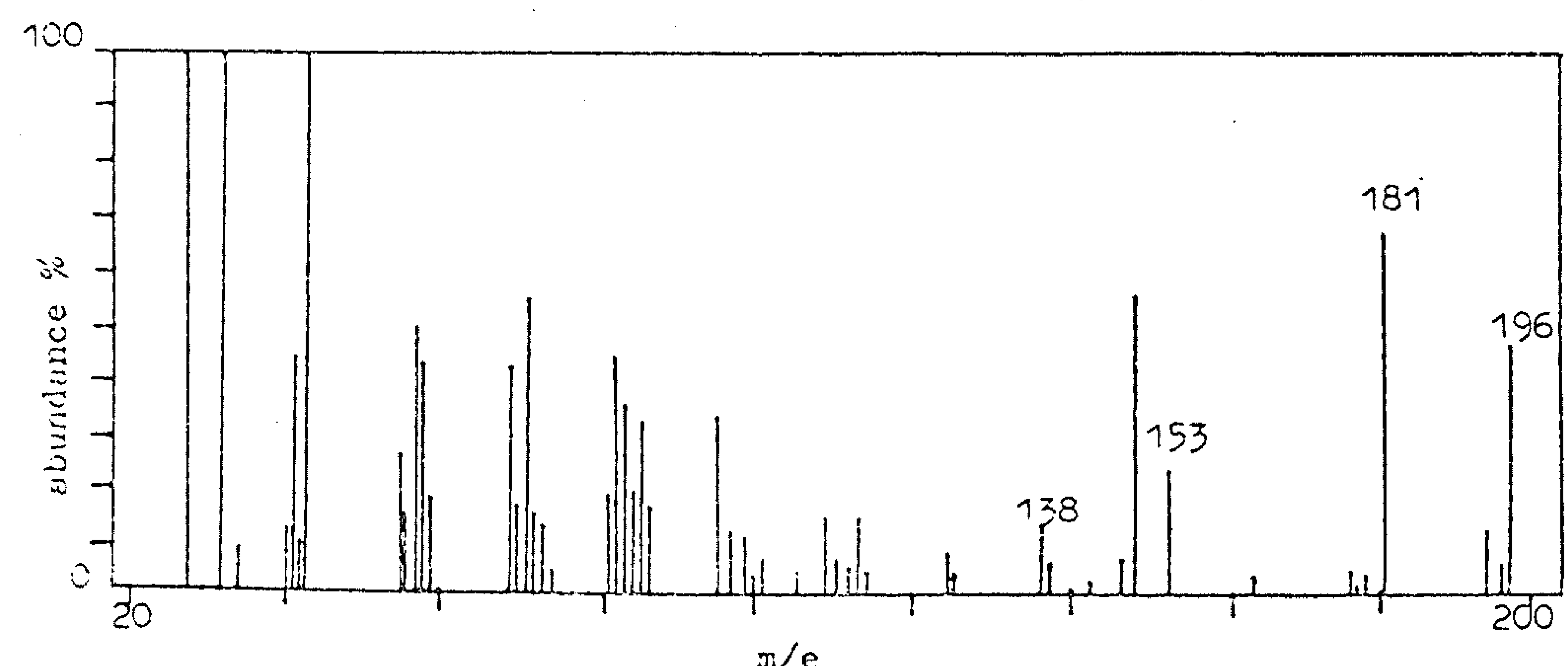


Figure (60) acetosyringone from Dennstaedtia bipinnata.



Equisetum and the Lycopods.

Table 12 shows that the lignin oxidation products obtained from Equisetum fluviatile were p-hydroxybenzaldehyde, vanillin, acetovanillone and syringic aldehyde. Syringic aldehyde and p-hydroxybenzaldehyde were present in relatively significant proportions, 20% of the total aldehyde content and 34% of the total aldehyde content respectively. Previously vanillin and p-hydroxybenzaldehyde have been reported from Equisetum hyemale (Towers and Gibbs, 1953 and Ibrahim, Towers and Gibbs, 1962) but no syringic aldehyde was observed. No secondary growth occurs in species of Equisetum and the xylem is mainly composed of tracheids. Equisetum species also consist of metaxylem elements which may be tracheids or may be true vessels (Sporne, 1975). Vessel elements do occur at the internodes but they do not form conducting channels as do vessels of the flowering plants (Bierhorst, 1958). Lignification may also occur in the endodermis, of which in Equisetum fluviatile each internodal bundle is surrounded by its own separate endodermis, or in the sclerenchyma cells of the cortex. Whether the presence of syringic aldehyde is due to the presence of vessel elements or the sclerified tissue is not answered here.

As early as 1953 Towers and Gibbs reported the presence of syringic aldehyde in three species of Selaginella while it was absent in Lycopodium complanatum. Since then several authors (Towers and Mass, 1965; White and Towers, 1967, and White, Tse and Towers, 1967) have found that the syringyl type lignin is present in species of Selaginella while guaiacyl type lignin was present in species of Lycopodium. No syringic aldehyde was detected in Lycopodium cernua or Lycopodium squarrosum in the present work (although both species were examined as young shoots). Bierhorst (1971) has reported that sclerenchyma is reduced or absent in smaller stems of the lycopods, which may be a factor in the absence of syringic aldehyde. El-Basyouni and Towers

(1964) reported that p-hydroxybenzaldehyde and vanillin residues were present during the early period of growth in plants and later as maturation proceeds, syringyl units are formed. This too could explain why syringic aldehyde was not detected from the young shoots of the *Lycopodium* species that were examined in this work. Unfortunately mature stems were not available to us at the time. Lycopods contain tracheids only and this can be cited as evidence for the association of vessels being necessary to obtain syringic aldehyde as an oxidation product of lignin.

Vessels have been found to be present in the Selaginellas and again a correlation between the detection of syringic aldehyde and the presence of vessels has been made (Sarkanen and Ludwig, 1971 and Lewis, [illegible]) However vessels have only been reported in certain species of the Homoeophyllum group including *Selaginella rupestris* and *Selaginella oregana* (Sporne, 1975). The majority of xylem tissue present in the Selaginellas is composed of tracheids. As in the lycopods, the *Selaginella* species do not have any secondary thickening except , according to Bruchmann (1897) in *Selaginella selaginoides*. *Selaginella* species do however possess a lignified endodermis and a zone of hypodermal sclerenchyma cells (Bierhorst, 1971 and Foster and Gifford, 1974). Interestingly *Selaginella wildenovii* belonging to the Heterophyllum, where no vessels have been reported so far, has a substantial amount of syringic aldehyde when examined here (54.8% of the total aldehyde content). Again it appears that it is not exclusively plants with vessels that give syringic aldehyde as a lignin residue.

Mosses and Liverworts.

Lignin and its derivatives obtained from mosses and liverworts have been reported in small amounts by several authors (Lindberg and Theander, 1952; Morrison, 1958; Freudenberg and Harkin, 1964; Bland, Logan, Menshun and Sternhell, 1968; Manskaya and Drozdova, 1968 and Siegel, 1969) using a variety of chemical criteria for lignin. Contradictory evidence has been provided by Sarkanen and Latif (1971) who failed to find any Hibbert's ketones in Rhytidiadelphus loerus. In 1974 Erickson and Miksche, using an oxidative degradation method, demonstrated that moss and liverwort species that they examined did not possess lignin but that they contained phenolic cell wall material.

Table 13 shows the type of 'lignin residues' obtained when moss and liverworts were subjected to alkaline cupric oxide oxidations. All three types of nuclei were detected from the species examined in this work. Polytrichum commune was found to contain the largest proportion of p-hydroxybenzaldehyde (78.7% of the total aldehyde content). The liverworts examined here were found to have less p-hydroxybenzaldehyde (31.4% of the total aldehyde content). The liverwort Conocephalum conicum contained larger amounts of syringic aldehyde than the other species examined(46% of the total aldehyde content).

p-Hydroxybenzaldehyde has been reported as the major lignin derivative from Sphagnum by Nilsson and Tottmar (1967) and Lindberg and Theander (1952) concluded that lignin from Sphagnum is built up chiefly of p-hydroxyphenyl units. In the bryophytes that have been examined here p-hydroxybenzaldehyde has been detected in substantial amounts, agreeing with these earlier observations. Infact p-hydroxybenzaldehyde has been shown to occur in high proportions in the

lignin oxidation products for Equisetum fluviatile and the Lycopsida examined in this present work. This accords with Manskaya's statement of 1958 "that the methoxyl content of primitive plants is lower than that of higher plants suggesting a slightly different lignin to that of angiospermous or gymnospermous lignin".

There have been no reports of mosses and liverworts possessing a vascular strand as amongst higher plants. However Proskauer (1960) reported the presence of spiral thickening in the columella cells of Dendroceros crispus. Watson (1964) has reported the presence of thick walled fibres from Coniocephalum conicum and Lorch (1931) has reported thick walled epidermal cells of the capsule but which he stated were unlignified. However because lignin oxidation products were observed in this work from mosses and liverworts it does seem likely that the epidermal cells do become lignified, as do the thick walled fibre cells.

CHAPTER 5.

LIGNIN DERIVATIVES FROM FOSSILS AND COALS.

Lignin derivatives from fossils and coals.

Organic residues have been used for geochemical purposes since the 1940's. Initially pigments such as haemin and chlorophyll (Fox and Anderson, 1941) and amino acids and sugars (Degens, Reuter and Shaw, 1964) were used as offshore indicators. More recently the distribution of aromatic aldehydes and polycyclic aromatics have been used (Clayton Swetland, 1978; Laflamme and Hites 1978 and Lam and Pedersen, 1978). Biochemical investigations of organic residues present in fossil material has just recently been explored. Initially the organic residues were interesting in themselves but these are now being used as characters for use in taxonomic and phylogenetic studies.

Leaf impressions obtained from the middle Eocene contained chlorophyll derivatives which were chromatographed (Dilcher,1967). One of the pigments absorption bands was indicative of methyl pheophorbidea, a derivative of chlorophyll. It is the oldest phorbin reported. Usually in fossil sediments further reduction and decarboxylation occurs producing highly stable porphyrins which are commonly reported in oil, coal and fossil rich shale.

Knoche and Ourison (1967) isolated hydrocarbons from the fossil _Equisetum bronginarti_ and compared them with those of living _Equisetum sylvaticum_. They isolated the same compounds from both the living and fossil _Equisetum_ proving that long chain hydrocarbons can remain unaltered in a fossil. Bonnett, Middlemiss and Noro (1972) investigated the presence of equisetolic acid in two fossil compressions _Equisetum columnare_ and _Equisetum lyelli_. Unfortunately no acid was detected in the compressions although it was present in certain contemporary _Equisetum_ species.

Coalified compressions and petrifactions of Palaezoic plants have been analyzed and the monosaccharide components galactose, glucose, mannose and xylose were extracted by Swain, Bratt and Kirkwood (1967, 1968). The wood of the Devonian plant _Callixylon_ a progymnosperm was found to have a higher ratio of galactose to glucose compared to that of _Cordaites_ a gymnosperm. Assumptions as to the

polysaccharides from the monosaccharides present were made, which correlated with a change from algal-like galactan rich polysaccharides to cellulose, in the evolution of Cordaites from the Callixylon ancestral group.

Niklas and co-workers in a series of papers (Niklas, 1976a; 1976b; Niklas and Chaloner, 1976 and Niklas and Gensel, 1977) examined a group of Devonian thalloid plants of the genera Protosalvina, Parka and Orestovia. These plants are only broadly understood so bichemical data is of great potential. Due to the hydrocarbon, amino acid and carbohydrate constituents ascribed to Protosalvinia, Parka and Orestovia Niklas and co-workers interpreted them as algal rather than of vascular plant affinity. Spongiophyton was interpreted as being of animal rather than plant-like affinity and Parka because it possessed stigmatane and ergostane was referable to the Chlorophyta.

Giannasi and Niklas in a series of papers investigated the flavonoid components of the fossil angiosperms, belonging to the Ulmaceae and classified this family into two main groups based on the classes of flavonoids (Niklas and Giannasi, 1977a; 1977b; Giannasi and Niklas, 1977; Giannasi, 1978a; 1978b and Niklas and Giannasi, 1978).

In 1978 Sigleo identified phenols, methyl phenols, alk ly substituted benzenes and benzofurans as all possible derivatives of lignin from a two hundred million year old silicified conifer, Araucarioxylon arizonium.

It must be stressed that during and after fossilization contamination of the original plant material components such as the amino acids and hydrocarbons can occur from overlying matrixes and percolating water. Lignin derivatives, which are less likely to become contaminated have been used by previous workers as markers of land derived organic matter in surface sediments (Leo and Barghoorn; 1970, Gardiner and Menzel, 1974 and Hedges and Parker; 1976).

In this work lignin derivatives have been examined from fossil woods and coals from various geological ages.

Primarily lignin derivatives have been examined in an attempt to apply phytochemical methods to compression fossil material and to use this information obtained taxonomically. Secondly the lignin derivatives obtained from the fossil wood and coal may elucidate some steps in the modification of coals that occur during the process of coalification. It therefore seems appropriate to outline briefly the main processes of coalification.

Coal is of considerable importance as a natural product derived primarily from organic material. It also contains mineral components such as uranium and vanadium. Therefore it is important to elucidate the stages of coal formation and to determine the transformation of the initial organic compounds that participate in the formation of coal. Coalification can be divided into two main phases, the first phase, the biological-bacterial process termed biological coalification and the second phase, metamorphism or geochemical coalification, in which chemical and physical influences lead from the brown coal (lignite) to higher degrees of coalification. There are many reviews of coalification processes (Manskaja and Drozdova, 1968; Flaig, 1968 and Teichmuller and Teichmuller, 1968). During coalification the original plant material loses oxygen, hydrogen, and nitrogen and the carbon content is increased.

Lignin is much more important than cellulose in the transformation of plant constituents into coal. Cellulose is linear and is depolymerized by many microorganisms into available glucose which is utilized as energy. The three dimensional lignin molecule is much more difficult to degrade and therefore participates in coalification. Lignin is degraded under anaerobic conditions or conditions in which oxygen can be transferred (Kirk, Higuchi and Chang, 1980).

Humification takes place in the upper part of the soil. The humic acids are primarily formed from lignin and its derivatives but also from other phenolic plant constituents and proteins. Microorganisms are involved and the plant constituents are decomposed to carbon dioxide, water and

ammonia. The first step in the process of coalification is considered to be peat formation which occurs under partly aerobic and partly anaerobic conditions. It is generally accepted that a decrease in cellulose and an increase in the modification of lignin and humic acids takes place at the peat stage up until the development of bituminous coal. Accompanying this a decrease in oxygen, hydrogen and nitrogen occurs with an increase in carbon and aromaticity. The process which leads from this stage to the formation of brown coal (lignite) occurs only when the organic material is covered by sediment and impervious to the air. In contrast to humification and peat formation the time factor plays an important part in the development of brown coal. In the formation of bituminous coal not only time but also an increase in temperature and pressure are necessary for decarboxylation, condensation and the rearrangement of compounds into polycyclic aromatic compounds. Hayatsu, Winans, McBeth, Scott, Moore and Studier (1978) identified organic acids from lignite and bituminous coal and found a greater proportion of aromatic structures and a greater degree of cross-linking than in the lignite. White and Lee (1980) identified polycyclic aromatic hydrocarbons from coals, some having biological origins. The aromatic hydrocarbons obtained indicated that coalification consists of dehydrogenation, aromatization and dealkylation of components in the original plant debris. To arrive at anthracite the end product of coalification many reactions must occur which lead to products of high aromaticity.

A large decrease in the methoxyl content of lignin takes place in the decomposition of the lignin to form coal (Stach, 1933; Haider, Lim and Flaig, 1962 and Leo and Barghoorn, 1970). Manskaya and Drozdova (1968) found that the quantities of aldehydes formed by oxidation with nitrobenzene decreased in fossil wood and lignites with increasing geological age. Kodina (1968) examined two coals and three lignites and reported the presence of vanillin and *p*-hydroxybenzaldehyde in the lignites. Bimer, Given and Raj (1978) using performic acid as an oxidant

isolated many degradation products from coal including lignin derivatives. Hayatsu, Winans, McBeth, Scott, Moore and Studier (1979) isolated phenolic acids showing a relationship between lignins and coals. Syringic groups were not detected in the coals.

Results with Quaternary Fossil Wood.

Samples from fossil wood of different geological ages were oxidized using the cupric oxide method described previously and chromatographed quantitatively and qualitatively. The Quaternary geological sites are listed fully in Chapter 2.

Figure (61).

A chromatogram of the lignin oxidation products of a gymnospermous wood from the Quaternary.

Pinus- two leaved type, sample 1, woodmeal, 2µl injection, (5% Pdegs column).

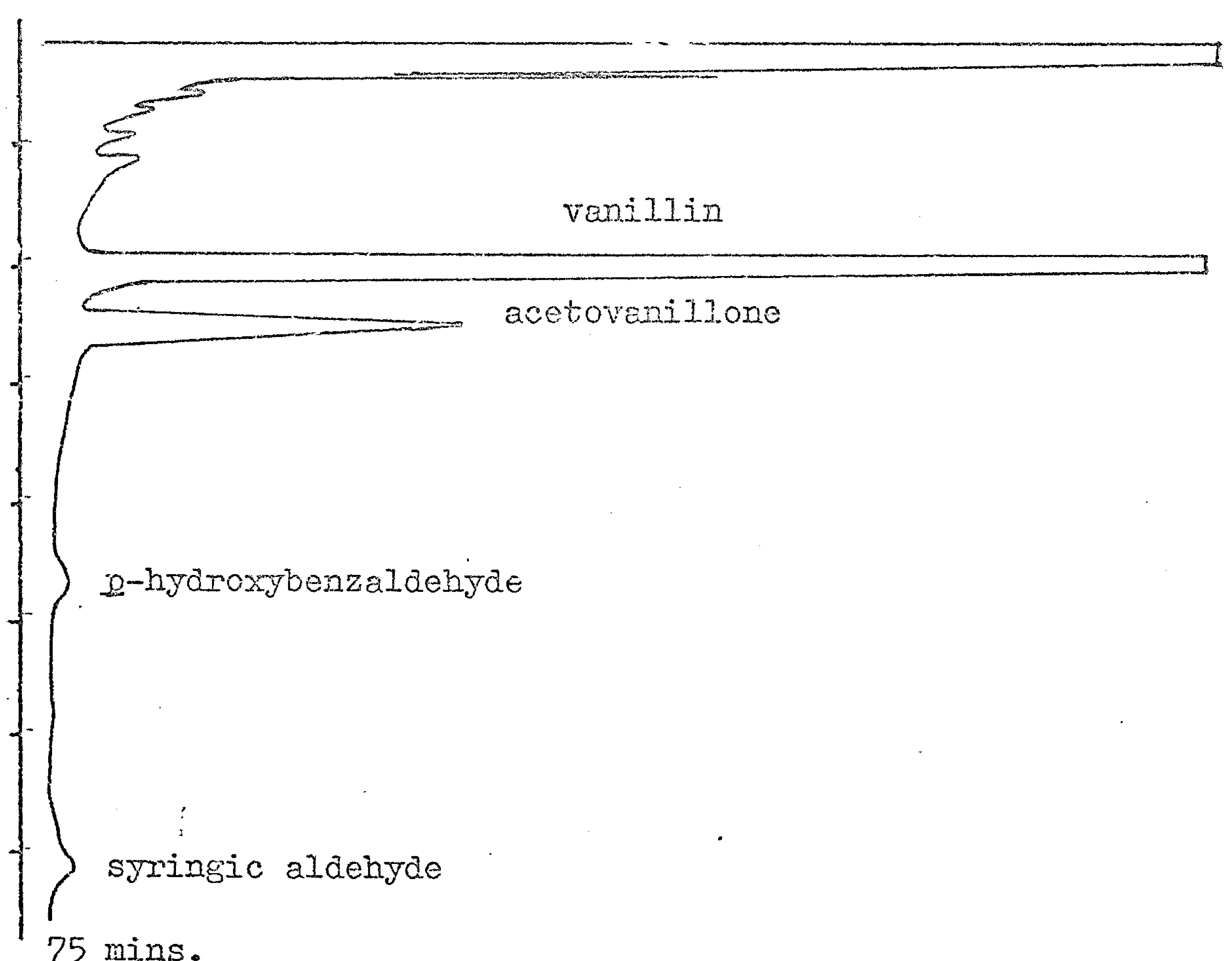


Figure (62).
A chromatogram of the lignin oxidation products of an angiospermous wood from the Quaternary.

Ulmus procera woodmeal, 2µl injection . (OV 101 column).

p-hydroxybenzaldehyde

vanillin

acetovanillone

syringic aldehyde

30 mins.

Table 15

The major lignin oxidation products from the fossil wood of the Quaternary period.

Unless otherwise stated 2µl injections were used.

			Major oxidation products. mg/gm of woodmeal			
		Quaternary Fossil Wood	p-OH	VAN	ACETO	SYR
FLANDRIAN HOLOCENE		Sample 1				
	1	Pinus sp.-2 leaved type	0.10	6.90	1.26	0.24
	2		0.09	6.97	1.26	0.25
		Sample 2				
	1	Betula pendula	absent	3.75	1.05	3.68
	2		absent	4.50	1.20	6.75
		Sample 3				
	1	Quercus sp.	absent	2.92	1.20	9.60
	2		absent	2.32	1.12	8.70
		Sample 4				
	1	Ulmus procera	trace	8.10	2.10	12.15
	2		trace	7.24	2.24	12.90
CROMER PLEISTOCENE		Sample 5				
	1	Pinus sp.-2 leaved type	0.09	3.45	0.69	absent
	2		0.08	3.45	0.69	absent
		Sample 6				
	1	Salixaceae ?	0.01	1.61	0.30	3.52
	2		0.01	2.21	0.45	5.40
	3		0.02	2.74	0.75	4.42

The Quaternary wood was hand sectioned using the light microscope and the scanning electron microscope for identification.

Anatomical identifications of the Quaternary samples.

Sample 1. (bog wood, Pinus sylvestris)
Site: Far Black Clough, Southern Pennines.

Plate (1) is a transverse section of the wood showing the axial tracheids as polygonal cells. The tracheids are thinner walled during the early wood and gradually become thicker walled and smaller in the late wood. Two circular axial resin canals can be seen which have been torn either in sectioning or in preservation. The rays are well preserved consisting of narrow parenchyma cells. Plate (2) shows a preserved circular intact resin duct.

In radial longitudinal section the large single bordered pits in the tracheids are well preserved as are shown in plate (3). The ray parenchyma cells have large window-like simple pits which suggest that this wood is Pinus. The ray tracheids, plate(3) are dentate, tooth like projections which are just visible in the lumen of the cell indicating that this species is of the two needled pine Pinus sylvestris.

The tangential longitudinal section plate (4) shows long chains of uniseriate rays. There is also a fusiform ray with a resin canal in the centre, the rays being uniseriate above and below the resin canal region.

Sample 1. (*Pinus sylvestris*)

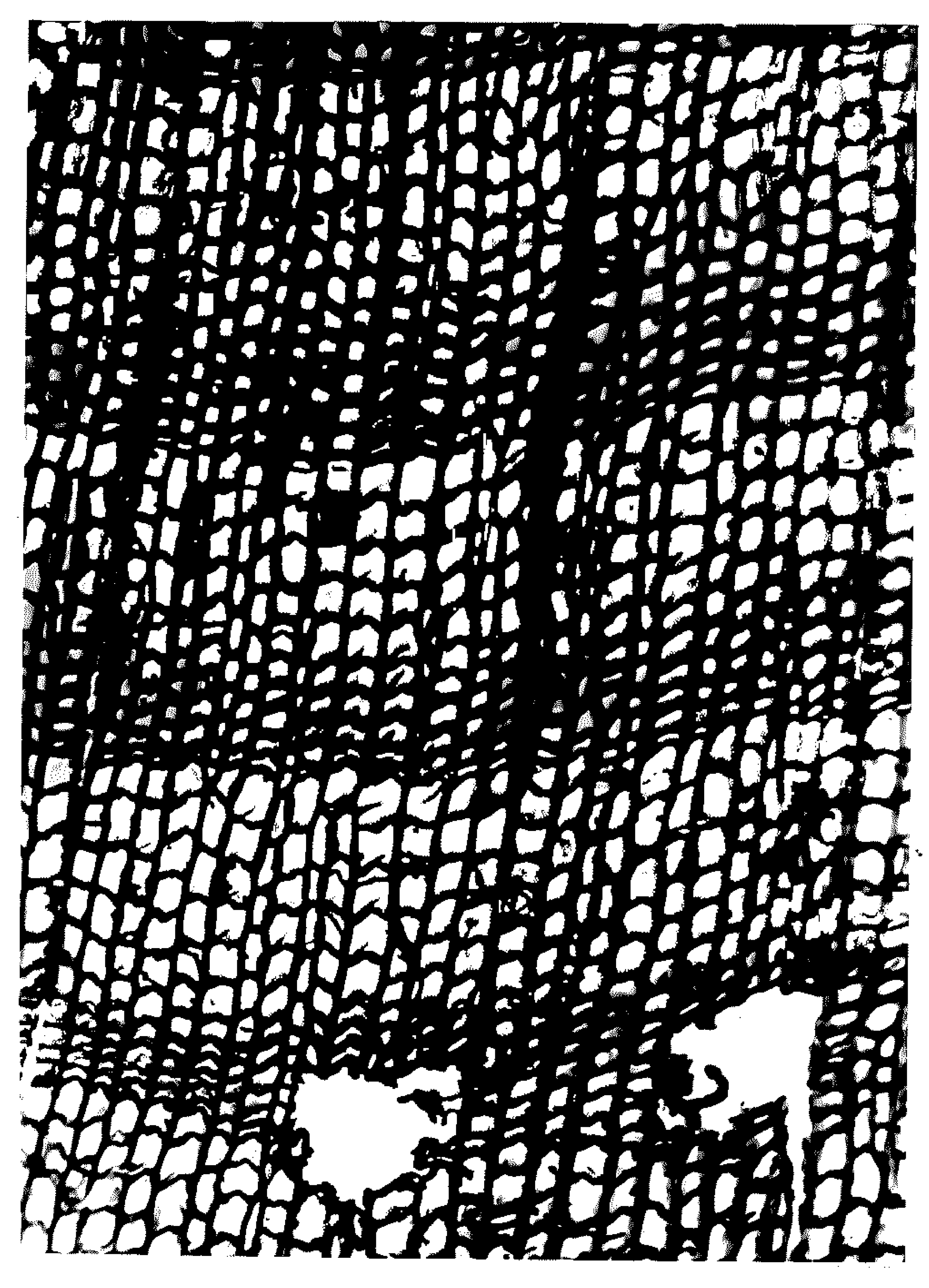
Site: Far Black Clough, Southern Pennines.

Plate (1).

T.S. (x100), showing the early and late wood. Two circular axial resin canals are present.

Plate (2).

T.S. (x250), showing an intact circular resin duct.



Sample 1 (*Pinus sylvestris*)

Site: Far Black Clough, Southern Pennines.

Plate (3).

R.L.S. (x250). A row of ray tracheids can be seen with peg-like dentations on the horizontal walls. Large window-like pits are present in the ray parenchyma cells and bordered pits are well preserved on the tracheids.

Plate (4).

T.L.S. (x100). Uniseriate and fusiform rays.

Sample 2. (bog wood, *Betula pendula*)
Site: Ringinglow Bog, Southern Pennines.

This sample was preserved with its bark intact. The bark was smooth with prominent horizontal lenticels characteristic of birch. Plate (5) shows a transverse section of a young stem showing a parenchymatous pith. The darkly stained areas in this photograph appear to be due to its preservation. The wood is diffuse porous with very narrow rays, mainly uniseriate but some multiseriate rays are shown in plate (5). Some of the vessels are clustered in two's or three's forming short radial chains. The solitary vessels are oval and slightly angular in shape, a characteristic of birch wood. Vessels were found to have alternate pitting and scalariform perforation plates, plate (5). Distinct fibre-tracheids were not present. These features are indicative of birch. Conway (1954) has identified substantial amounts of *Betula pendula* pollen grains from this site. Although *Betula pendula* and *Betula pubescens* cannot be differentiated on the basis of anatomical features it seems reasonable to assume that it is *Betula pendula* when pollen counts are taken into consideration.

Sample 2. (Betula pendula)

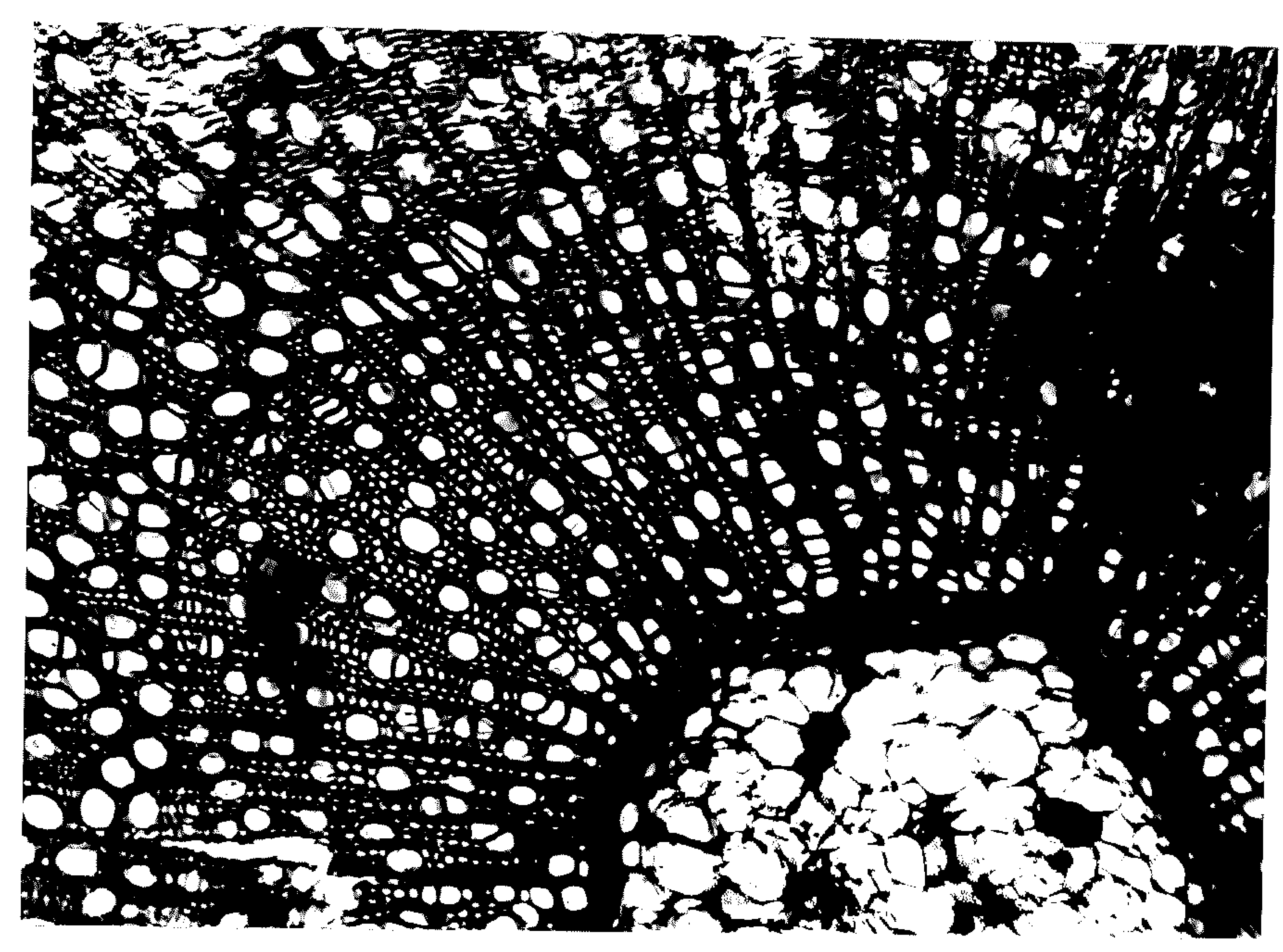
Site: Ringinglow Bog, Southern Pennines.

Plate (5).

T.S. (x100), showing a diffuse porous wood with uniseriate and multiseriate rays.

Plate (6).

R.L.S. (x50), showing vessels with alternate pitting and scalariform perforation plates.



Sample 3. (bog wood, *Quercus species*)
Site: Mildenhall Fen, Cambridgeshire.

This wood was very brittle and partially petrified. It was also extremely discoloured, that is black and so was bleached carefully in 10% hydrogen peroxide to remove this staining. The wood was identified from the transverse sections made. It is a ring porous wood with very large multiseriate rays and some small uniseriate rays as shown in plate (7). The multiseriate rays are obvious to the naked eye, a characteristic of oak. The change from early to late wood is abrupt. Apotracheal parenchyma stretches between the rays. Large solitary vessels show the remains of tyloses, plate (7). These are all features of English oak either *Quercus robur* or *Quercus petraea*. According to Jane (1970) neither species can be distinguished with certainty. The presence of *Quercus* pollen grains has been reported from this site by Godwin and Clifford (1938).

Sample 4. (*Ulmus procera*)
Site: Broxbourne, Hertfordshire.

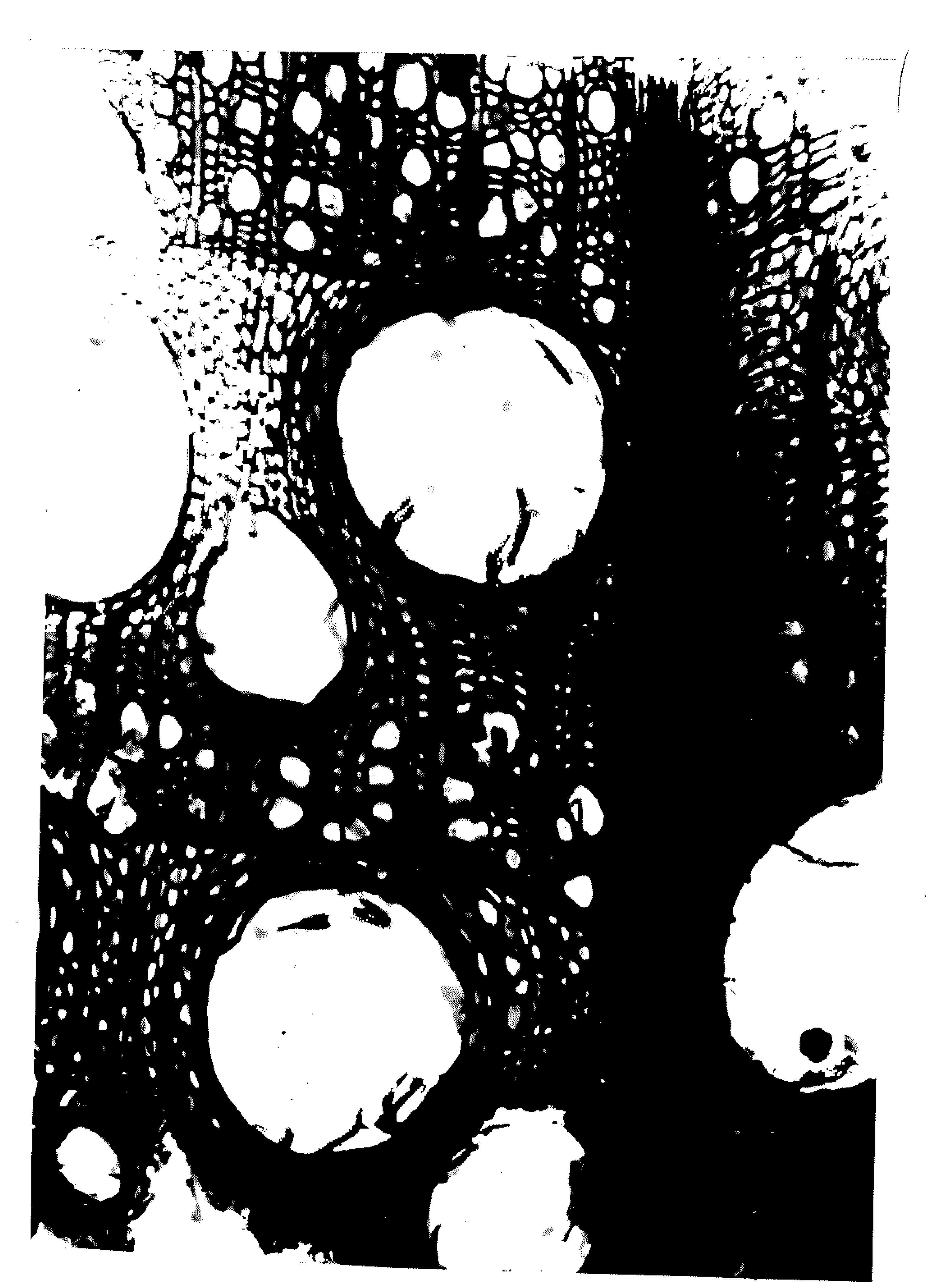
This wood was identified from the transverse sections made. It is ring porous with large vessels in the early wood with tyloses, plate (8) and smaller and more numerous vessels in the late wood. The late wood is arranged in undulating tangential bands in a zig-zag arrangement, plate (9). There is an abrupt change from the large vessels to the closely packed tangentially arranged vessels of the late wood. Fairly large rays are present in this wood as shown in plates (8) and (9). These features indicate this wood is an *Ulmus* species, that of *Ulmus procera*. The results of pollen analysis from the peat at Broxbourne show high values of *Ulmus* pollen (Warren, Clark, Godwin and MacFayden, 1934).

<u>Sample 3.</u> (<u>Quercus</u> species)

Site: Mildenhall Fen, Cambridgeshire.

Plate (7).

T.S. (x100), showing the early and late wood, large multiseriate rays and solitary vessels with the remains of tyloses.



Sample 4. (*Ulmus procera*)

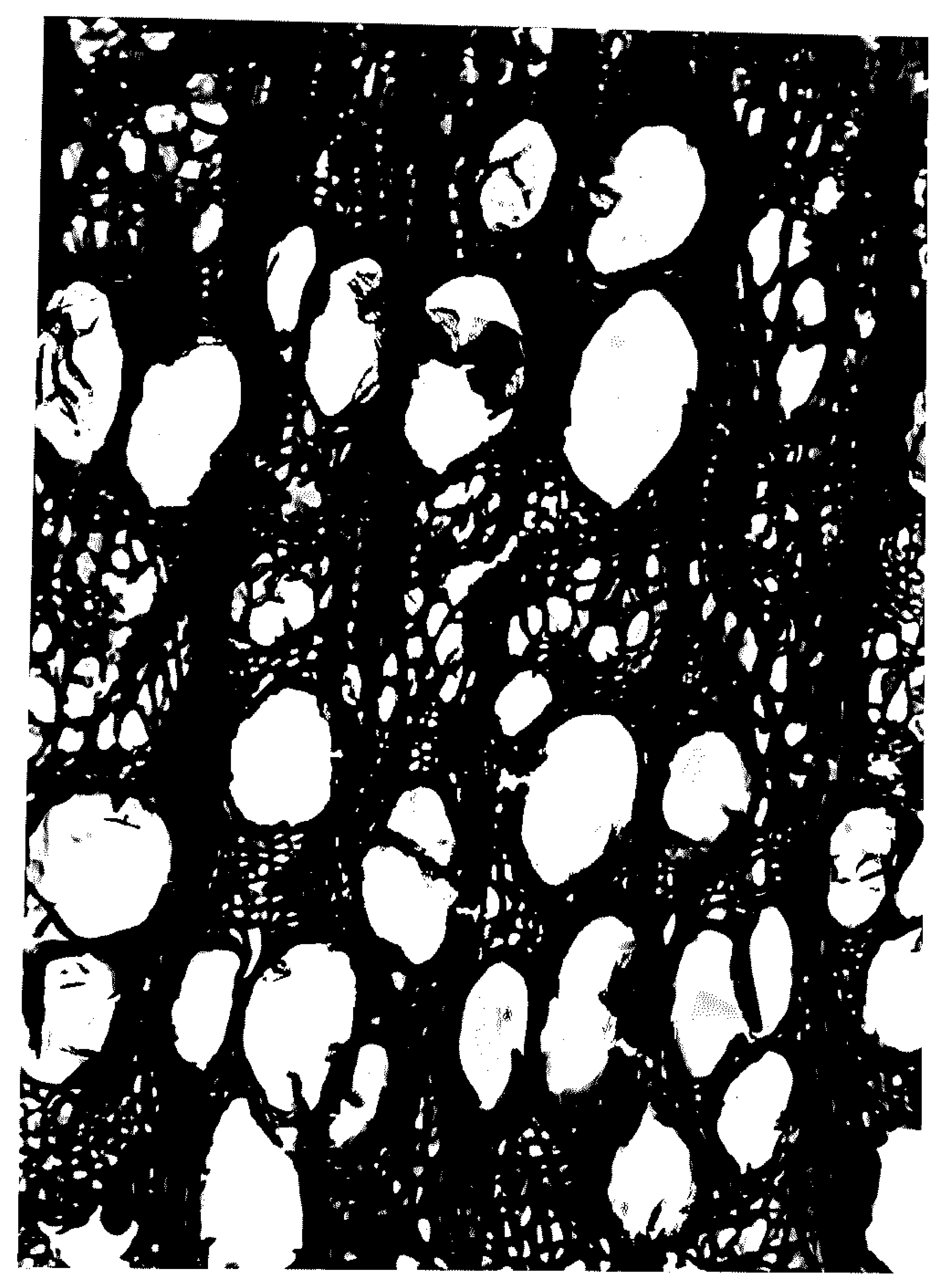
Site: Broxbourne, Hertfordshire.

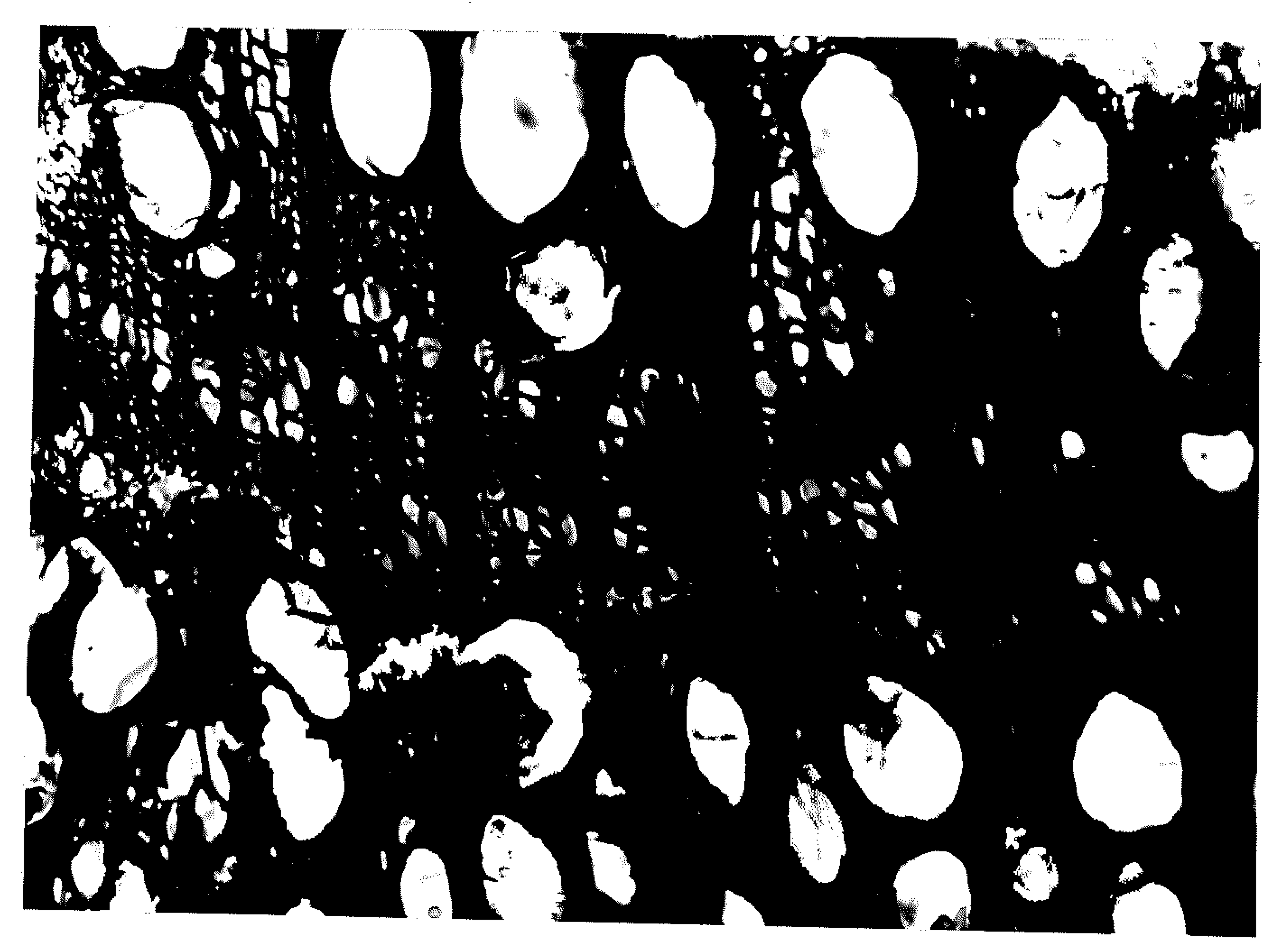
Plate (8).

T.S. (x100), showing a ring porous wood with large vessels in the early wood with tyloses and smaller vessels in the late wood.

Plate (9).

T.S. (x100), showing the late wood arranged in undulating tangential bands. Fairly large rays are present.





Sample 5. (compressed wood, *Pinus sylvestris*)
Site: Freshwater Bed, West Runton, Norfolk.

This wood is not as well preserved and is more compressed than the wood of sample 1. Plate (10) is a transverse section of the wood showing the axial tracheids as polygonal shaped cells. The tracheids become thicker walled and smaller in the late wood. Axial resin canals can be seen which have been torn in the preservation. In the radial longitudinal section large window-like simple pits are present in the ray parenchyma together with ray tracheids which are dentate and have small bordered pits, plate (11), both features of which are characteristic of the two needled type of pine. The tracheids show large single bordered pits which are well preserved, plates (12) and (13). In plate (13) the torus is prominent on both the bordered pits shown. The biostratigraphy of this area is described by West (1980). West (1980) has reported the presence of *Pinus* pollen from this site but no macrofossils of *Pinus* were reported.

Sample 5 (*Pinus sylvestris*)

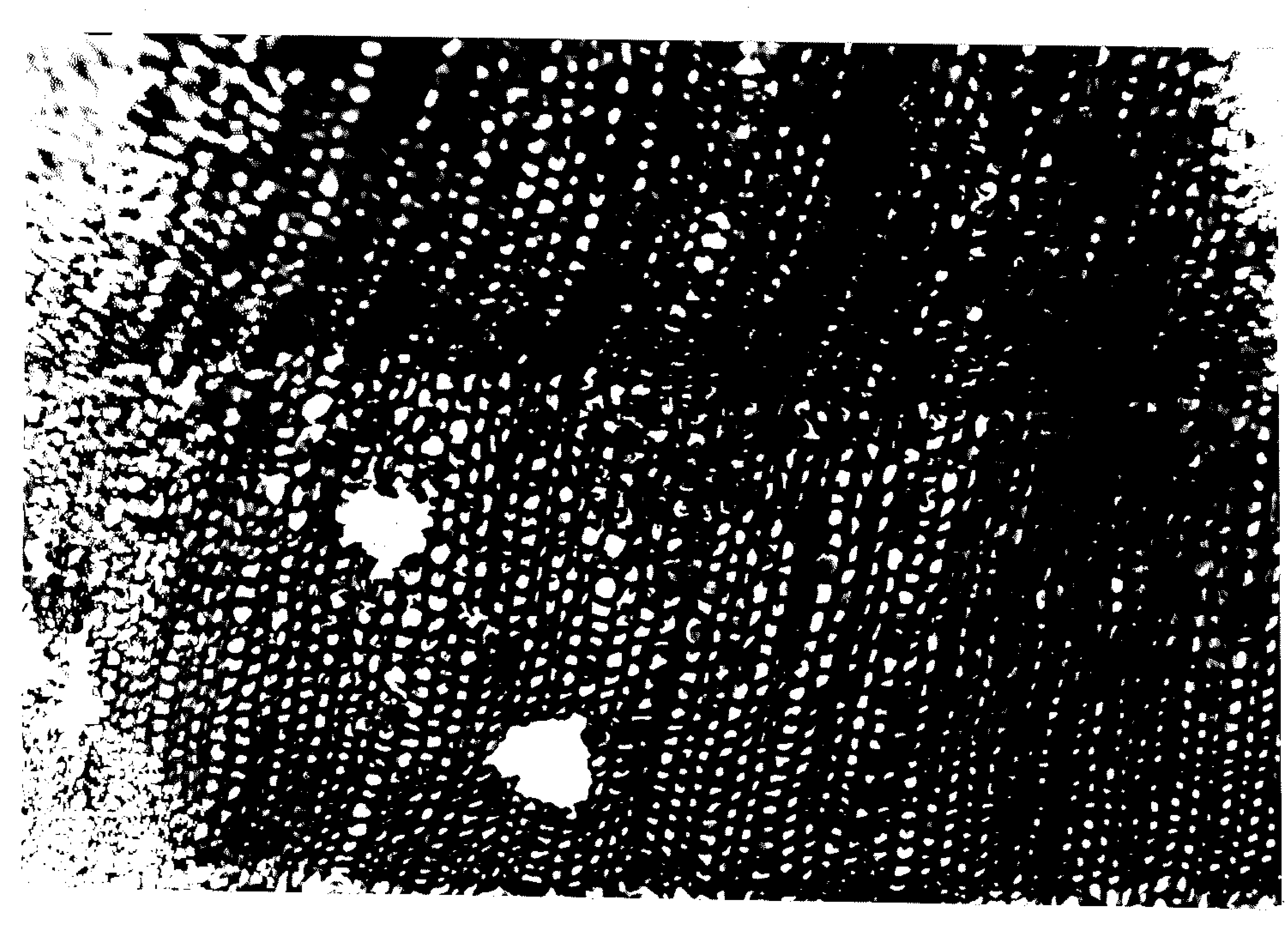
Site: Freshwater Bed, West Runton, Norfolk.

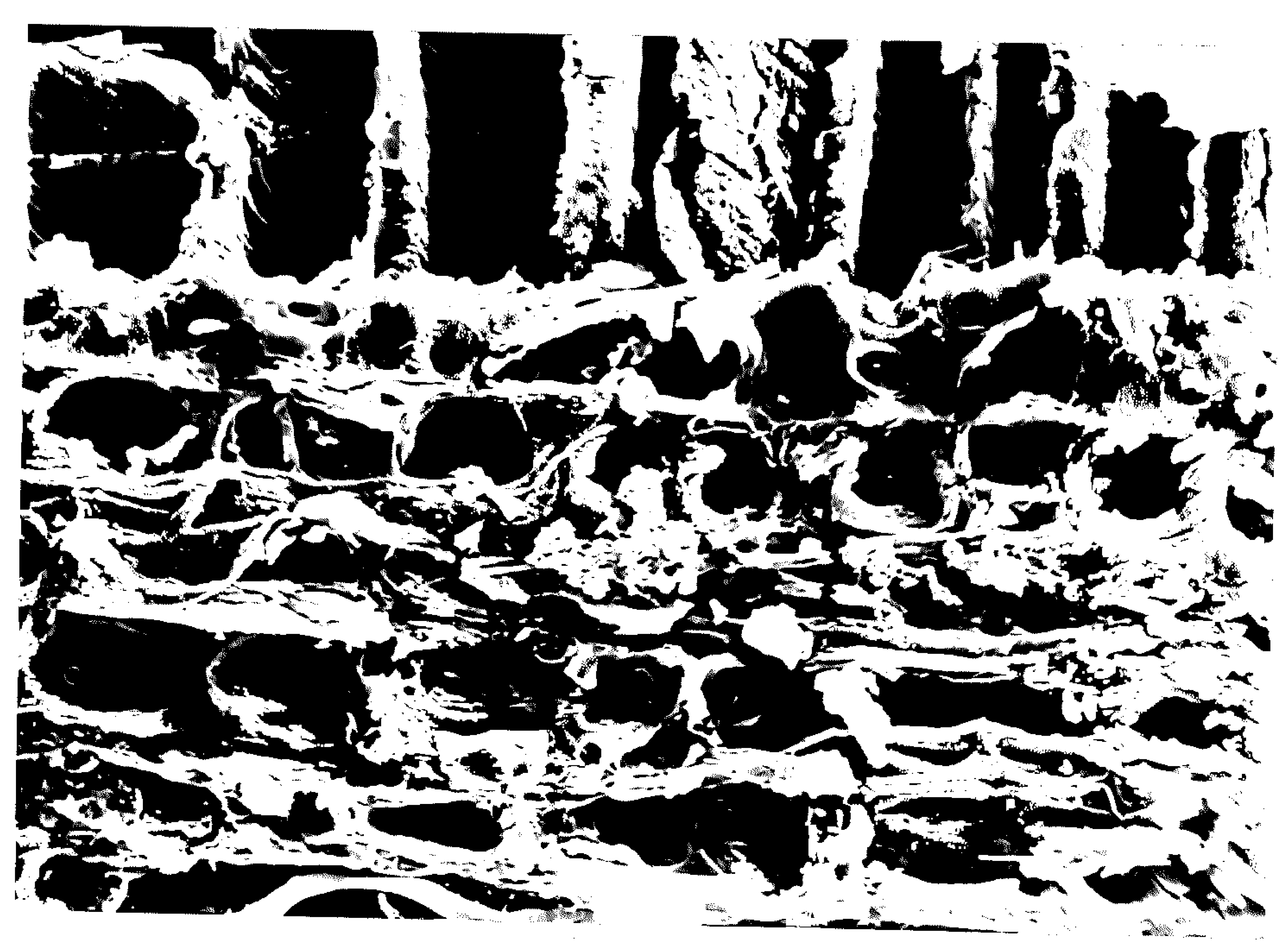
Plate (10).

T.S. (x100), showing compressed axial tracheids, thicker walled and smaller in the late wood. Two axial resin canals can be seen.

Plate (11).

R.L.S. (x2130), showing window-like simple pits in the ray parenchyma together with dentate ray tracheids. A small bordered pit is present above the ray tracheid.





Sample 5. (Pinus sylvestris)

Site: Freshwater Bed, West Runton, Norfolk.

Plate (12).

R.L.S. (x1680), showing tracheids with large single bordered pits.

Plate (13).

R.L.S. (x5790), showing bordered pits in which the torus is prominent.

Sample 6.(compressed wood, Salixaceae ?)

Site: Freshwater Bed, West Runton, Norfolk.

Plates (14) and (15) are transverse sections of an angiospermous wood which is poorly preserved and compressed. The fibre and parenchyma tissues have collapsed leaving shrunken, angular shaped vessels, plate (15), the wood is diffuse porous and a growth ring is present in plate (14). There is no evidence of multiseriate rays being present. Simple vessel perforations were observed in longitudinal sections. No spiral thickening was observed in these sections. Fairly large alternate pitting on the walls of the vessels were observed, plate (16). In plate (17) the pitting on the vessels were oval in shape. These characteristics are reminiscent of the family Salixaceae, but due to the severity of the collapsed walls this is only a tentative identification. In 1980 West reported a number of micro and macro-fossils from this area, and these included the following species; Alnus, Betula, Carpinus, Prùnus, Rubus, Rosa, Ilex, Quercus, Ulmus, Tilia and Salix. Most of these species can be discounted apart from the Salixaceae, on the basis of possessing one or more of the following characters, ring porous, scalariform perforations, spiral thickening, small pitting and to an extent aggregate or multiseriate rays.

Sample 6. (Salixaceae ?)

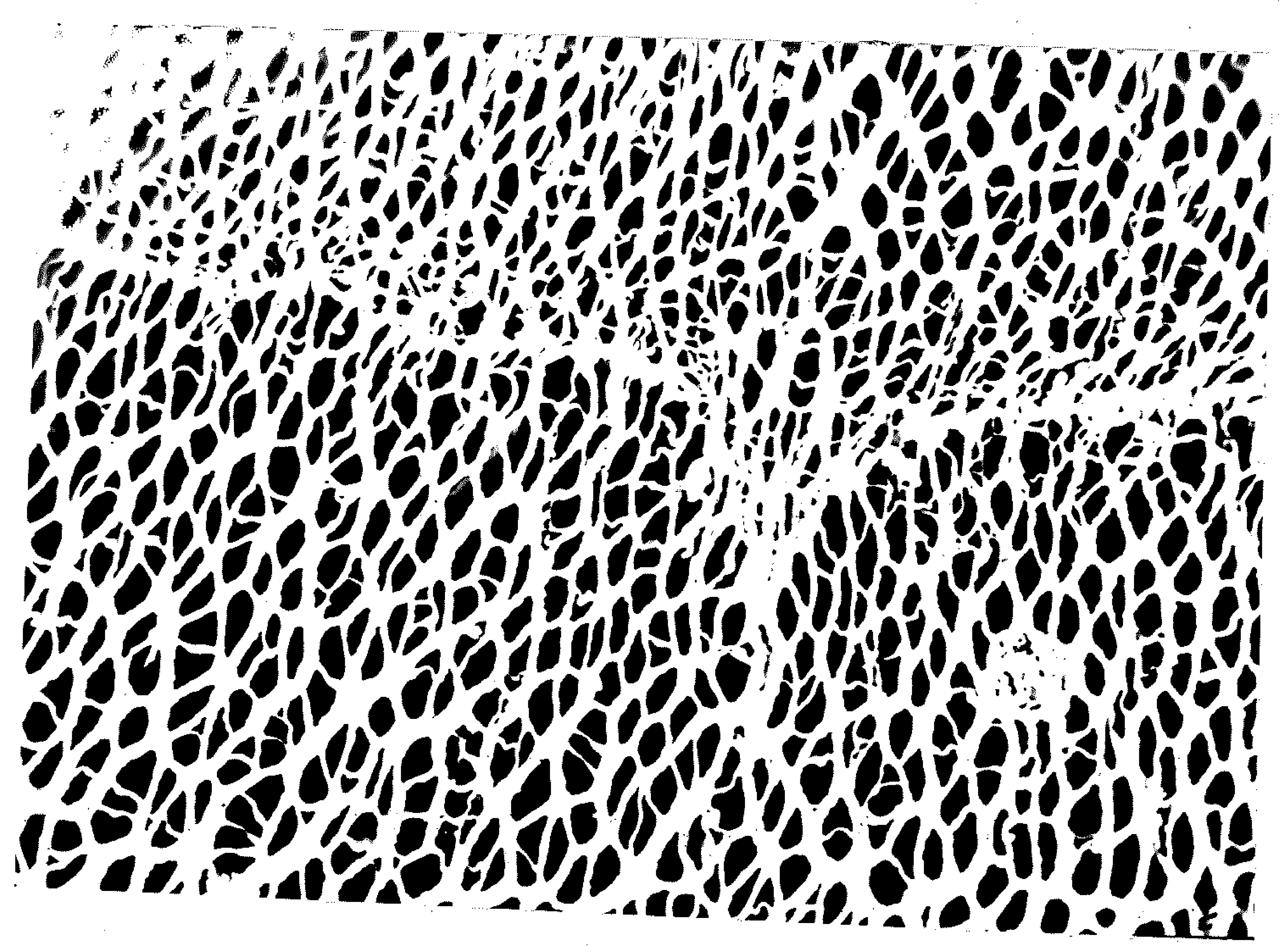
Site: Freshwater Bed, West Runton, Norfolk.

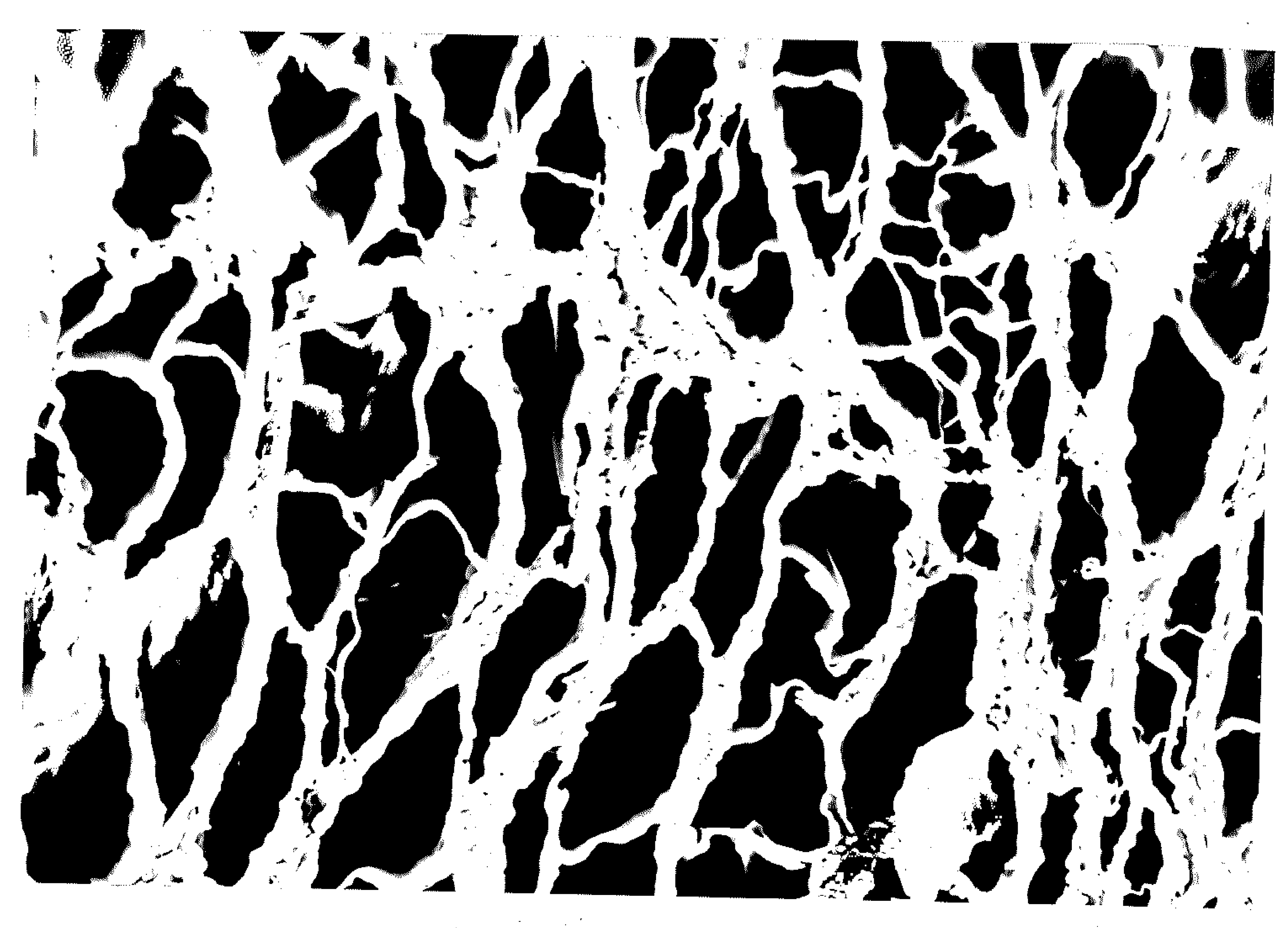
Plate (14).

T.S. (x280), showing a diffuse porous wood and a growth ring.

Plate (15).

T.S. (x1200), showing the collapsed nature of this wood, the angular vessels remaining.





Sample 6. (Salixaceae ?)

Site: Freshwater Bed, West Runton, Norfolk.

Plate (16).

L.S. (x3110), showing a vessel with large alternate pitting on the wall.

Plate (17).

L.S. (x2070), showing pitting on the vessel wall which is oval in shape.

Results with Tertiary Fossil Wood.

Samples from fossil wood of different geological ages were oxidized using the cupric oxide method described previously and chromatographed quantitatively and qualitatively. The Tertiary geological sites are listed more fully in Chapter 2. The state of the preservation varied enormously in the samples of Tertiary wood examined in this work. Some of the samples were extremely coalified and showed little anatomical detail whereas other woods were very well preserved. The injection size therefore varied considerably, from 2µl to 10µl. A 10µl injection was the largest size used because if greater injections were used overloading of the column occurred.

Figure (63).
A chromatogram of the lignin oxidation products of a gymnospermous wood from the Tertiary.

Coniferous wood from the Astarte tenera Bed, sample 14, woodmeal, 10µl injection. (OV 101 column).

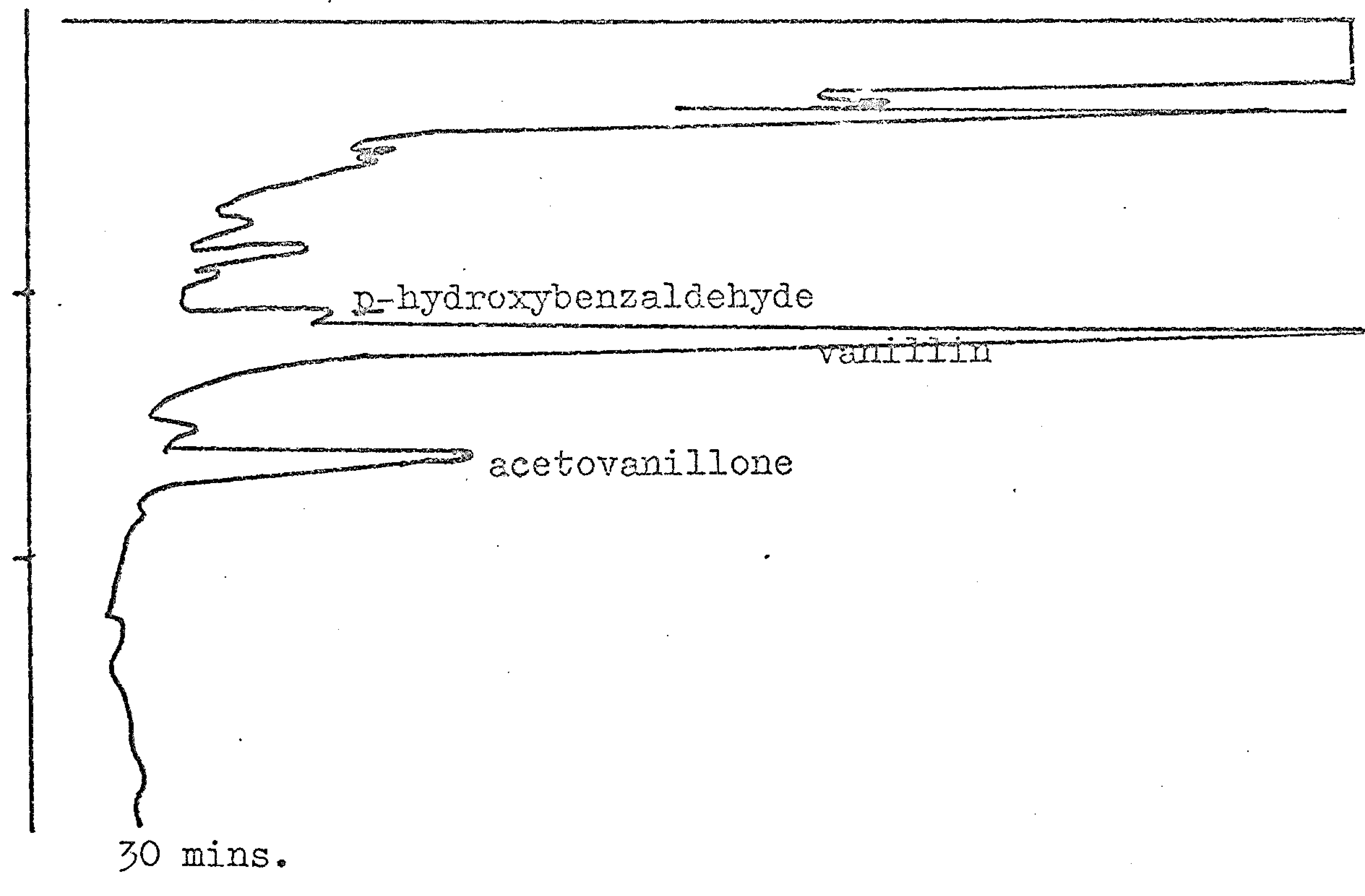


Figure (64).
A chromatogram of the lignin oxidation products of an angiospermous wood from the Tertiary.

Laurinoxylon endi nd oides, sample 10, woodmeal, 2µl injection, (5% Pdegs column).

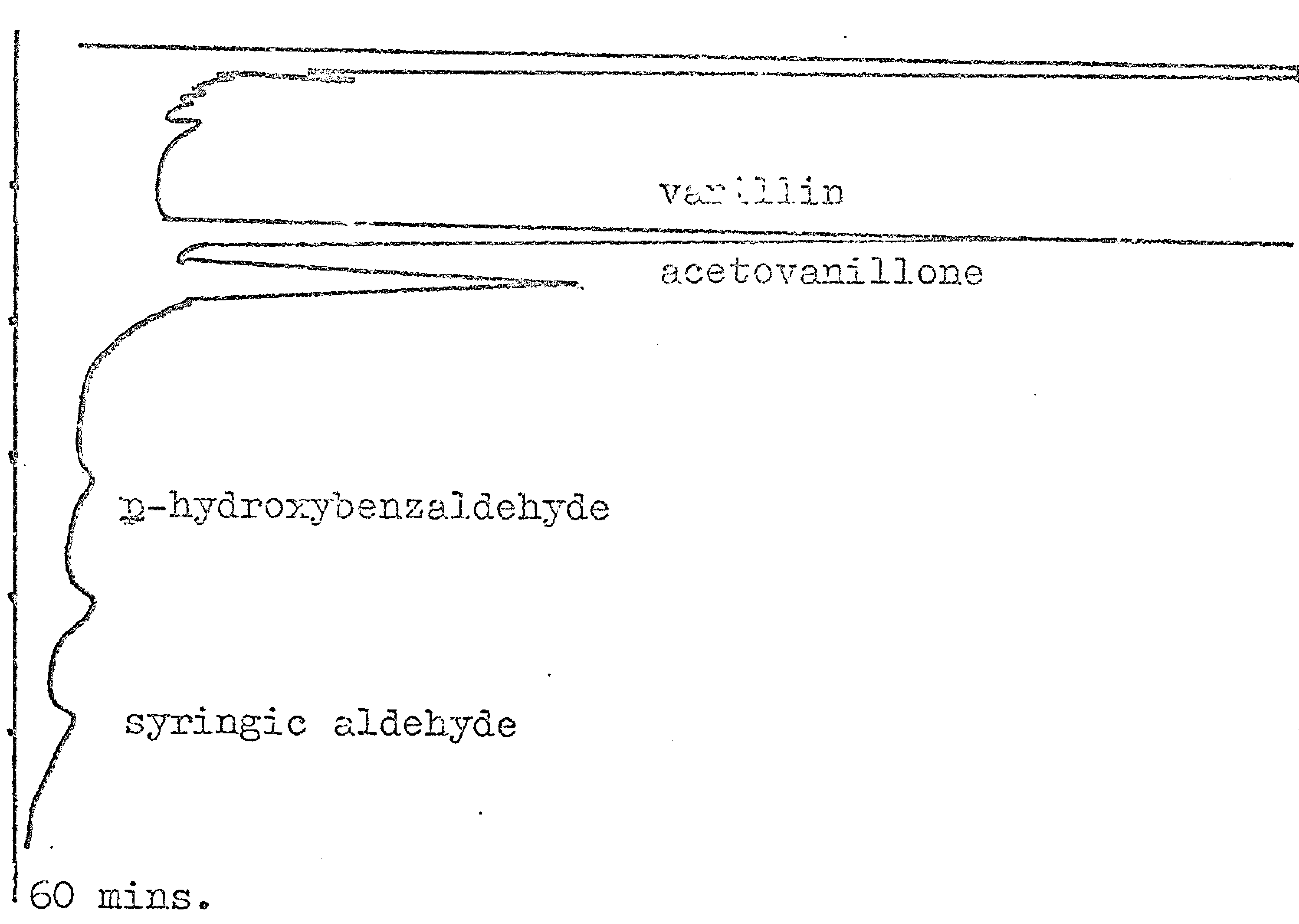


Table 16

The major lignin oxidation products from the fossil wood of the Tertiary period.

Unless otherwise stated 10µl injections were used.

		Tertiary Wood.	Major oxidation products. mg/gm of woodmeal			
			p-OH	VAN	ACETO	SYR
MIOCENE		Sample 7				
	1	Pinus sp.	*0.03	0.17	0.12	trace
	2	Taxodiaceae	*0.02	0.14	0.12	trace
		Sample 8				
	1	Taxodiaceae	*0.32	1.35	0.22	trace
	2		*0.35	1.45	0.25	trace
		Sample 9				
	1	Taxodiaceae	*0.11	2.26	0.25	trace
	2		*0.20	2.67	0.37	trace
		Sample 10				
	1	L. endiandroides	*0.04	2.07	0.82	0.32
	2		*0.05	2.37	0.81	0.39
EOCENE		Sample 11				
	1	coniferous	0.04	0.18	0.06	absent
	2		0.07	0.25	0.17	absent
		Sample 12				
	1	angiospermous	absent	0.39	0.22	0.18
	2		absent	0.29	0.22	0.23
		Sample 13				
	1	Taxodiaceae?	0.09	1.13	0.70	0.04
	2		0.05	0.90	0.53	0.03
PALAEOCENE		Sample 14				
	1	coniferous	0.04	0.22	0.08	absent
	2		0.04	0.20	0.10	absent

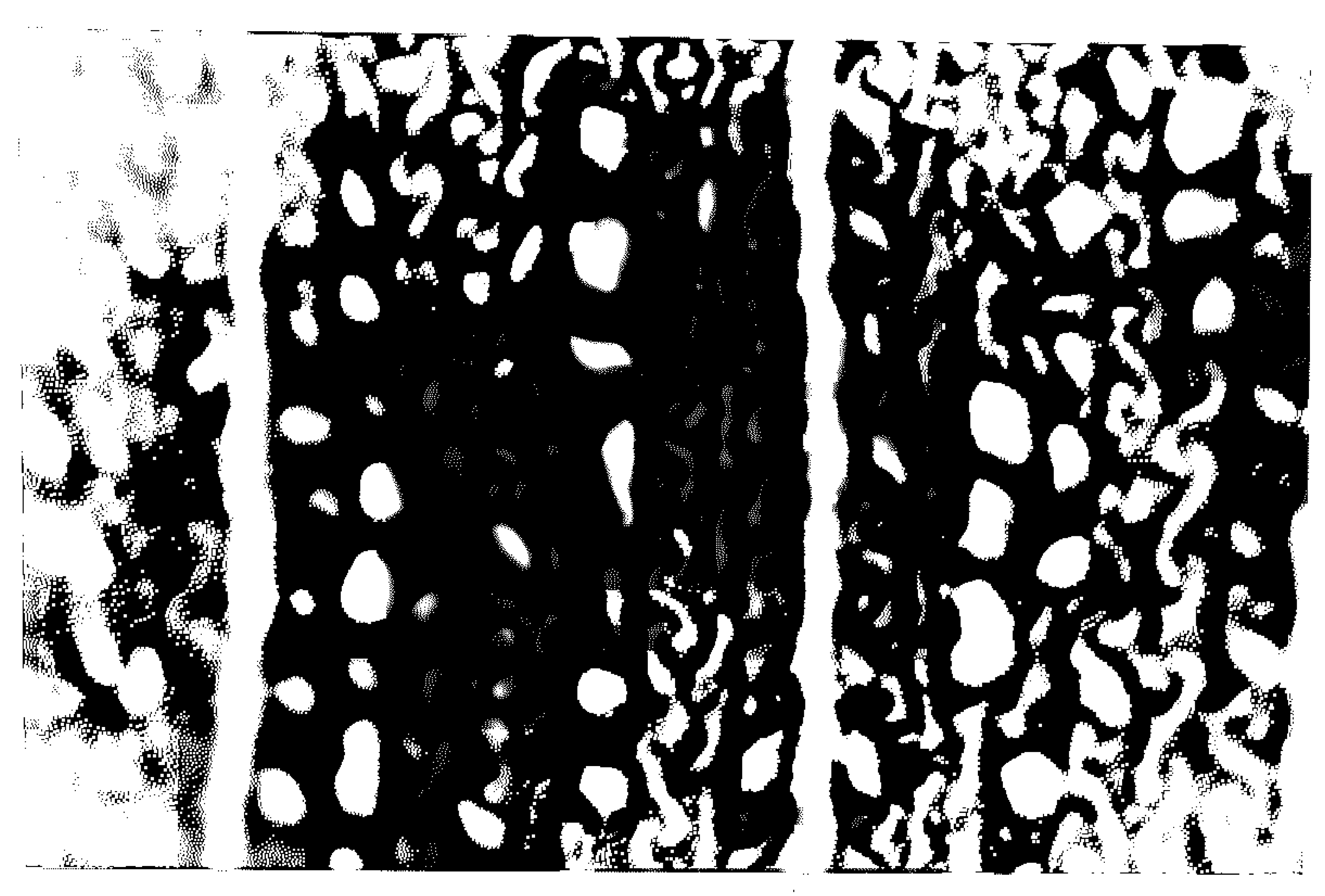
*2µl injections

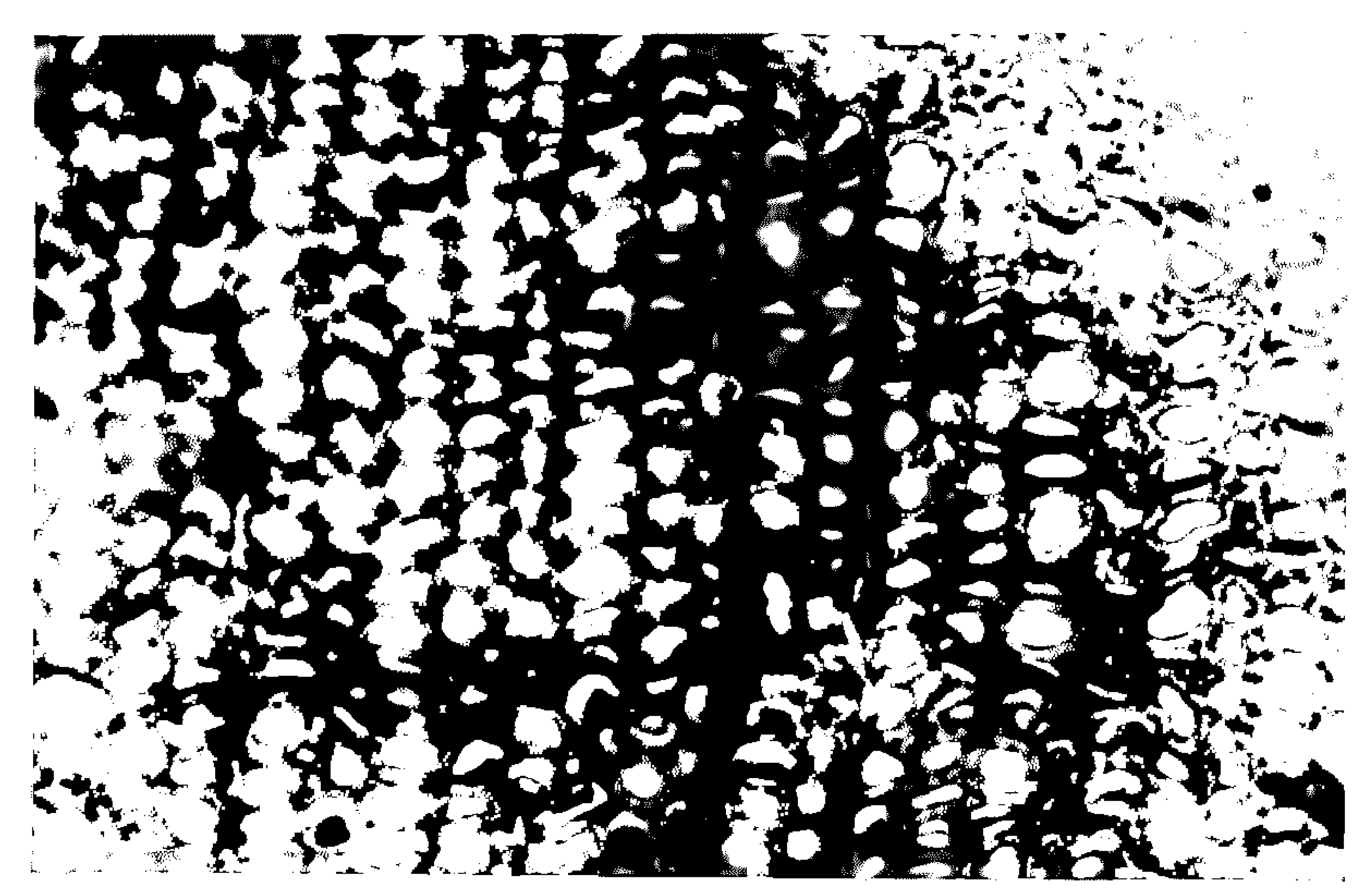
The Tertiary wood was hand sectioned using the light microscope and the scanning electron microscope for identification.

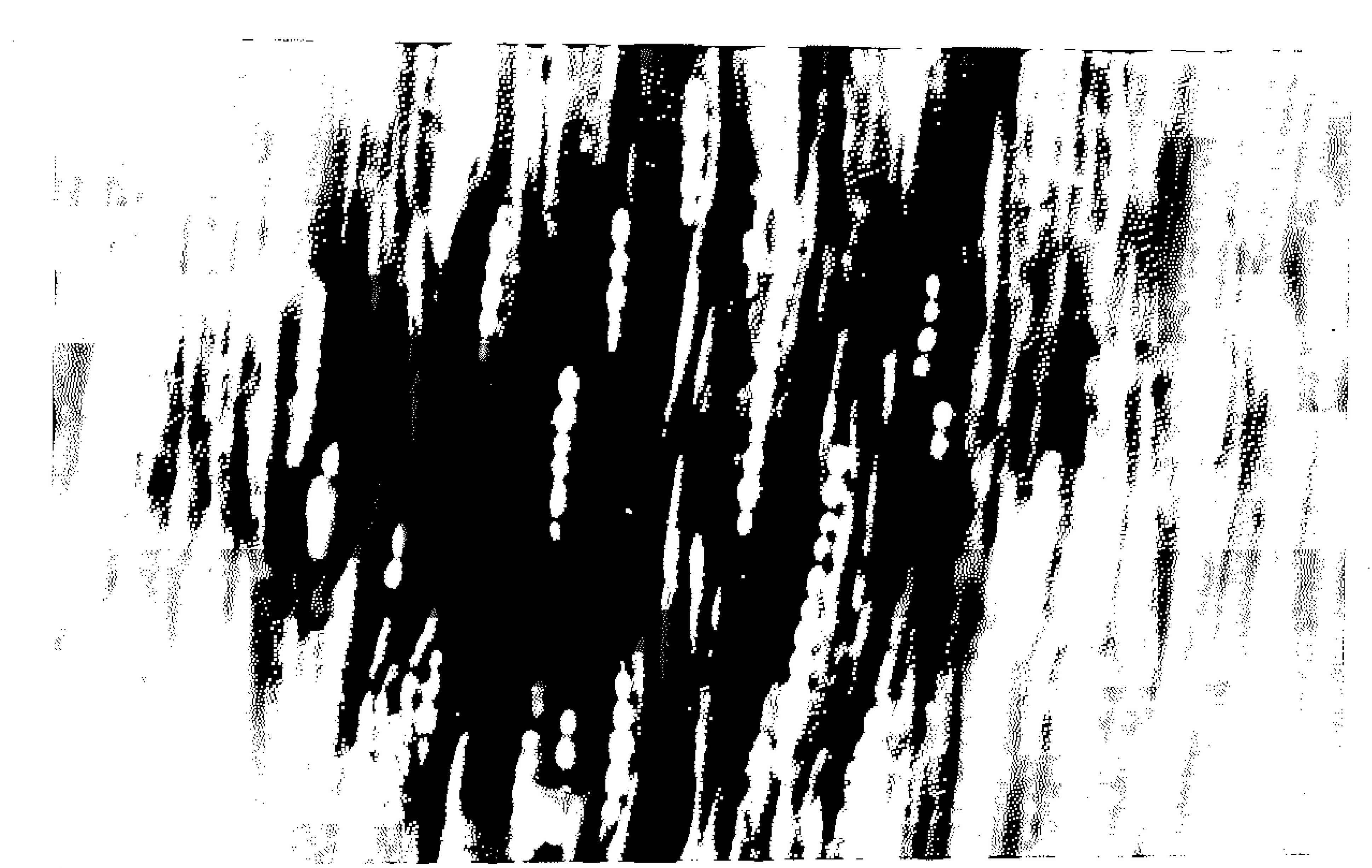
<u>Anatomical identifications of the Tertiary samples.</u>

<u>Sample 7.</u> (compressed wood, ~~Pinus~~ Taxodiaceae species?)
Site: Bees Nest Pit, Brassington Formation, Derbyshire.

This wood is very compressed and dense in comparison to the Quaternary coniferous wood that has been examined. Plates (18) and (19) are transverse sections of the wood showing the compressed tracheids with no visible differences between the cells of the late and early wood. In the transverse sections and the tangential sections made no resin canals were observed. The wood when cut tangentially, plate (20) shows the rays to be preserved and they are either uniseriate or fusiform. In the radial longitudinal sections large, single bordered pits in the tracheids are well preserved as shown in plates (21) and (22). The ray parenchyma cells have large window-like simple pits suggestive of <u>Pinus</u>, plates (23) and (24). There appears to be the remains of some ray tracheids, plate (23) and (25) with dentate projections visible in the lumen of the cell. A small bordered pit is present in the ray tracheid, plate (25). <u>Pinus</u> species normally have resin canals but in this wood there appears to be no resin canals, this may be due to the highly compressed nature of the wood. The window-like pits of the ray parenchyma and the ray tracheids are features of <u>Pinus</u> species. Boulter (1971) has reported several macrofossils including the wood of Taxodiaceae, <u>Picea</u> and <u>Pinus</u> as well as leaves of <u>Abies</u> species. <u>Podocarpus</u>-type pollen has also been reported from the Brassington Formation. One can discount members of the Taxodiaceae, <u>Picea</u>, <u>Abies</u> and <u>Podocarpus</u> because they have either taxodioid or cupressoid pits on the ray cells.







Sample 8. (compressed wood, Taxodiaceae)

Site: Vilettwitz, Senftenberg, East Germany.

The transverse section, plate (26) shows a very compressed and unorganised wood. Plate (27) shows the presence of uniseriate rays and parenchyma cells. The bordered pits of the axial tracheids are well preserved, plate (28) and are opposite in arrangement. They are generally found in rows of two's. The pits of the ray cells are taxodioid, two to five in the cross-field, plates (29) and (30). These features are characteristic of the family Taxodiaceae. Further identification to the genus and species level is difficult particularly when dealing with fossil wood. Jane (1970) separates Sequoia from Taxodium on the basis of Sequoia possessing more frequently biseriate rays and in possessing two to three taxodioid pits to the cross-field, compared with three to four taxodioid pits to the cross-field in Taxodium. While Philips (1948) separates Cryptomeria, Sequoia and Taxodium on the following features:

From Philips (1948)

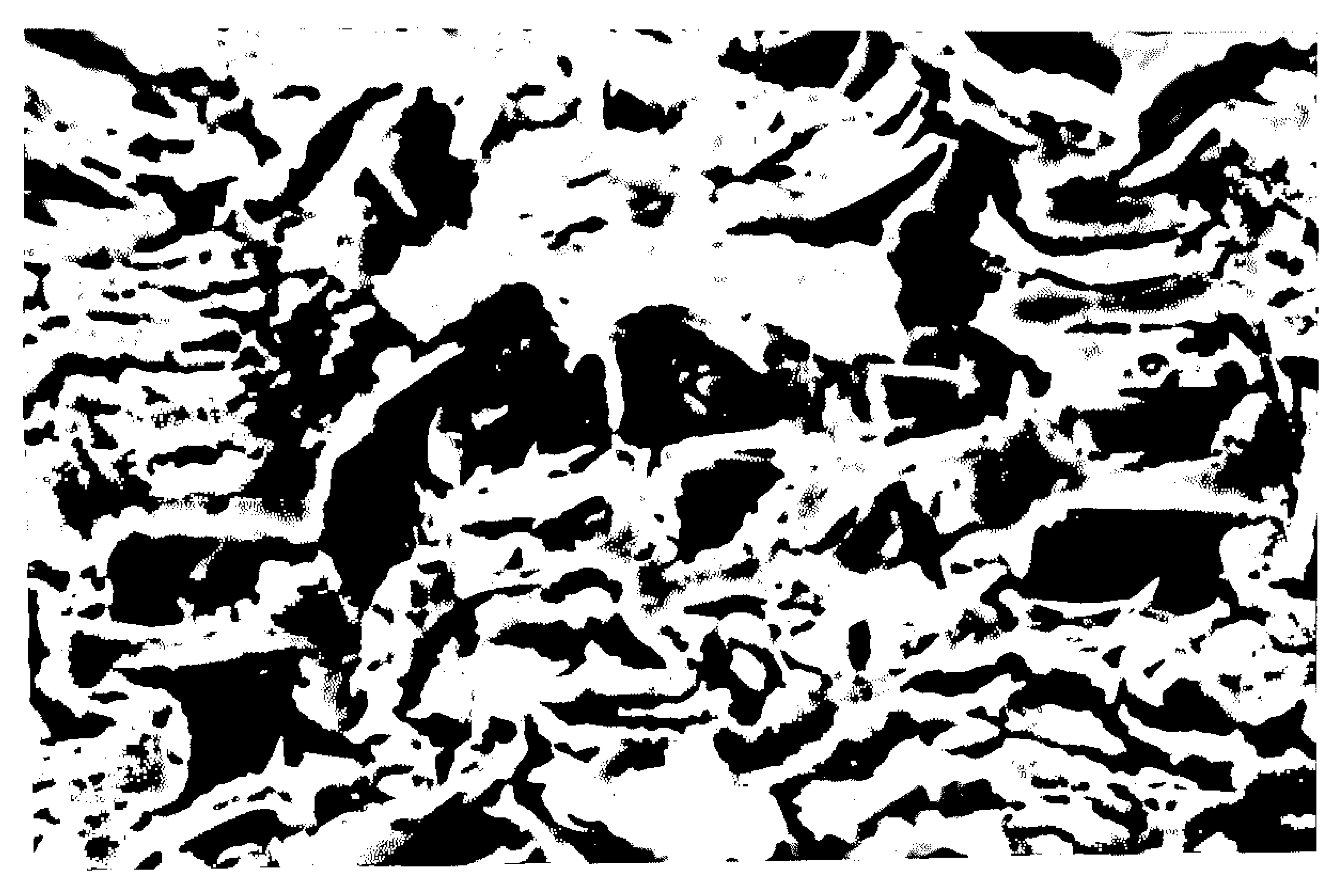
	Cryptomeria japonica	Taxodium distichum	Sequoia sempervirens	Sequoia giganteum
ray parenchyma T.L.S.	1seriate	1seriate	1-2seriate	1seriate
bordered pits: tracheid	1-2	1-3	1-2	1
no. of pits/cross-field	2-3	1-6	2-8	1-3
cross-field pits	taxodioid for all species			
end walls on vertical parenchyma	smooth + nodular	pitted	±smooth	smooth

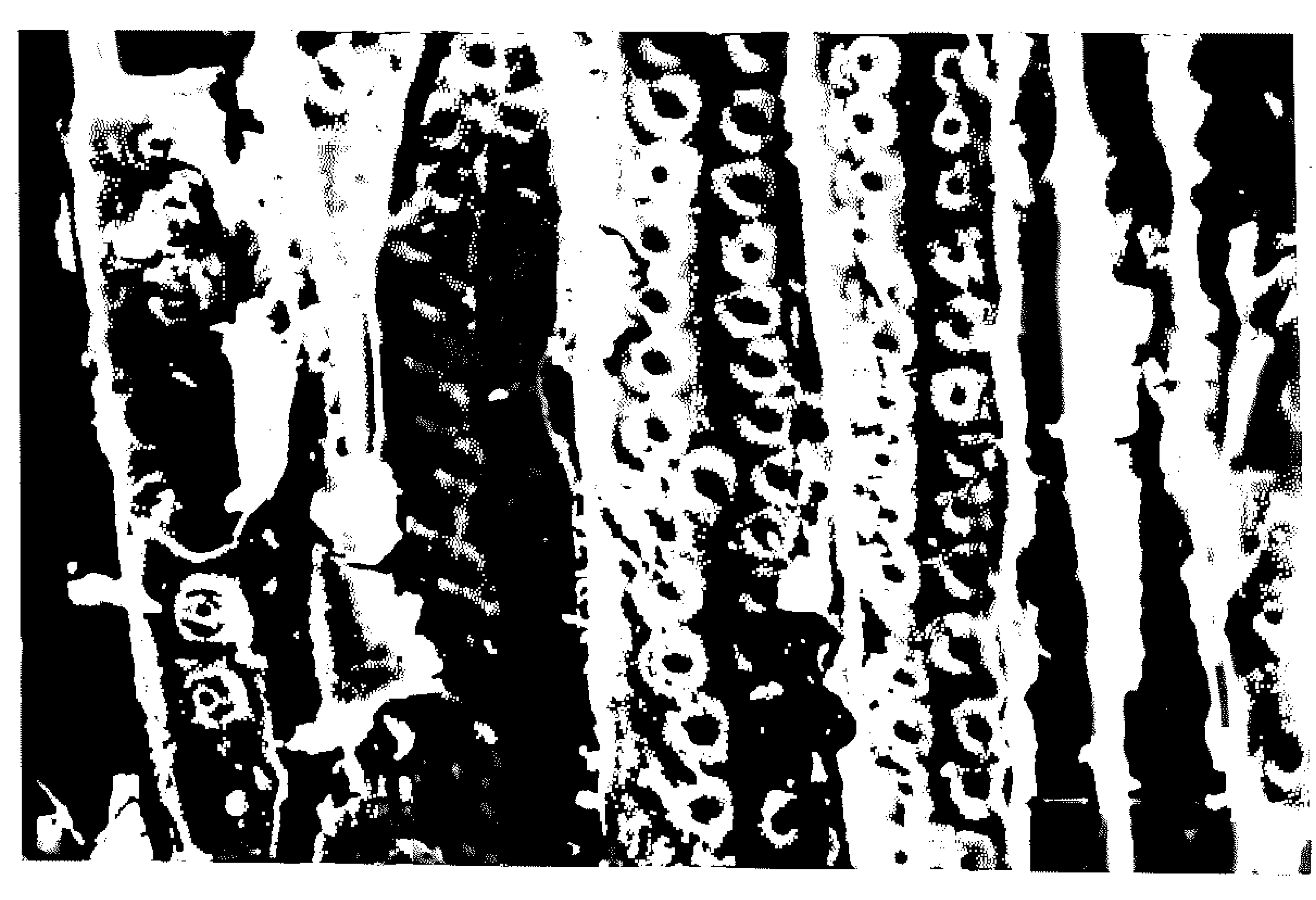
In this wood examined, the end walls of the parenchyma cells appear to be smooth but for fossil woods this would not be in general a very reliable character due to preservation and microbiological deterioration. The number of

taxodioid pits in this wood varied from two to five as shown in plates (29) and (30). It therefore seems appropriate to identify this wood only as far as its family and therefore to place it in the Taxodiaceae.

Sample 9. (compressed wood, Taxodiaceae)
Site: Upper Miocene, East Germany.

The transverse section, plate (31) shows the marked differences between the early and the late wood. The tracheids of the early wood are large and thin walled where as those of the late wood are thick walled. The change from the early to the late wood is abrupt. This wood is not as compressed as that of sample 8. The rays are uniseriate, plate (32). The bordered pits of the axial tracheids are well preserved, plates (33) and (34). The bordered pits are generally in rows of two's and are opposite in arrangement. The pits of the ray cells are taxodioid, two to three in the cross-field, plates (35) and (36). These features are characteristic of the family Taxodiaceae. Further identification to genus and species level of this wood is not attempted for similar reasons to those outlined in sample 8.





Sample 8. (Taxodiaceae)

Site: Vilettwitz, Senftenberg, East Germany.

Plate (29).

R.L.S. (x130), showing the ray cells with taxodioid pits, 2-5 pits in a cross-field.

Plate (30).

R.L.S. (x130), showing the ray cells with taxodioid pits, 3 pits in a cross-field.

Sample 9. (Taxodiaceae)

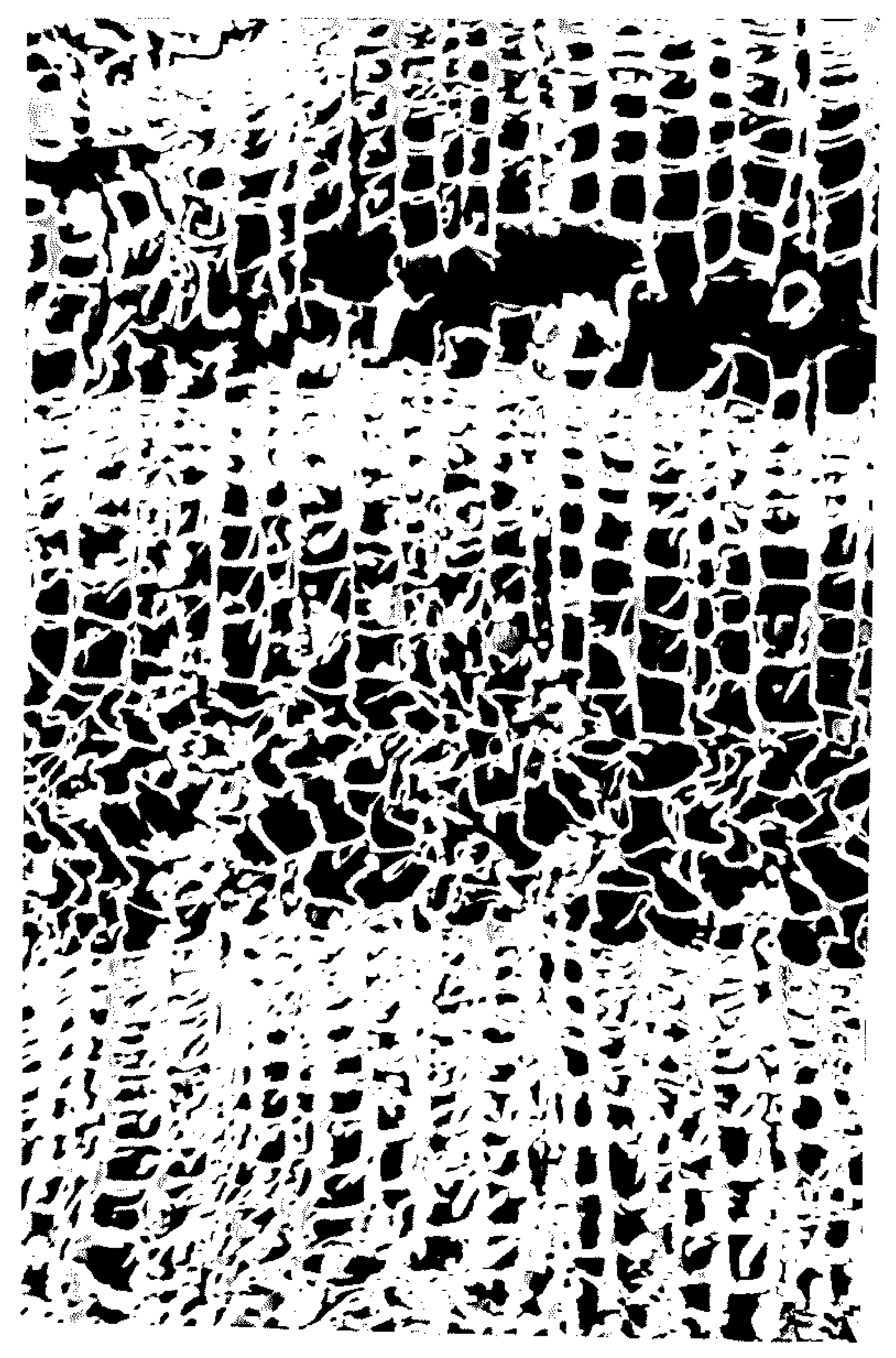
Site: Upper Miocene, East Germany.

Plate (31).

T.S. (x100), showing the thin walled early wood and the thicker walled late wood.

Plate (32).

T.L.S. (x130), showing uniseriate rays and parenchyma cells.

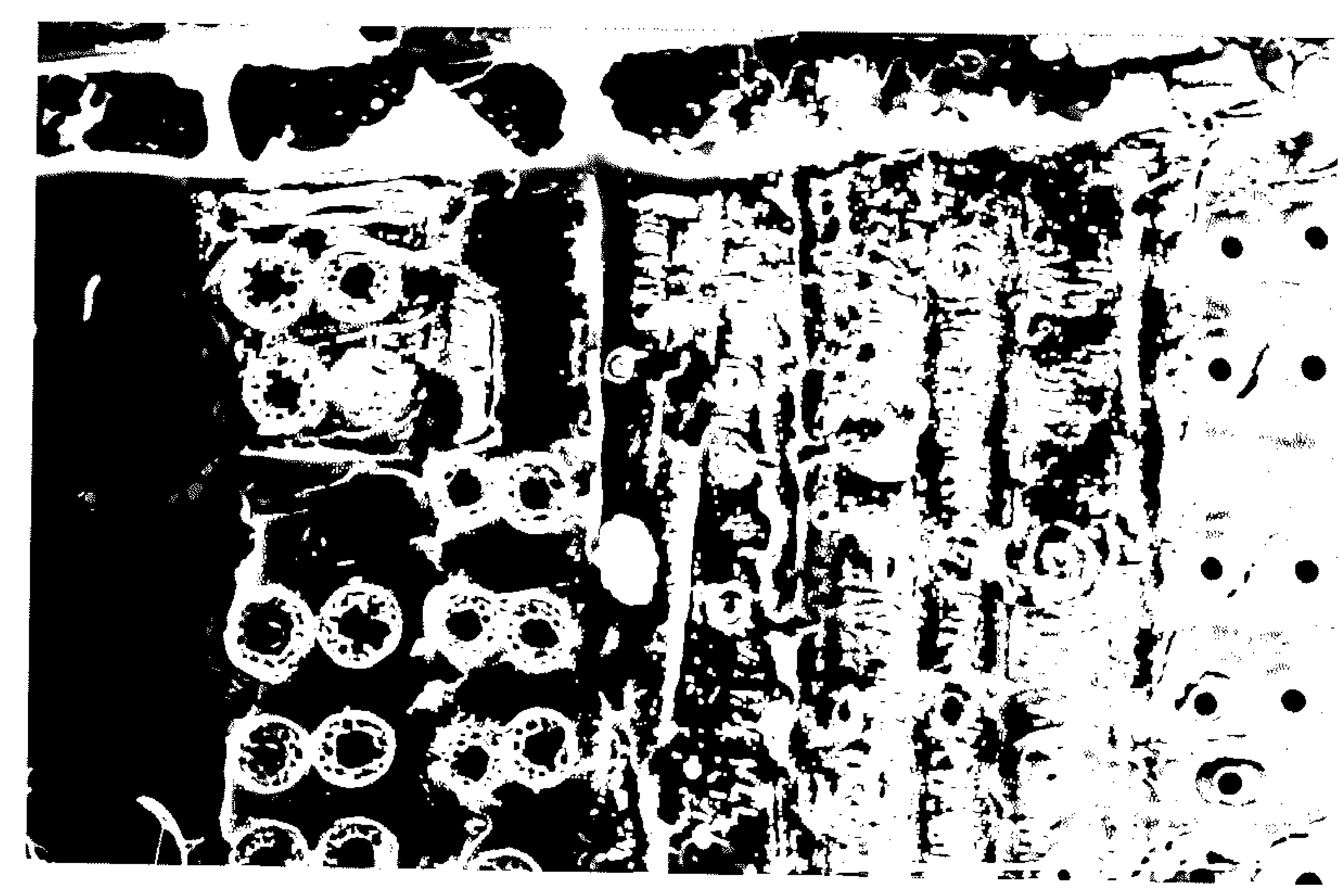


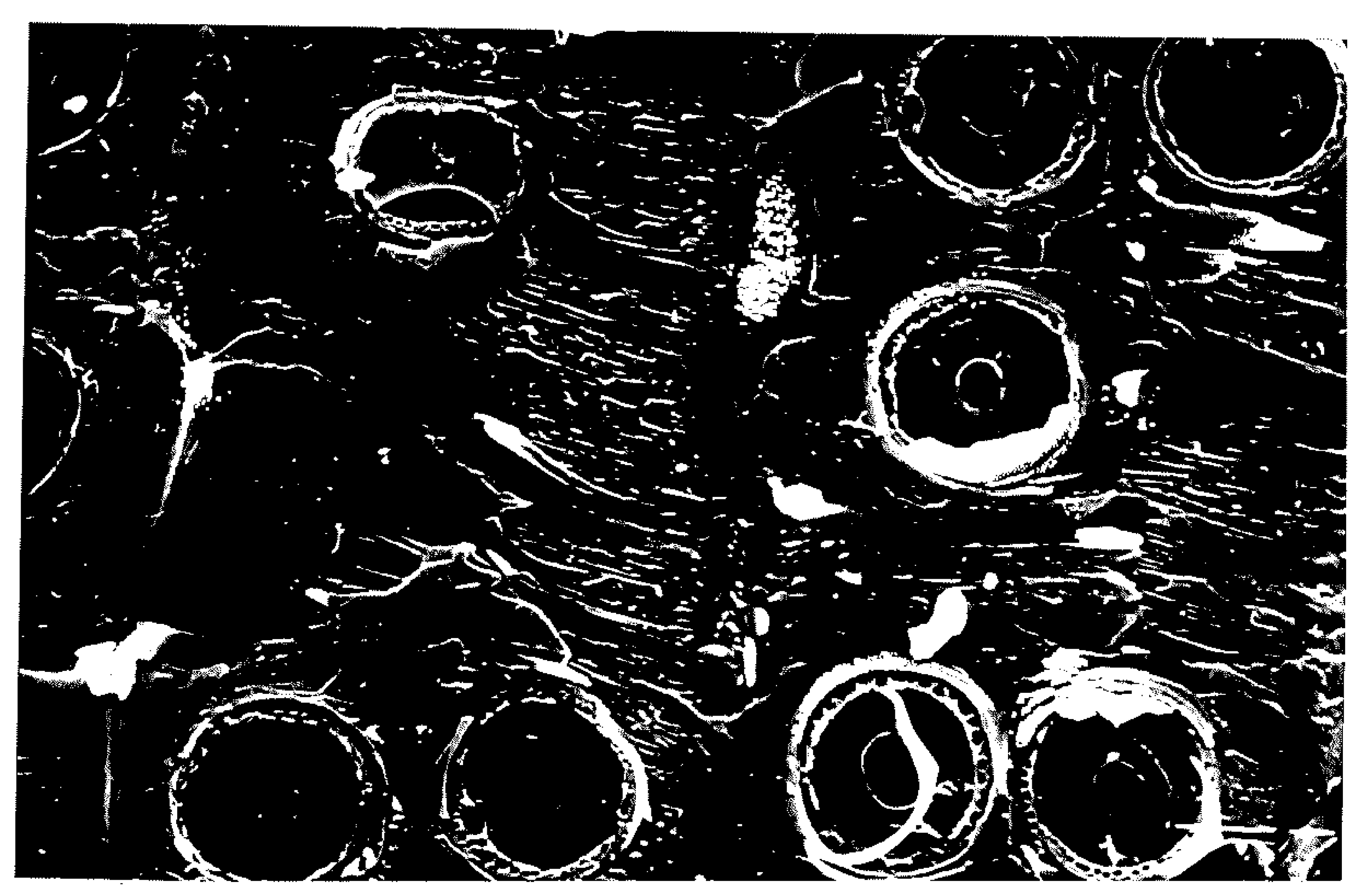
Sample 9 (Taxodiaceae)

Site: Upper Miocene, East Germany.

Plates (33) and (34).

R.L.S. (x880) and (x2140), showing the bordered pits arranged oppositely in the tracheids.





Sample 9. (Taxodiaceae)

Site: Upper Miocene, East Germany.

Plates (35) and (36).

R.L.S. (x1280) and (x1300), showing the ray cells with taxodicid pits, 2-3 pits in a cross-field.

Sample 10. (compressed wood, *Laurinoxylon endiandroides*)
Site: Upper Miocene, East Germany.

This compressed piece of wood was recieved from East Germany, labelled *Laurinoxylon endiandroides*. The sections that were made here conform to the descriptions made by Süss (1956) of *Laurinoxylon endiandroides*. It is a ring porous wood with large solitary vessels, plate (37). The medullary rays are very wide and are heterogeneous as shown in plates (38) and (39), with procumbent cells and above them upright cells.

Sample 10 (Laurinoxylon endiandroides)

Site: Upper Miocene, East Germany.

Plate (37).

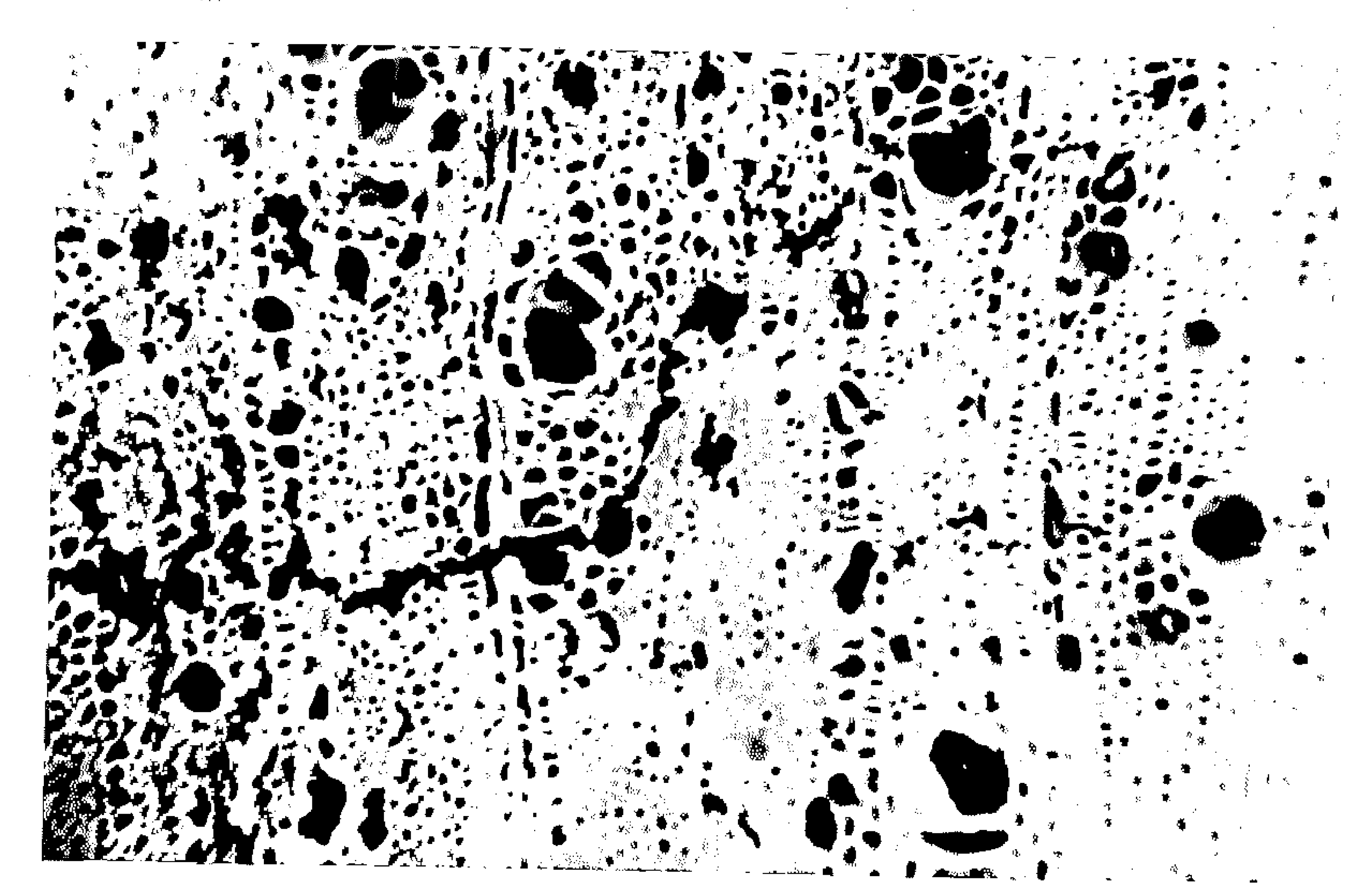
T.S..(x250), showing the large solitary vessels.

Plate (38).

T.L.S. (x750), showing the wide rays.

Plate (39).

R.L.S. (x750), showing the heterogenous rays with procumbent and marginal or upright cells.



Sample 11. (coalified wood, coniferous)
Site: Bovey Basin, South Devon.

This wood is extremely coalified and therefore only certain anatomical features are preserved. Plate (40) shows the presence of a uniseriate ray, the surrounding tissue is coalified, no cellular detail is preserved. Several uniseriate rays are preserved, plate (41) and a fusiform ray, plate (42) can be seen. Chandler (1964) has reported small broken pieces of *Sequoia* wood from this site. However no taxodioid pits were seen in the ray parenchyma cells. Infact the ray parenchyma cells are poorly preserved and coalified, plate (43) and the pits give the appearance of being window-like indicative of pine. Unfortunately no bordered pits were found to be preserved on the tracheids. Due to the absence of key characteristics this wood can only be identified as being a coniferous wood.

<u>Sample 11</u>. (coalified wood, coniferous)

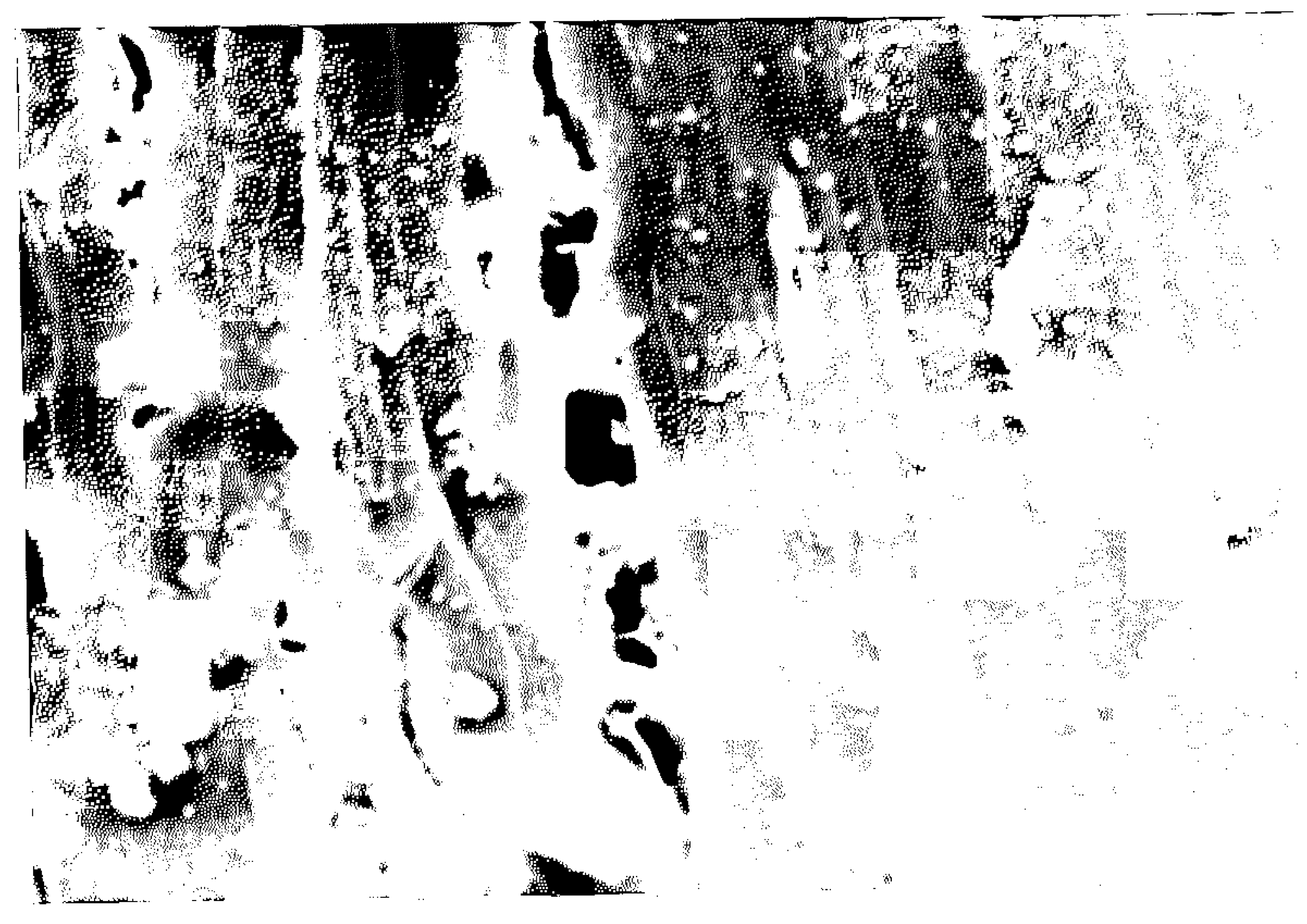
Site: Bovey Basin, South Devon.

Plate (40).

T.L.S. (x2400), showing a uniseriate ray, the remaining tissue is coalified.

Plate (41).

T.L.S. (x330), showing several uniseriate rays.



Sample 11. (coalified wood, coniferous)

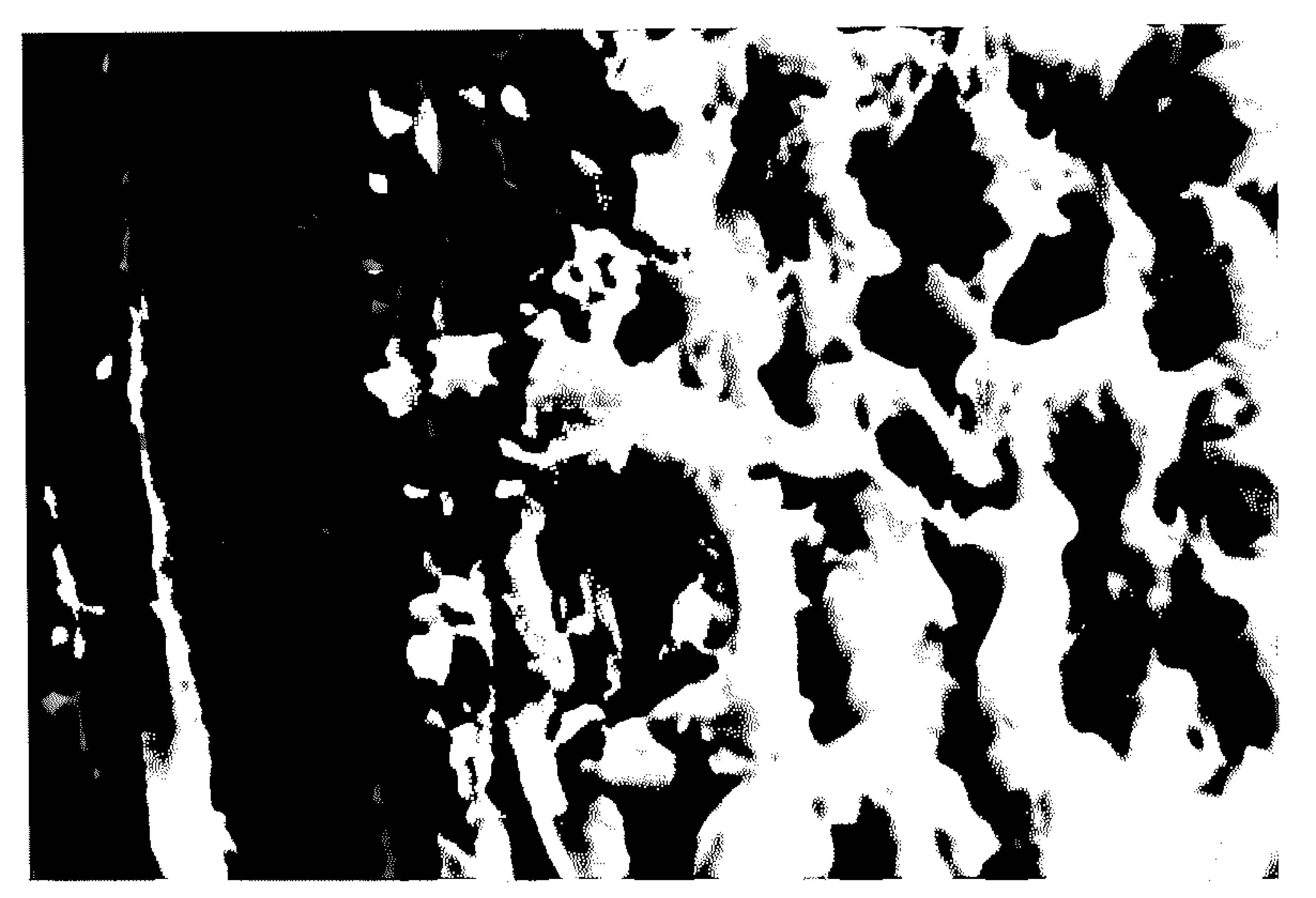
Site: Bovey Basin, South Devon.

Plate (42).

T.L.S. (x1280), showing a preserved fusiform ray.

Plate (43).

R.L.S. (x5300), showing poorly preserved ray parenchyma cells and tracheids without any pitting preserved.



Sample 12. (coalified wood, angiospermous)
Site: *Chara* Shell Bed, Lower Headon, Hordle Cliff, Hampshire.

This angiospermous wood is extremely coalified and therefore only certain anatomical features are preserved. No transverse sections were obtained with good cellular detail. Plates (44) and (45) show coalified vessels which have spiral thickenings on their walls. Plate (46) and (47) are tangential longitudinal sections showing compressed fibre-tracheids and multiseriate rays. In radial longitudinal sections the rays are poorly preserved, plate (48) with procumbent cells, plate (49) and tile cells, plate (50). The stratigraphy and plant fossil remains of the Lower Headon are detailed by Chandler (1961) and include numerous angiosperms.

Sample 13. (coalified wood, Taxodiaceae?)
Site: Lower Headon Bed, Hordle, Hampshire.

This coniferous wood is extremely coalified and therefore only certain anatomical features are preserved. Some of the bordered pits are preserved on the tracheids, plates (51) and (52). The torus can just be made out as shown on plate (52). Very few bordered pits were found to be preserved in this wood and there is no indication of whether the tracheids have multiseriate pitting or not. Plate (53) shows part of a uniseriate ray and tracheids. Plate (54) is a radial longitudinal section showing the ray cells with pits. Plate (55) shows the pits to be taxodioid but the number of taxodioid pits per cross-field is unknown due to the preservation. Taxodioid pits occur in species of *Abies*, *Juniperus*, *Pseudotsuga* and in the family Taxodiaceae. Chandler (1961) has reported the presence of *Sequoia* and *Pinus* from this site, therefore it is possible that this wood is a member of the Taxodiaceae rather than the other species mentioned.

Sample 12. (coalified wood, angiospermous)

Site: *Chara* Shell Bed, Lower Headon, Hordle, Hampshire.

Plates (44) and (45).

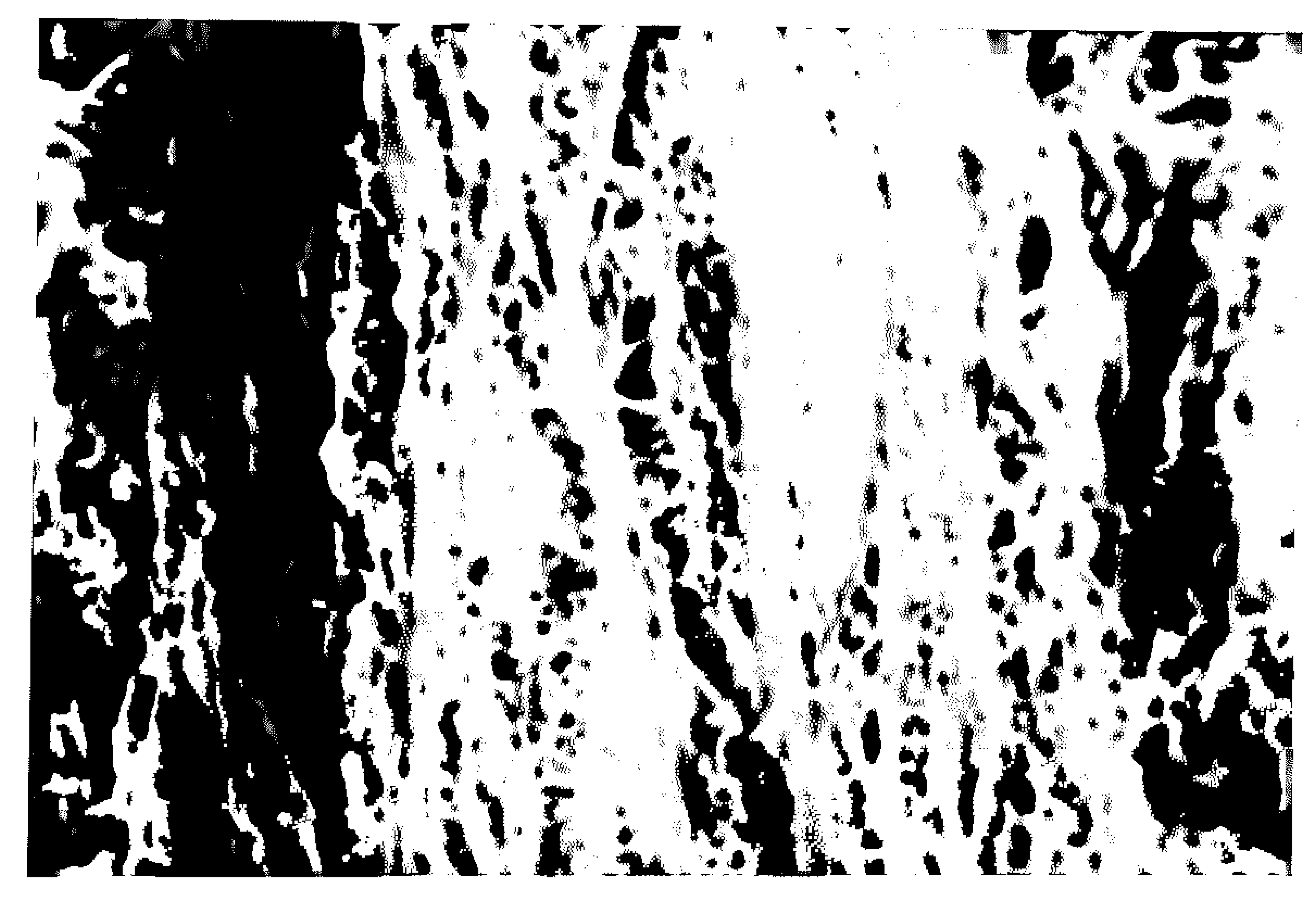
T.L.S. (x1240) and (x4050), showing coalified vessels which have spiral thickenings.

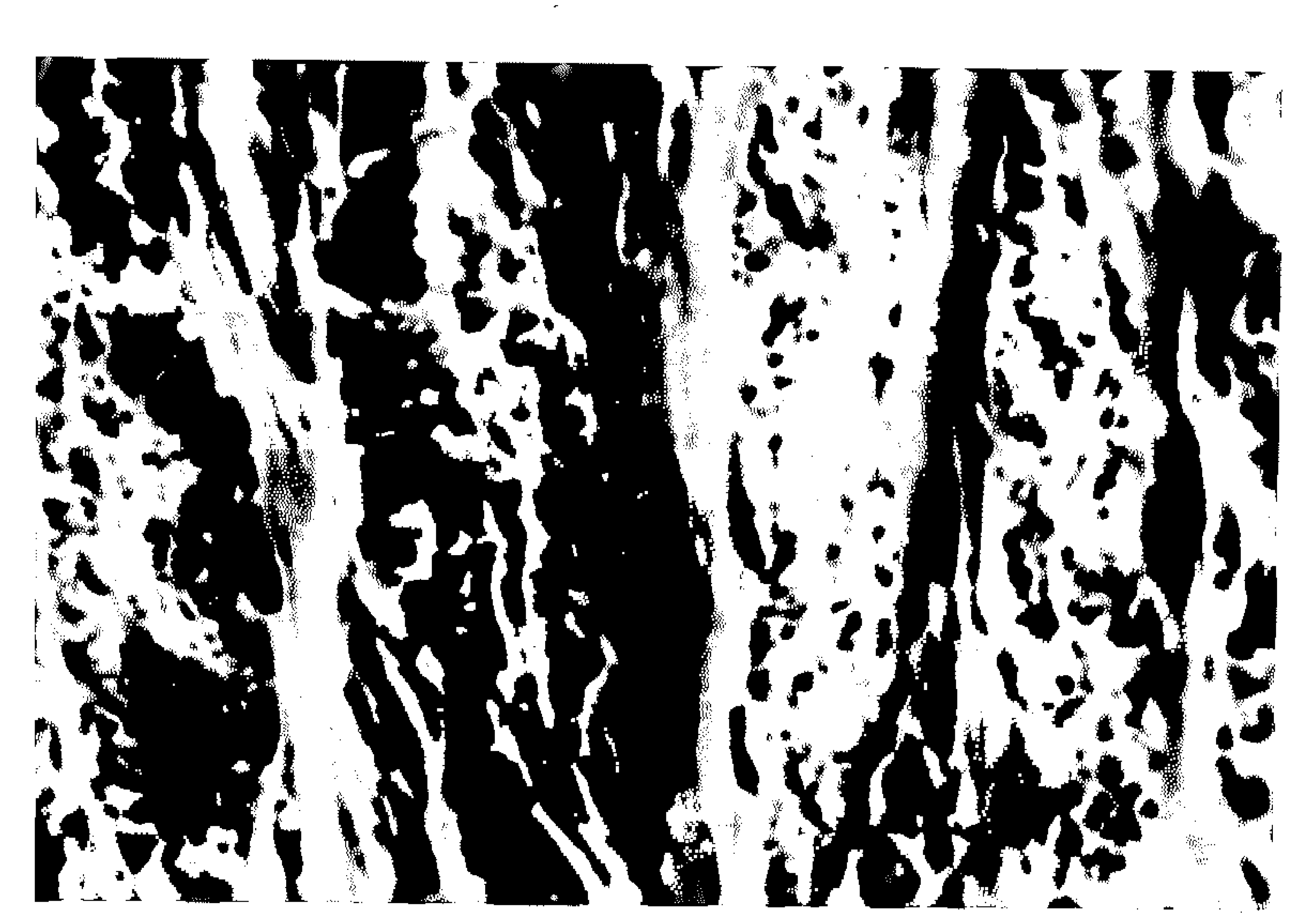
Sample 12. (coalified wood, angiospermous)

Site: *Chara* Shell Bed, Lower Headon, Hordle, Hampshire.

Plates (46) and (47).

T.L.S. (x690) and (x1770), showing compressed fibre-tracheids and multiseriate rays.





Sample 12. (coalified wood, angiospermous)

Site: Chara Shell Bed, Lower Headon, Hordle, Hampshire.

Plate (48).

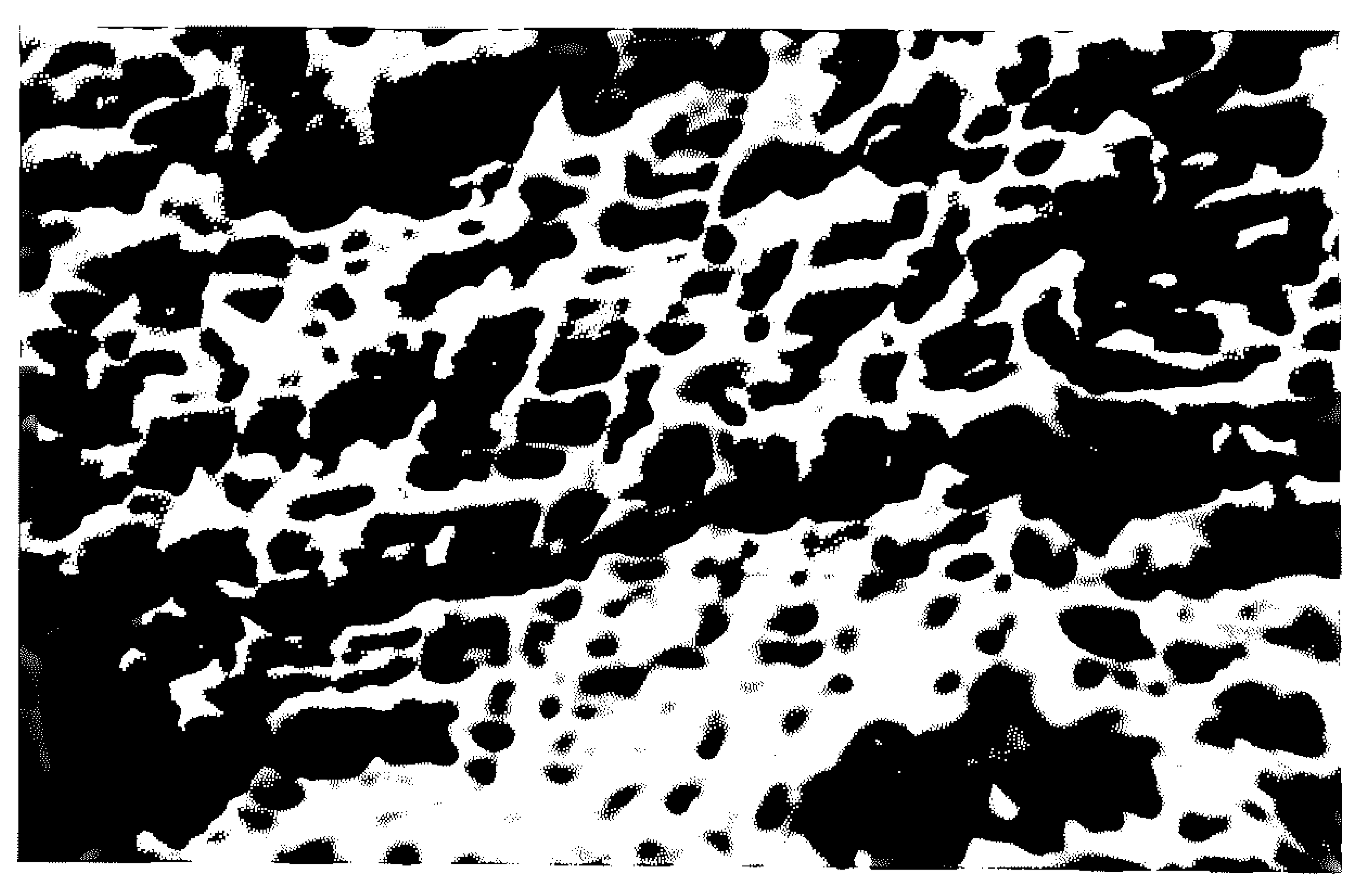
R.L.S. (x840), showing poorly preserved rays.

Plate (49).

R.L.S. (x2150), showing procumbent cells of the ray.

Plate (50).

R.L.S. (x1800), showing the tile cells of the ray.



Sample 13. (coalified wood, Taxodiaceae?)

Site : Lower Headon Bed, Hordle, Hampshire.

Plate (51).

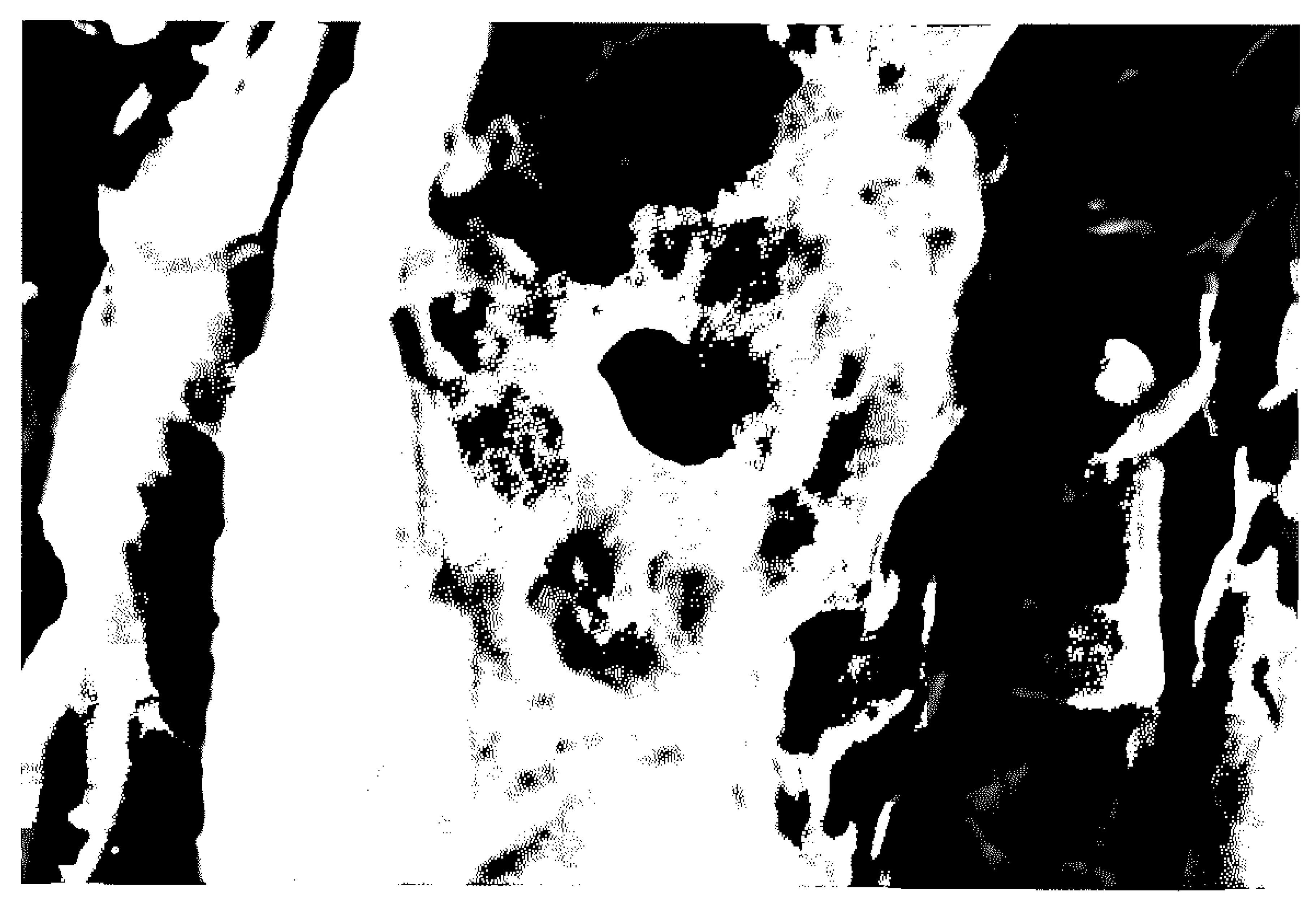
R.L.S. (x1020), showing some bordered pits on the tracheids.

Plate (52).

R.L.S. (x1840), showing the torus of the bordered pit.

Plate (53).

T.L.S. (x1100), showing part of a uniseriate ray and tracheids.



Sample 13. (coalified wood, Taxodiaceae?)

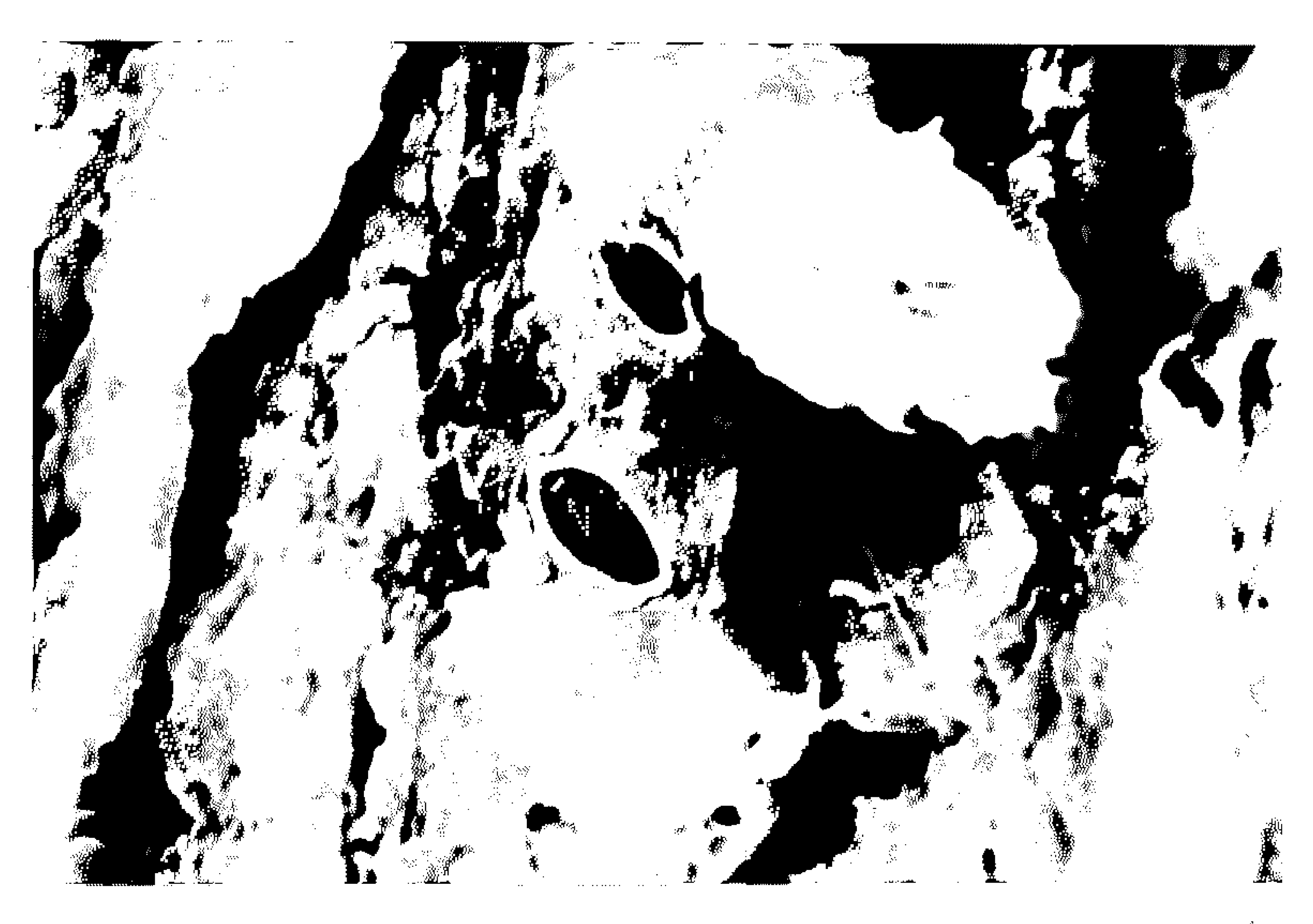
Site: Lower Headon Bed, Hordle, Hampshire.

Plate (54).

R.L.S. (x2020), showing the ray cells, poorly preserved, with some pits.

Plate (55).

R.L.S. (x7080), showing the pits to be taxodioid.



Sample 14. (coalified wood, Coniferous)

Site: *Astarte tenera* Bed, Herne Bay, Kent.

This wood is extremely coalified and therefore only certain features are preserved. Plates (56) and (57) are transverse sections showing compressed tracheids, many of which are filled with sediment. Some uniseriate rays are visible, plate (58). The axial tracheids are preserved with bordered pits which are opposite in arrangement, plate (59). The ray parenchyma cells are coalified and have one or two pits, possibly piceoid, per cross-field, plates (60) and (61). Ward (1978) has reported the presence of pine cones from this bed but this wood has no ray tracheids or window-like pits therefore it is not pine. Due to the absence of characteristic features of this wood and the difficulty of interpreting the types of pits because they are blocked with sediment, this wood can only be identified as a coniferous wood.

Sample 14. (coniferous wood)

Site: *Astarte tenera* Bed, Herne Bay, Kent.

Plate (56).

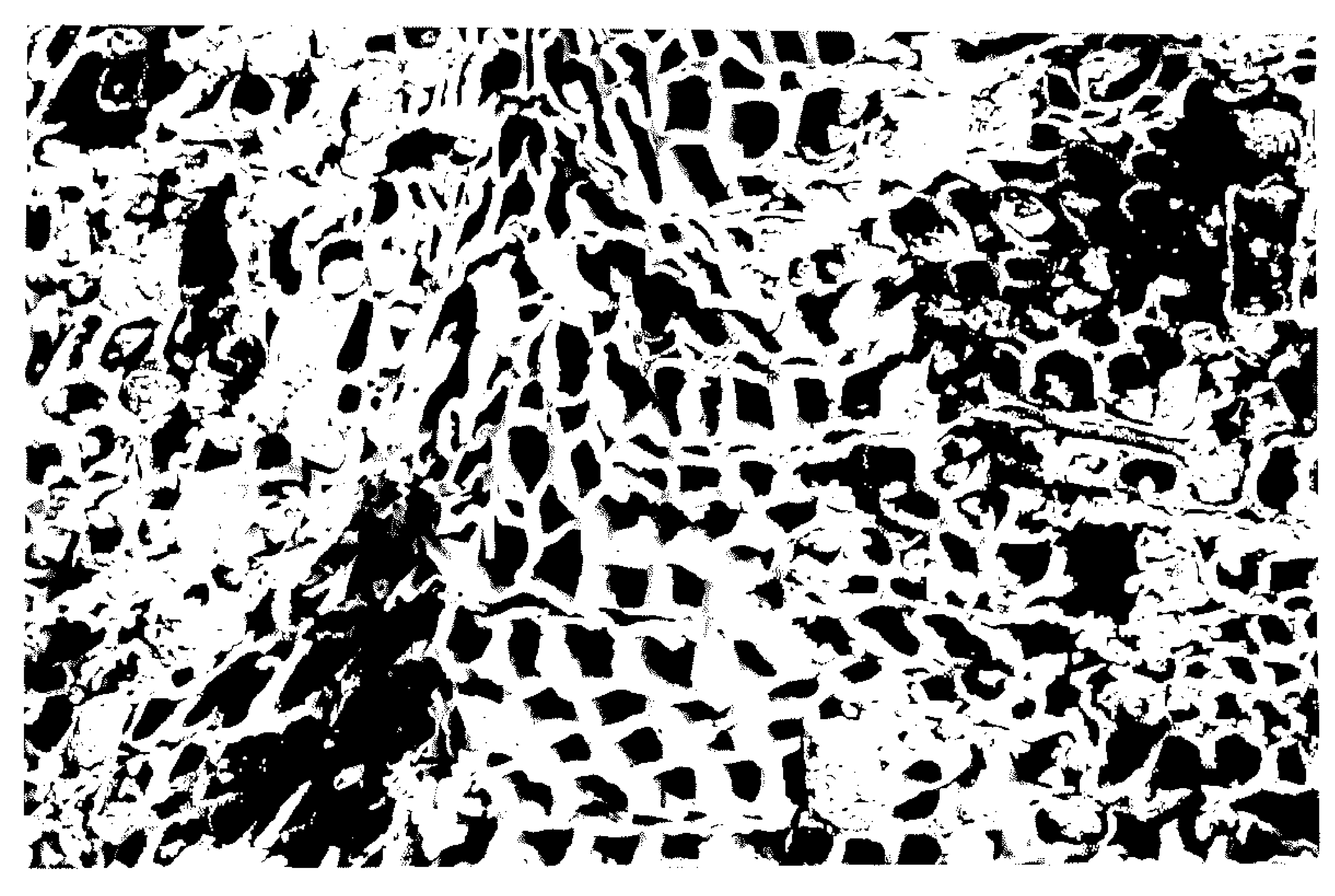
T.S. (x690), showing compressed tracheids.

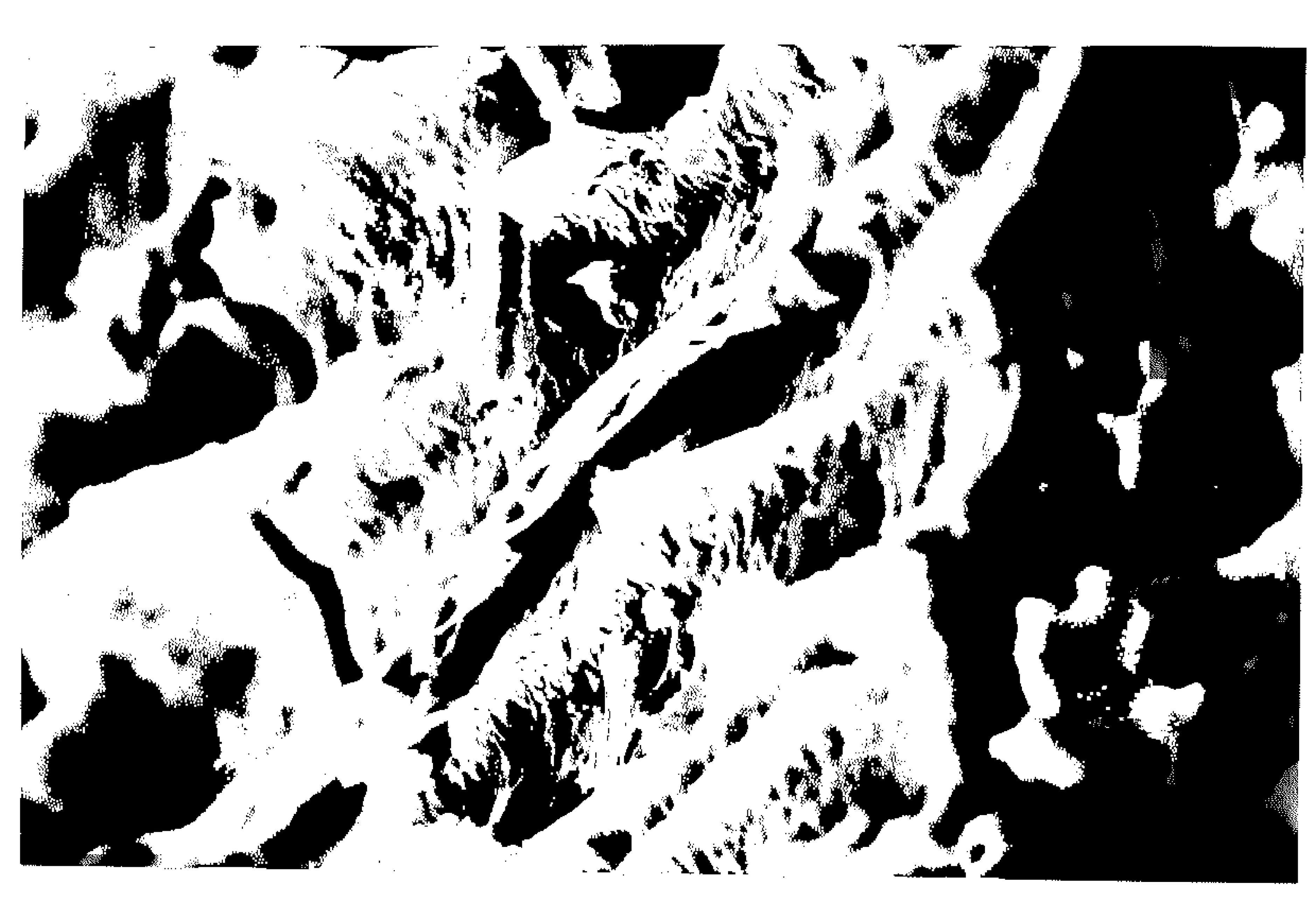
Plate (57).

T.S. (x10600), showing a tracheid with a plug of sediment.

Plate (58).

T.L.S. (x3400), showing a compressed and coalified uniseriate ray.





Sample 14. (coniferous wood)

Site: *Astarte tenera* Bed, Herne Bay, Kent.

Plate (59).

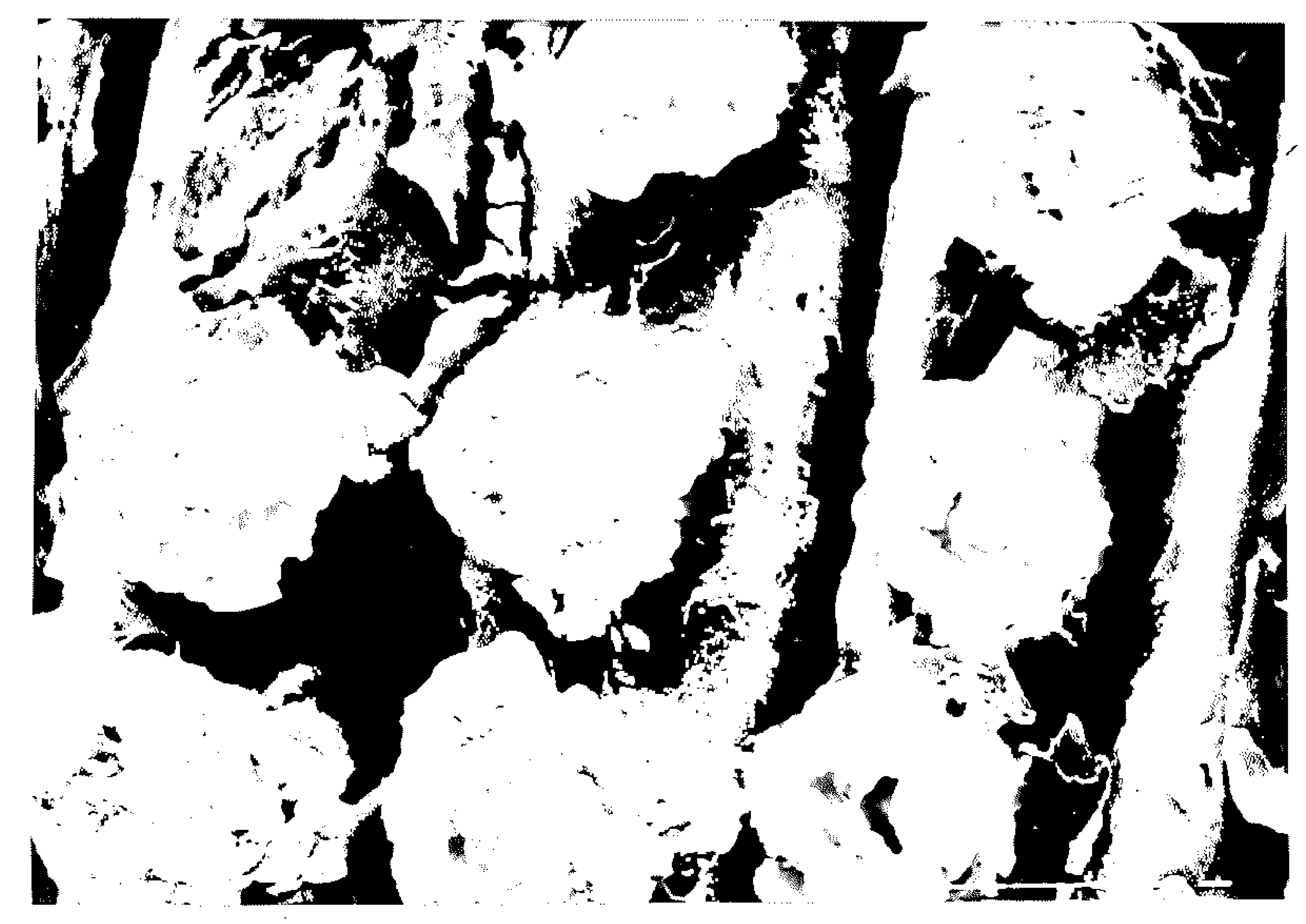
R.L.S. (x4900), showing the axial tracheids with opposite bordered pits.

Plate (60).

R.L.S. (x1216), showing the ray parenchyma cells. There are one or two pits per cross-field.

Plate (61).

R.L.S. (x14700), showing one of the cross-field pits magnified. The pit is clogged with sediment, it may be piceoid in shape.



Results with Coals.

A range of coals from the brown coal stage to anthracite were oxidized using the cupric oxide method described previously and chromatographed quantitatively and qualitatively. Very small amounts of lignin derivatives were found to occur. Therefore the samples to be injected were rotary evaporated to dryness and 0.25ml. of solvent were injected using a syringe to concentrate the products. It was found that the coal derivatives obtained from the oxidation procedure when chromatographed often polymerized on the column and so shortened the life of the column.

Figure (65).
A chromatogram of the lignin oxidation products of a brown coal.

German Brown Coal, 4µl injection, (5% Pdegs column).

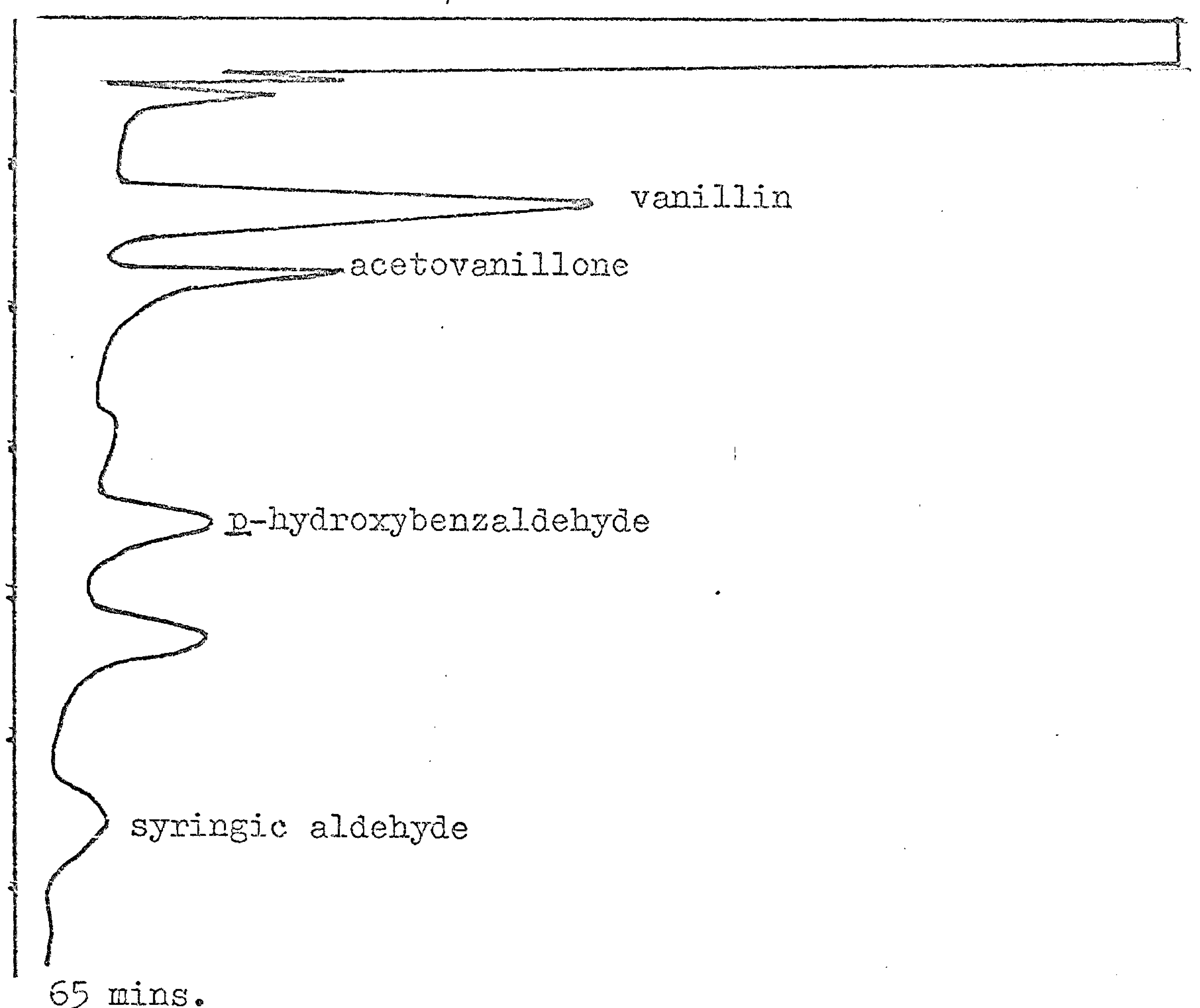
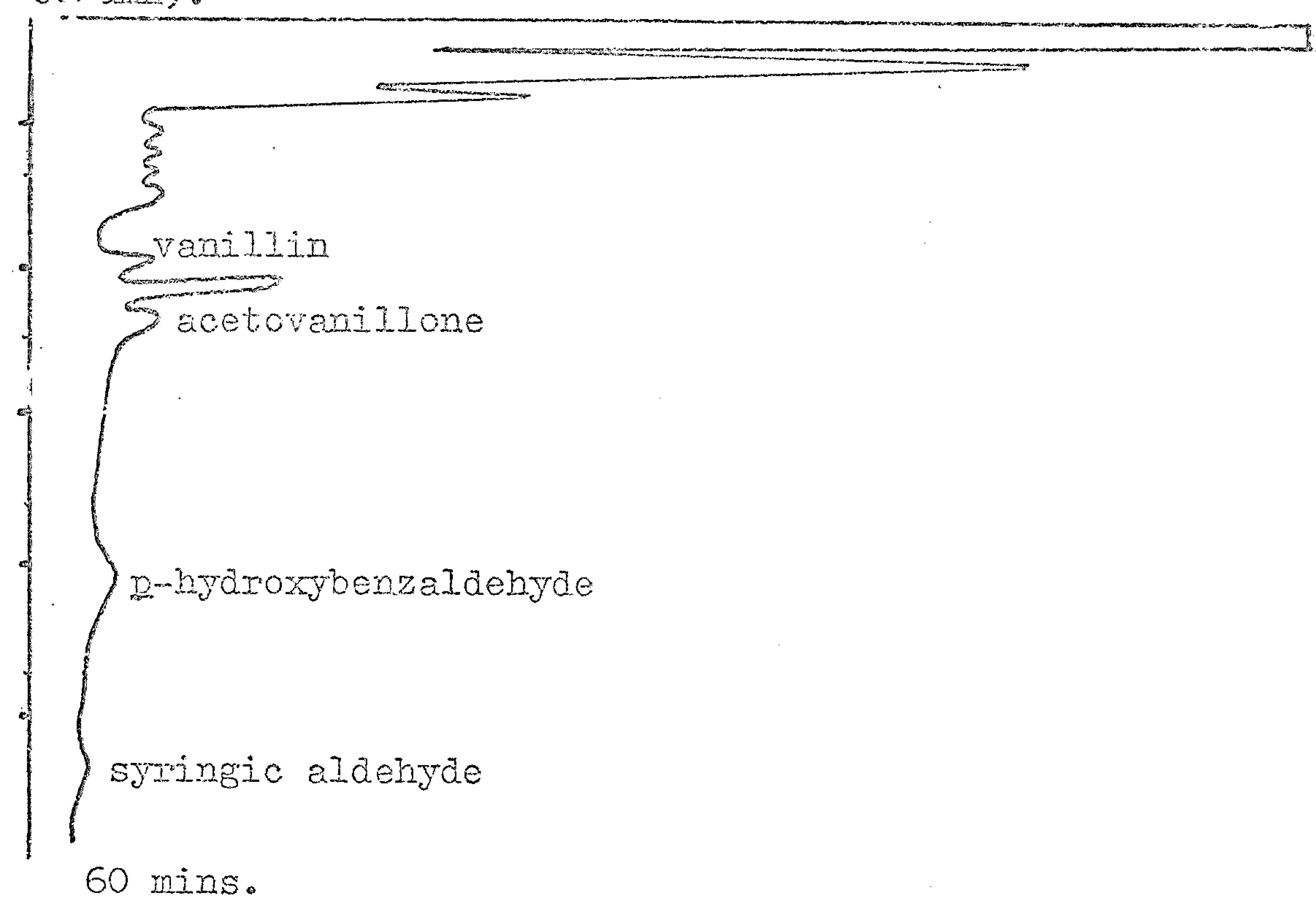


Figure (66).
A chromatogram of the lignin oxidation products of a bituminous coal.

British Daw Mill Bituminous Coal, 4μl injection, (5% Pdegs column).



Mixed chromatography using the authentics, p-hydroxybenzaldehyde, vanillin, acetovanillone and syringic aldehyde were added to the Daw Mill coal to confirm the presence of small amounts of the lignin oxidation products as shown in figure (67).

Figure (67).

A chromatogram of the lignin oxidation products of the Daw Mill coal with the added authentics.

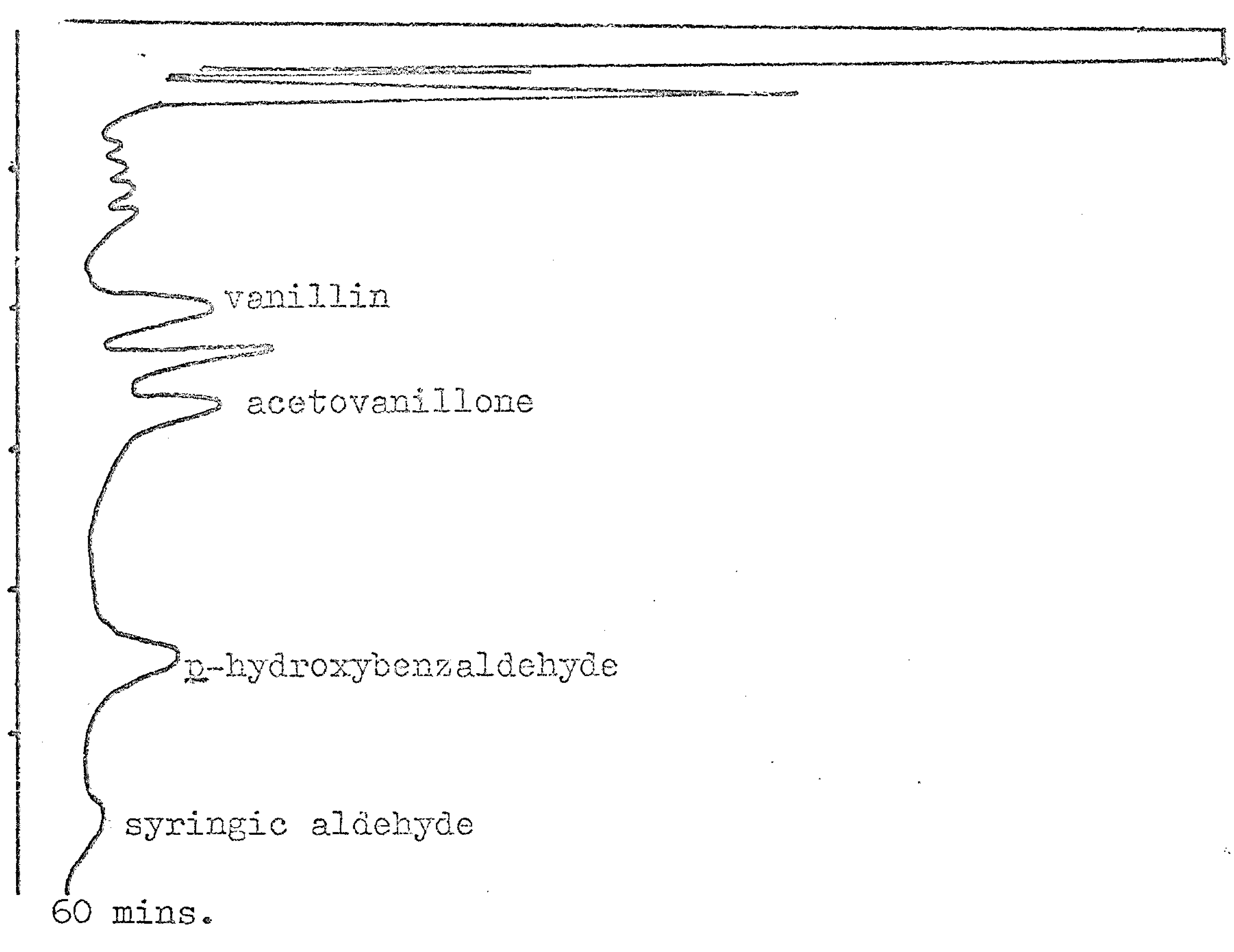


Table 17

The lignin oxidation products from a range of coals.
Unless otherwise stated 4µl injections were used. Larger injections were found to burst the glass liner at the injection point and to rapidly shorten the life of the column.

	Coal	Lignin oxidation products. µg/gm of coal			
		p-OH	VAN	ACETO	SYR
	Brown Coals.				
1	Hengistbury coal	490.00	120.00	40.00	5.26
2		310.00	80.00	32.00	7.34
1	German coal	28.00	12.50	41.56	6.35
2		17.50	10.30	65.00	7.50
1	Konin Patnów coal	81.20	600.00	662.50	25.00
2		99.90	537.50	437.50	16.25
1	Canakkale coal	28.00	12.50	41.56	6.25
2		17.50	10.30	65.00	7.50
	Bituminous Coal.				
1	Daw Mill coal	6.25	12.50	32.50	trace
2		8.56	15.32	32.76	trace
	Anthracite Coal.				
1	Bettws Beans coal	4.68	32.81	18.75	absent
2		2.96	31.66	16.21	absent

Five bituminous coals from the Middle Coal Measures in the British Carboniferous were oxidized using the cupric oxide method described previously. These coals contain abundant plant spores. The spores occur in characteristic associations, implying the occurrence of different plant communities associated with the deposition of the peat.

Smith (1962) recognises four possible types of spore assemblages within the British Carboniferous, each in association with its characteristic coal microlithotype. The five coals that were examined here belong to two of Smith's assemblages, either:-

A. Lycospore phase: usually dominated by microspores of arborescent lycopods such as Lepidodendron (Chaloner, 1953 and Felix, 1954), microspores of the Lycospora type, and associated with coals rich in the microlithotypes vitrite and clarite, thereby giving the coal a bright shiny appearance.

or

B. Densospore phase: usually characterized by spores of various other lycopods and thought to be herbaceous (Chaloner, 1958), microspores of the Densosporites type, and associated with coals entirely confined to durite, thereby giving the coal a dull appearance.

Plates (62) and (63) show a Lycospora type and a Densosporites type of miospore.

The coals were oxidized to see if the presence of any of the lignin oxidation products could be used as additional evidence for the type of vegetation, that is herbaceous or arborescent and as an indicator of phylogenetic relationships. The preparation of the spores has been discussed in Chapter 2. One thousand spores were counted and each spore was put into one of three categories, Densosporites type, Lycospora type and others. This was to confirm that the dull coal had a dominant Densosporites

species and that the *Lycospora* species was the dominant spore type in the bright coal, so agreeing with the work of Smith (1962).

Table 18

Percentage occurrence of the species found in dull and bright coals from the Middle Coal Measures.

COALS	LYCOSPORA TYPE	DENSOSPORITES TYPE	OTHERS
Bright Coals.			
Kiverton Park coal.	58.0%	15.0%	40.5%
Gelding Top coal.	65.7%	3.2%	31.1%
Dull Coals.			
Peckfield coal.	0.9%	65.5%	33.6%
Gelding Top coal.	2.2%	72.0%	25.8%
Flockton Orgreave coal.	3.8%	64.2%	32.0%

Plate (62).

(x300), a <u>Lycospora</u> type of miospore obtained from the Kiverton Park coal.

Plate (63).

(x300), a <u>Densosporites</u> type of miospore obtained from the Peckfield coal.

Table 19

The lignin oxidation products from the bituminous coal from the Middle Coal Measures of the British Carboniferous.

Unless otherwise stated 4µl injections were used.

		Lignin oxidation products. µg/gm of coal			
	Bituminous Coals.	p-OH	VAN	ACETO	SYR
	Bright coal.				
1	Kiverton Park coal	12.50	3.12	12.50	absent
2		6.25	1.88	11.30	absent
3		8.50	1.25	6.25	absent
1	Gelding Top coal	15.00	1.25	5.60	absent
2		26.00	1.25	4.40	absent
	Dull coal.				
1	Peckfield coal	4.37	18.10	12.50	18.75
2		2.50	15.60	7.50	12.50
1	Gelding Top coal	8.60	12.50	9.37	10.56
2		7.32	8.35	11.22	11.24
1	Flockton Orgreave coal	3.13	9.37	15.60	4.68
2		5.25	10.11	14.26	3.25

Results with Compression Fossils.

A range of compression fossils were oxidized using the cupric oxide method described previously and chromatographed. As only minute amounts of lignin derivatives were found to occur in certain species, the presence or absence of the derivative only was recorded. In species where more of the lignin derivatives were obtained , quantitative chromatography was used. Mixed chromatography using the authentics p-hydroxybenzaldehyde, vanillin, acetovanillone and syringic aldehyde were added to the fossils to confirm the presence of small amounts of the lignin oxidation products obtained from the fossils.

Figure (68).
A chromatogram of the lignin oxidation products of Ginkgo huttoni.

Ginkgo huttoni, 4µl injection, (5% Pdegs column).

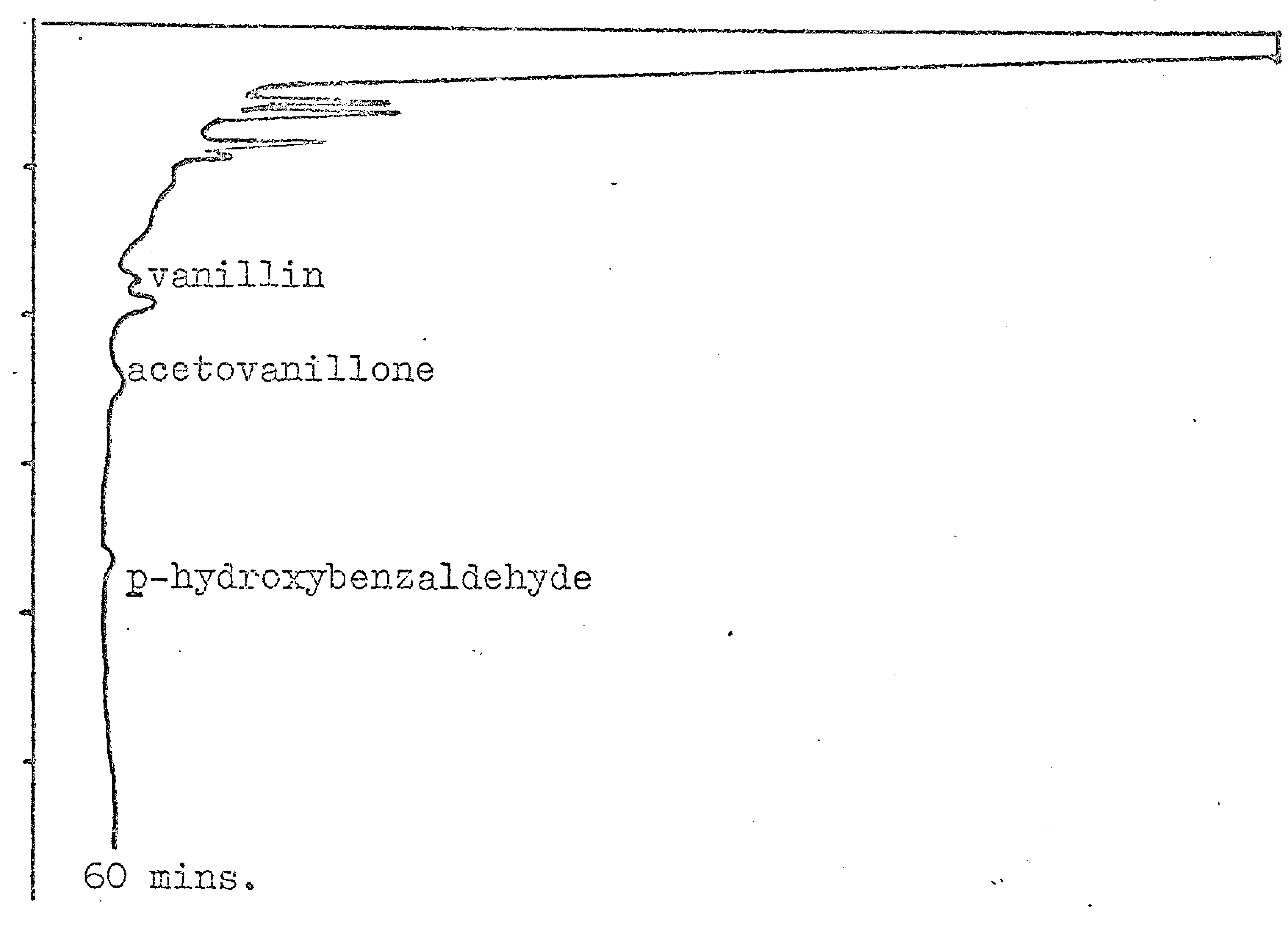


Figure (69).
A chromatogram of the lignin oxidation products of
Sigillaria ovata.

Sigillaria ovata (116), 4µl injection, (5% Pdegs column).

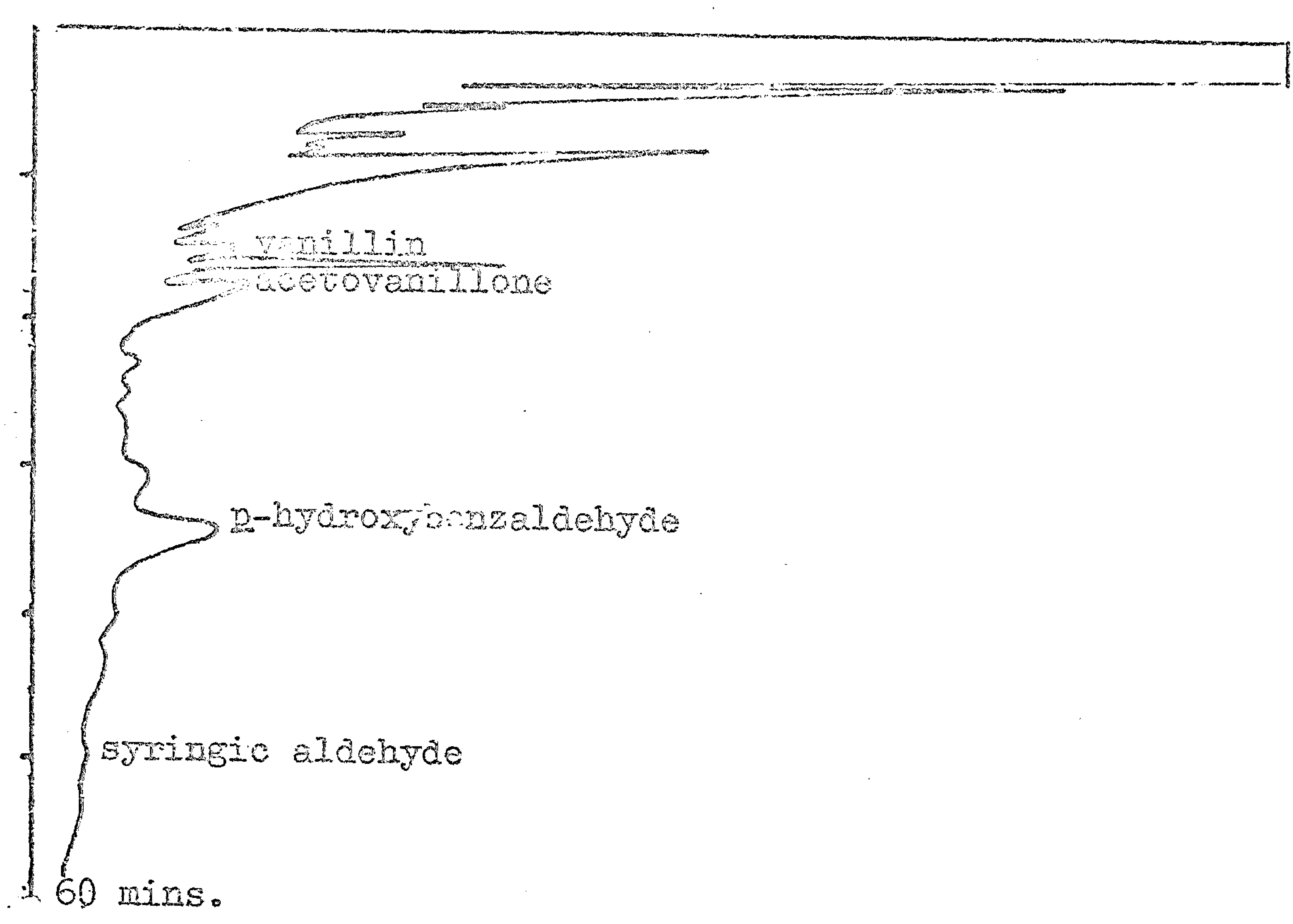


Table 20

The lignin oxidation products from Compression Fossils.

Unless otherwise stated 4µl injections were used.

			Lignin oxidation products. µg/gm of coal			
		COMPRESSION FOSSILS	p-OH	VAN	ACETO	SYR
CRETACEOUS	1	Pseudofrenelopsis parceramosa - shoot	54.00	6.30	5.60	absent
	2		37.00	3.80	2.90	absent
	1	Chierolepidaceae - wood	115.62	106.25	28.12	absent
	2		81.25	87.50	14.37	absent
JURASSIC	1	Ginkgo huttoni - leaf	present	present	present	absent
	2		present	present	present	absent
	3		present	present	present	absent
	1	Ptilophyllum pectinoides - leaf	present	present	present	absent
	2		present	present	present	absent
	1	Pachypteris lanceolata - leaf	present	present	present	absent
	2		present	present	present	absent
CARBONIFEROUS	1	Lepidodendron sp. - bark	present	present	present	absent
	2		present	present	present	absent
	3		present	present	present	absent
	1	Lepidophlois sp. - bark	present	present	present	absent
	2		present	present	present	absent
	1	Sigillaria ovata 116 - bark	45.87	21.87	16.87	trace
	2		57.50	20.62	29.37	trace
	1	Sigillaria ovata 104 - bark	40.62	18.75	15.62	trace
	2		39.37	34.37	13.12	trace

Plates (64- 70) show the fossils that were used and their state of preservation.

Plate (68).

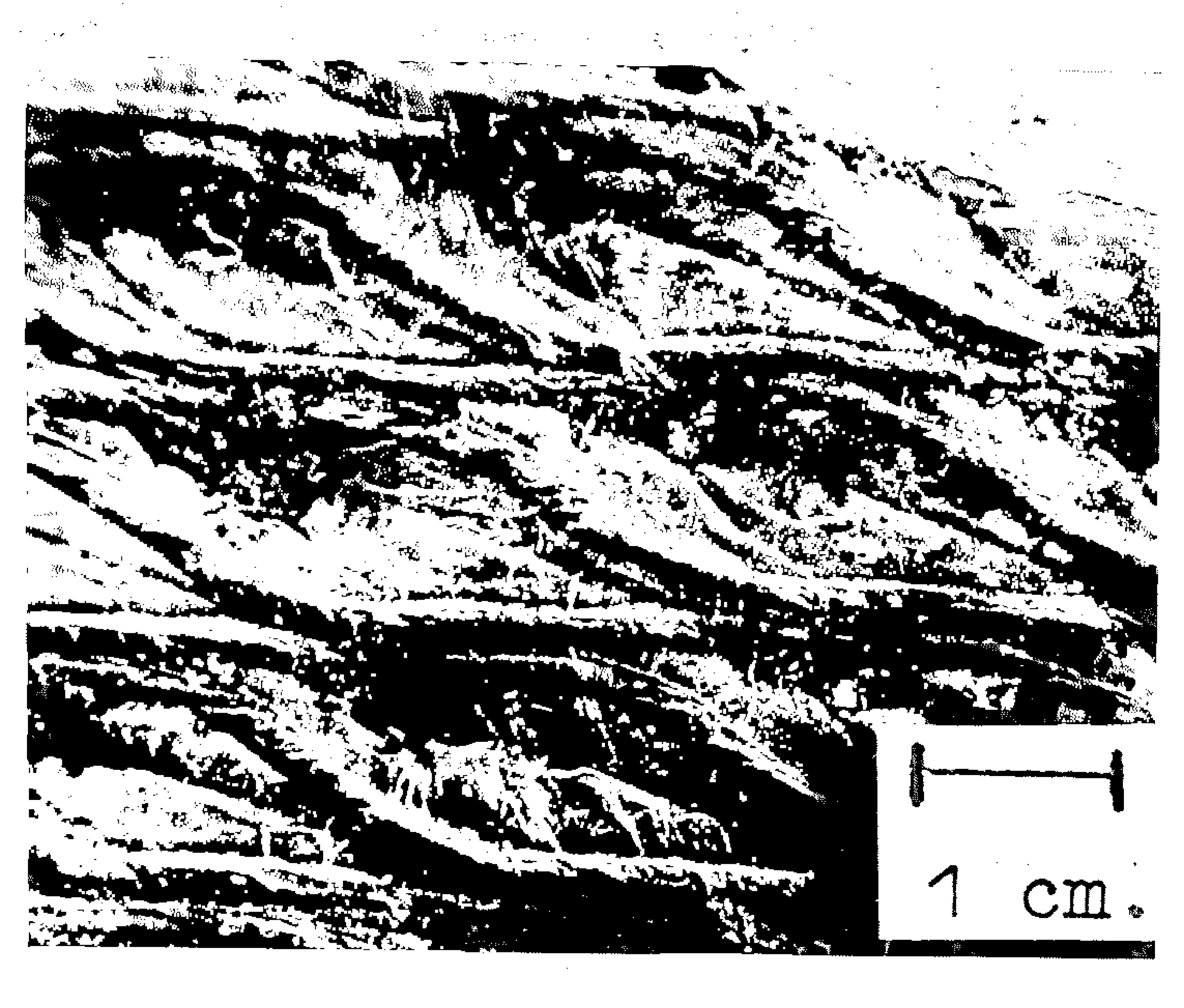
Lepidodendron species - showing the leaf bases.

Plate (69).

Lepidophlois species - showing the leaf bases.

Plate (70).

Sigillaria ovata Sauveur - showing the leaf bases.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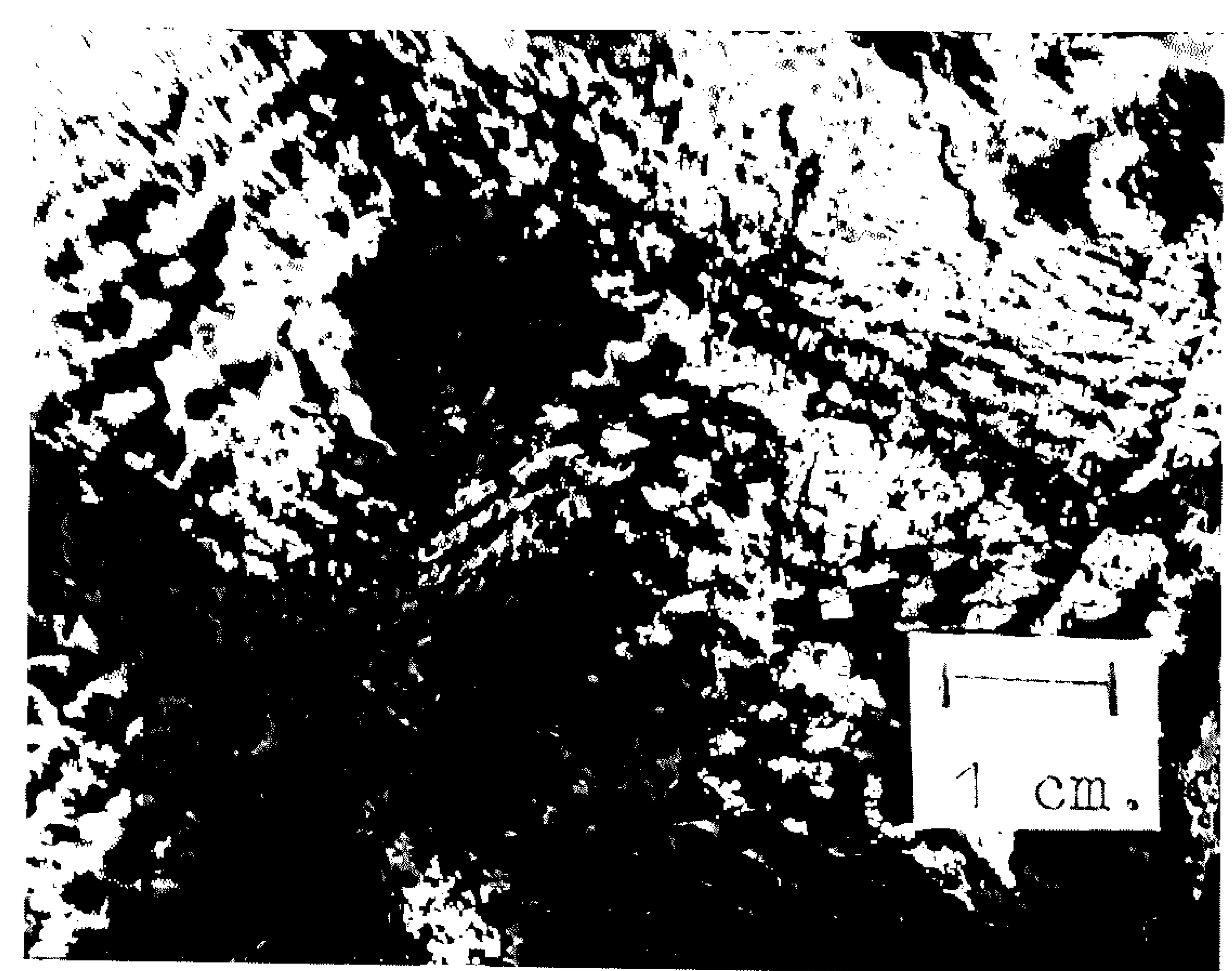

1 cm.

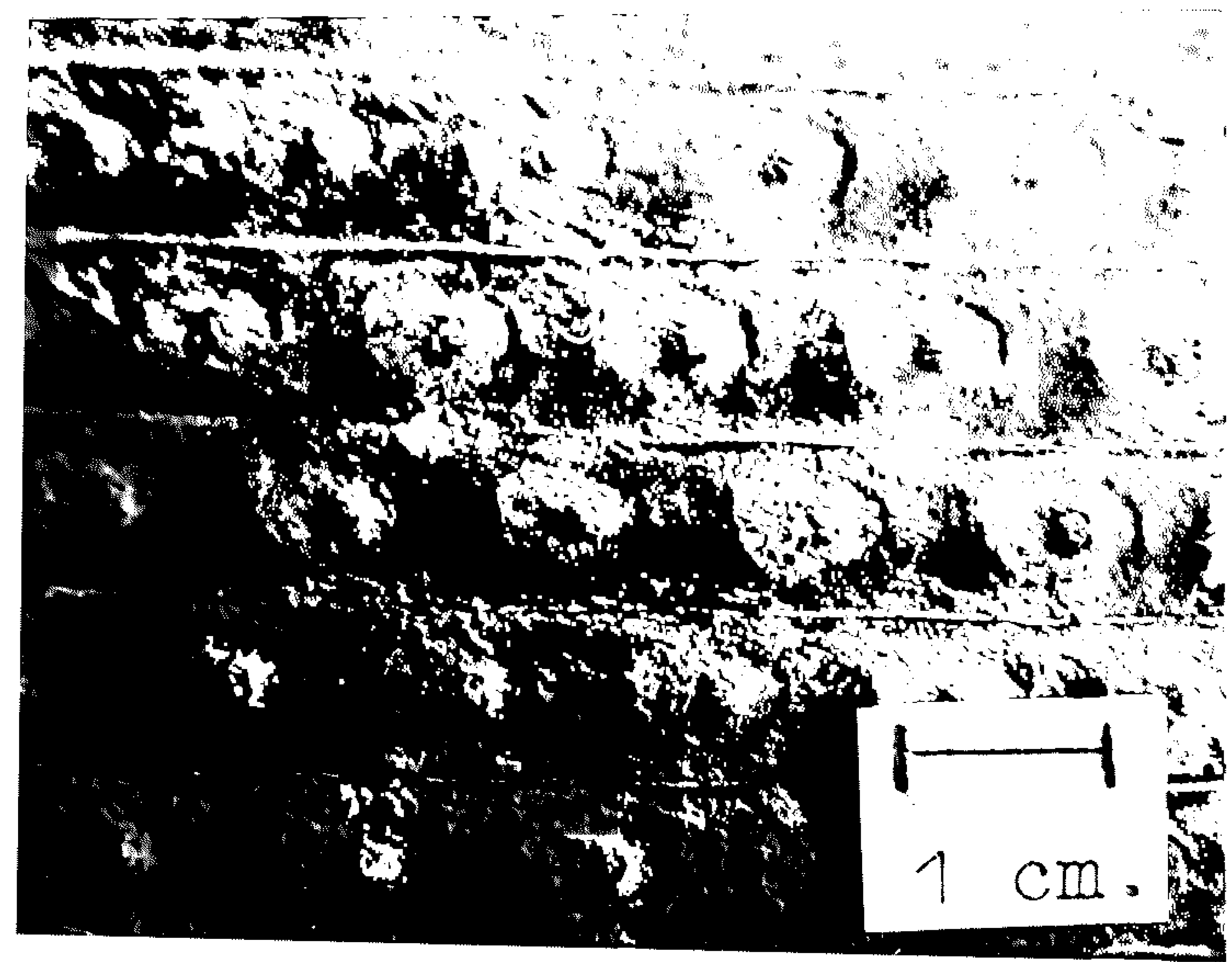
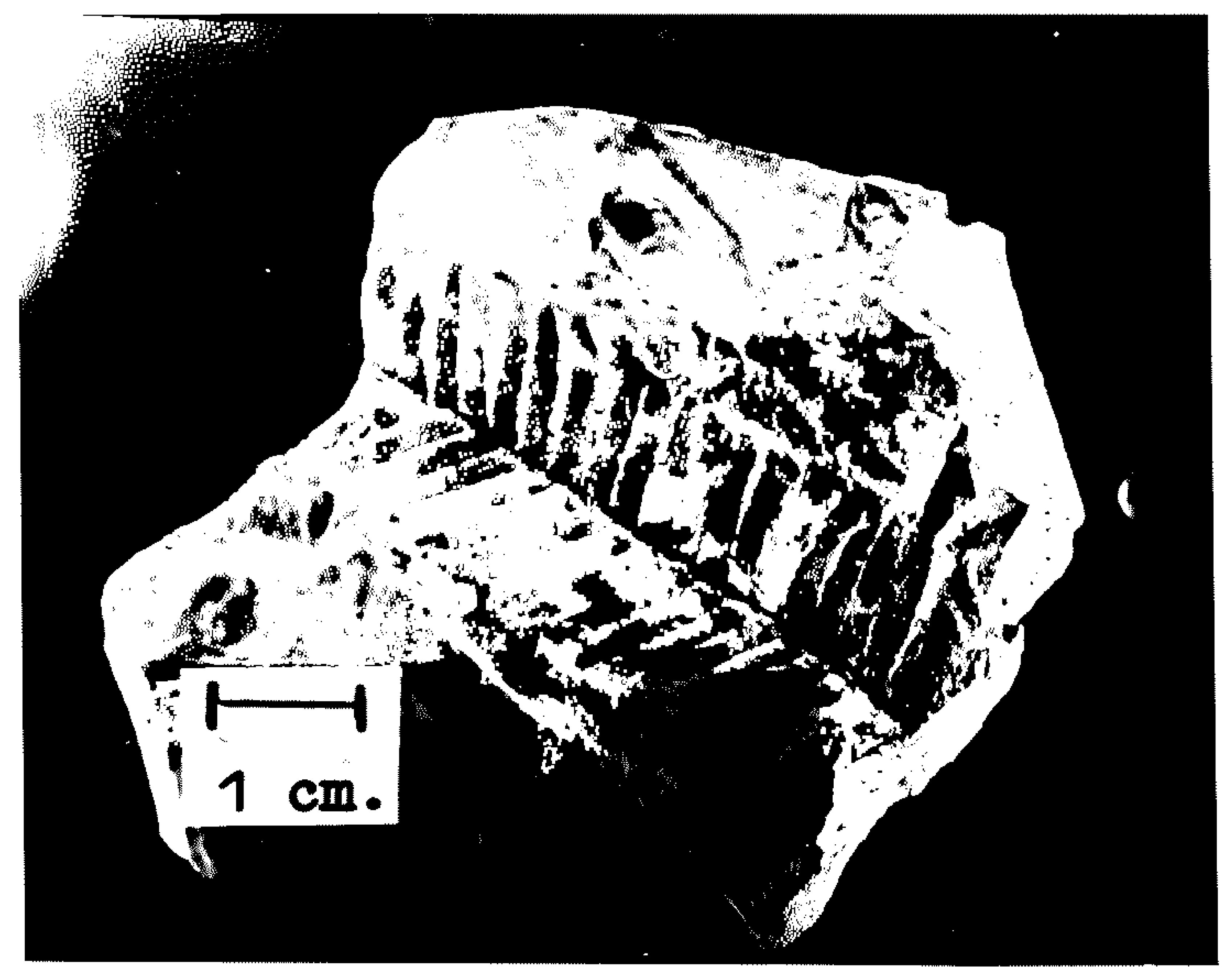

1 cm.

Plate (66).

Ptilophyllum pectinoides (Phillips) Morris - portion of the leaf.

Plate (67).

Pachypteris lanceolata Brongniart. - portion of the leaf.


1 cm.

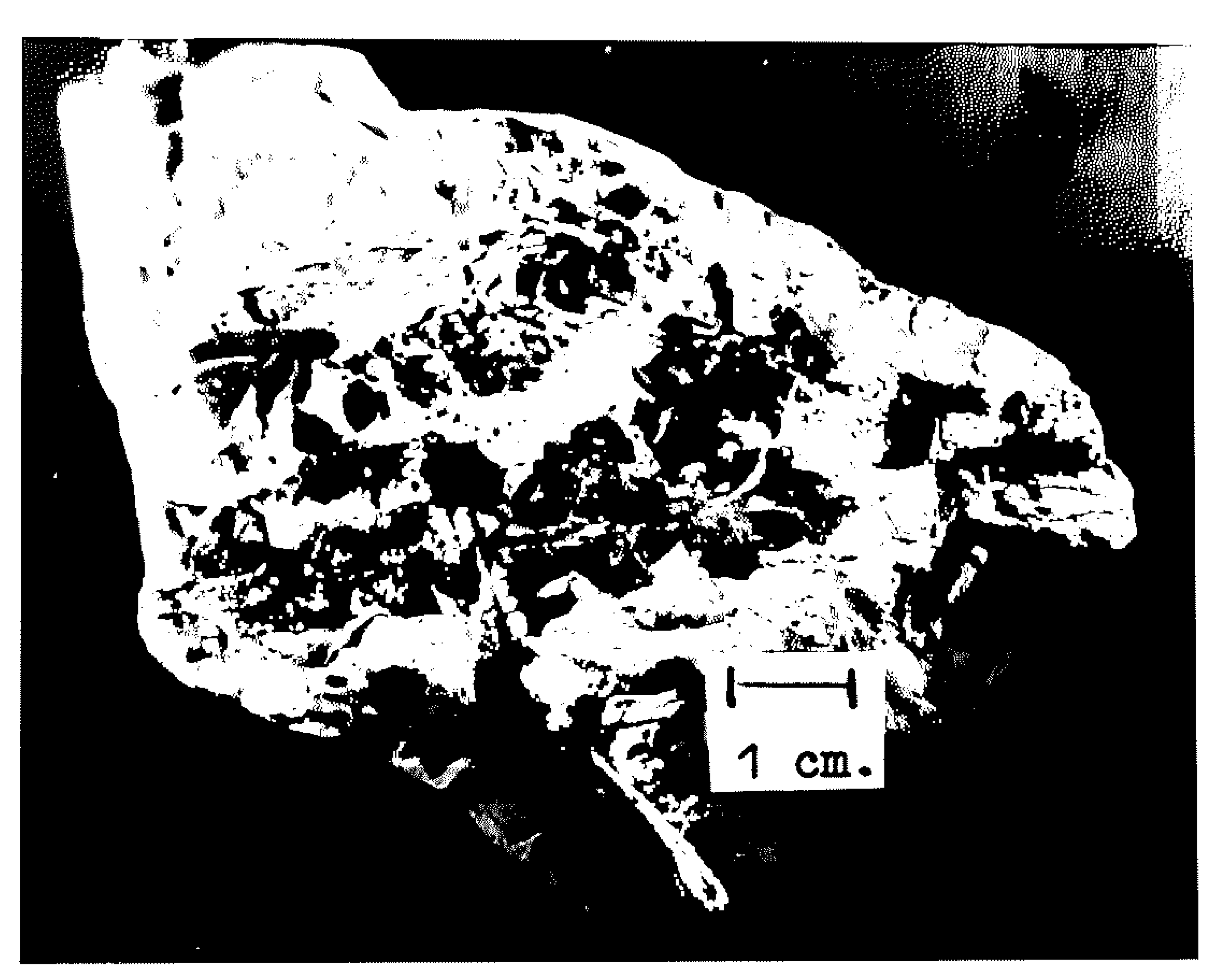

1 cm.

Plate (64).

Pseudofrenelopsis parceramosa (Fontaine) Watson - a shoot fragment.

Plate (65).

Ginkgo huttoni (Sternberg) Heer - leaves.

1 cm.

Discussion : Lignin derivatives from fossils and coals.

Quaternary wood.

Phenolic aldehydes and ketones were obtained from a variety of Quaternary fossil wood samples which were oxidized using the reaction described earlier (Chapter 2). Examples of typical chromatograms are shown in figures (61) and (62). The results are tabulated in table 15.

Sample 1 (a Pinus species of the two leaved type) is well preserved, and the resin ducts and ray tracheids with peg-like dentations are still intact as shown in plates (2) and (3). The lignin oxidation products obtained from Sample 1, when compared with those from its contemporary wood, Pinus sylvestris contained similar amounts of vanillin, acetovanillone and p-hydroxybenzaldehyde. However the syringic aldehyde content of Sample 1, showed differences when compared in this way with the contemporary species. In Sample 1 syringic aldehyde gave 3.3% of the total aldehyde content whereas for the contemporary wood the corresponding value was 0.3%.

Sample 5, again a Pinus species of the two leaved type has undergone more deterioration than has Sample 1, as shown in plates (10) to (13). It is also a more compressed wood than is Sample 1. Vanillin, acetovanillone and p-hydroxybenzaldehyde were detected from Sample 5 in smaller amounts than those obtained from Sample 1 and its contemporary species Pinus sylvestris. In terms of relative proportions the phenolic aldehydes from Sample 5 were found to be similar to those of Sample 1 and its contemporary wood. However syringic aldehyde was not detected in Sample 5. As syringic aldehyde was found to be present in all the contemporary coniferous wood examined it is likely that Sample 5 did initially contain syringyl units in its lignin. The absence of this aldehyde can be accounted for in two ways. Either demethoxylation or the preferential break-down of the syringic aldehyde due to the presence of more oxysubstituents occuring. The preferential loss of syringic aldehyde was demonstrated in Chapter 3.

Manskaya and Drozdova (1968) reported the presence of vanillin from four fossil wood samples and p-hydroxybenz-aldehyde from a sample of fossilized *Pinus sylvestris* wood. No syringic aldehyde was reported from the fossil wood of *Pinus sylvestris*. p-Hydroxybenzaldehyde, vanillin and vanillic acid have also been reported by Kodina (1968) from fossilized *Pinus* wood.

Over all the chromatograms obtained from the Quaternary coniferous woods that were examined were remarkably similar to those obtained from contemporary coniferous woods, as are shown in figures (32) and (61).

When the Quaternary angiospermous woods were oxidized, Sample 4 (*Ulmus procera*) a mature wood, was found to give small amounts of p-hydroxybenzaldehyde, as shown in the chromatogram, figure (62). No p-hydroxybenzaldehyde was detected in the mature wood of the contemporary species *Ulmus procera*. The wood of Sample 6, possibly a member of the Salixaceae, was also found to contain small amounts of p-hydroxybenzaldehyde. No p-hydroxybenzaldehyde was detected in Sample 2 (*Betula pendula*) or Sample 3 (*Quercus* species). The presence of p-hydroxybenzaldehyde in Samples 4 and 6 suggests that during burial and degradation, demethoxylation has occured. Demethoxylation has been suggested to occur in fossil woods and coals by several authors (Sen and Basak, 1957; Flaig, 1968; Kodina, 1968 and Leo and Barghoorn, 1970).

Sample 2 (*Betula pendula*) and Sample 4 (*Ulmus procera*) both show proportionally more vanillin and less syringic aldehyde than in their contemporary woods. This is particularly noticeable with Sample 2 which has vanillin as 45.3% of the total aldehyde content and syringic aldehyde as 54.7% of the total aldehyde content. For comparison, the contemporary species *Betula pendula* gave vanillin as 32.8% of the total aldehyde content and syringic aldehyde as 67.2% of the total aldehyde content. Again this accords with the view that loss of methoxyl groups is occurring from the syringic aldehyde to form vanillin.

Sample 6, possibly a member of the Salixaceae, shows

a substantially lower aldehyde content compared to the other samples of Quaternary wood that were examined. Perhaps this is not surprising as most of the cellular structures from Sample 6 have collapsed and decayed as shown in plates (14) to (17). It is also one of the oldest samples to be examined from the Quaternary period. The preservation of the other Quaternary angiosperm samples is better, as shown in plates (5) to (9) and this is reflected in the amount of lignin derivatives obtained from these samples.

There appears to be an increase in the amount of lignin obtained from most of the Quaternary angiospermous woods when they are compared to their contemporary woods. This may be due to there being relatively more lignin per gram oxidized in the fossil wood, the cellulose and hemicelluloses having been decomposed more rapidly by micro-organisms. The increase of lignin in the Quaternary angiospermous woods may explain why p-hydroxybenzaldehyde was detected in certain angiospermous samples. Many authors have reported an apparent increase in lignin from fossil wood using both anatomical and chemical analysis (Jahn and Harlow, 1942; Cudy, 1946; Varossieau and Breger, 1952; Sen and Basak, 1957 and Farmer and Morrison, 1964).

The Quaternary woods examined remain relatively unchanged with respect to their contemporary woods. Small differences in their relative abundance of the phenolic aldehydes and ketones are observed. The Quaternary coniferous woods are still recognizable as coniferous from the substantial amounts of vanillin that is obtained (an average of 96.4% of the total aldehyde content). Although more changes in the composition of the angiospermous woods have occurred, the Quaternary angiospermous woods are still recognizable by the retention of their syringic aldehyde residue. In the present work the vanillin to syringic aldehyde ratio of the Quaternary angiosperms has varied from 1:2.5 in Sample 3 (Quercus species) to 1:1 in Sample 2 (Betula pendula).

Tertiary Wood.

The preservation of the woods examined from the Tertiary period varies enormously as shown in the plates. Samples 8,9 and 13 have a 'woody' appearance and show some detailed anatomical features, as shown in plates (26) to (39). Other wood samples, particularly those found from the Eocene and Palaeocene are very coalified with few anatomical features, such as Samples 12 and 13, plates (44) to (55).

All the Tertiary wood samples that were examined showed a decrease in the amount of phenolic aldehydes detected as tabulated in table 16, when compared to the contemporary woods examined tabulated in tables 3 and 5.

Vanillin was the major aldehyde recovered from the Tertiary coniferous woods, ranging from 90.7% in Sample 13 (possibly a member of the Taxodiaceae) to 79.9% in Sample 11.

Sample 7, a coniferous sp. , shows an increase in the amount of p-hydroxybenzaldehyde (13.7% of the total aldehyde content) compared to its contemporary species Pinus sylvestris (2.8% of the total aldehyde content). The vanillin content of this species was found to be lower (86.2%) compared to that of the contemporary wood (94.8% of the total aldehyde content). Trace amounts of syringic aldehyde were present in Sample 7 (coniferous sp.) suggesting that not all demethoxylation proceeds differentially. The results obtained from two fossil woods led Leo and Barghoorn (1970) to believe that preferential demethoxylation proceeds from syringic aldehyde to vanillin and then from vanillin to p-hydroxybenzaldehyde.

Samples 8 and 9 are both members of the Taxodiaceae. Sample 8 is more compressed and degraded than is Sample 9 as shown in plates (26) and (31) and consequently Sample 8 is likely to possess less lignin than Sample 9. The p-hydroxybenzaldehyde content of the Taxodiaceae examined in the contemporary woods ranges from 0.34% of the total aldehyde content in Sequoia semperivirens to 9.5% of the

total aldehyde content in Taxodium distichum. Sample 8, the more compressed wood, possessed more p-hydroxybenzaldehyde (19.3% of the total aldehyde content) whereas Sample 9 has less (5.8%). Trace amounts of syringic aldehyde were present in both samples. Sample 13 which may be a member of the Taxodiaceae had a high proportion of vanillin (90.7% of the total aldehyde content). In this wood the syringic aldehyde detected was 3.1% of the total aldehyde content, surprisingly high for a wood of Eocene age, (that is between 38 and 63 million years old).

Samples 11 and 14 show little anatomical detail but their chromatograms are similar to the chromatograms obtained from contemporary coniferous woods. This can be seen when figure (63), a chromatogram of Sample 14, is compared to a chromatogram of a coniferous wood such as Juniperus communis, figure (32). Both Samples 11 and 14 possess higher amounts of p-hydroxybenzaldehyde and lower amounts of vanillin than any of the contemporary coniferous woods that have been examined. No syringic aldehyde was detected in these samples. Leo and Barghoorn (1970) compared a Miocene wood Cedrus penhallowii with a contemporary species Cedrus deodera and found no syringic aldehyde present in the fossil wood. They found that the vanillin and p-hydroxybenzaldehyde obtained were similar to the proportions obtained from the contemporary wood.

Only two of the Tertiary woods examined in this work were found to be angiospermous, initially by observing their lignin derivatives and subsequently using microscopy. Sample 10, Laurinoxylon endiandroides, a fossil genus, was 'woody' and well preserved as shown in plates (37) to (39) whereas the angiospermous wood from the Chara-shell Bed, Sample 12 was coalified and showed few anatomical features, plates (44) to (50). Both samples contained vanillin in higher proportions than that found in lignin from any contemporary angiospermous woods that have been examined while the syringic aldehyde content was less than that found in any lignin from contemporary angiospermous woods examined.

Sample 12 had less syringic aldehyde (0.2 mg/gm) than had Sample 10 (0.35 mg/gm) but Sample 12 which was very coalified had proportionally more syringic aldehyde (37.9% of the total aldehyde content) compared to Sample 10 (13.5% of the total aldehyde content). Both angiospermous lignins had more syringic aldehyde than that observed from lignin of coniferous woods from the Tertiary.

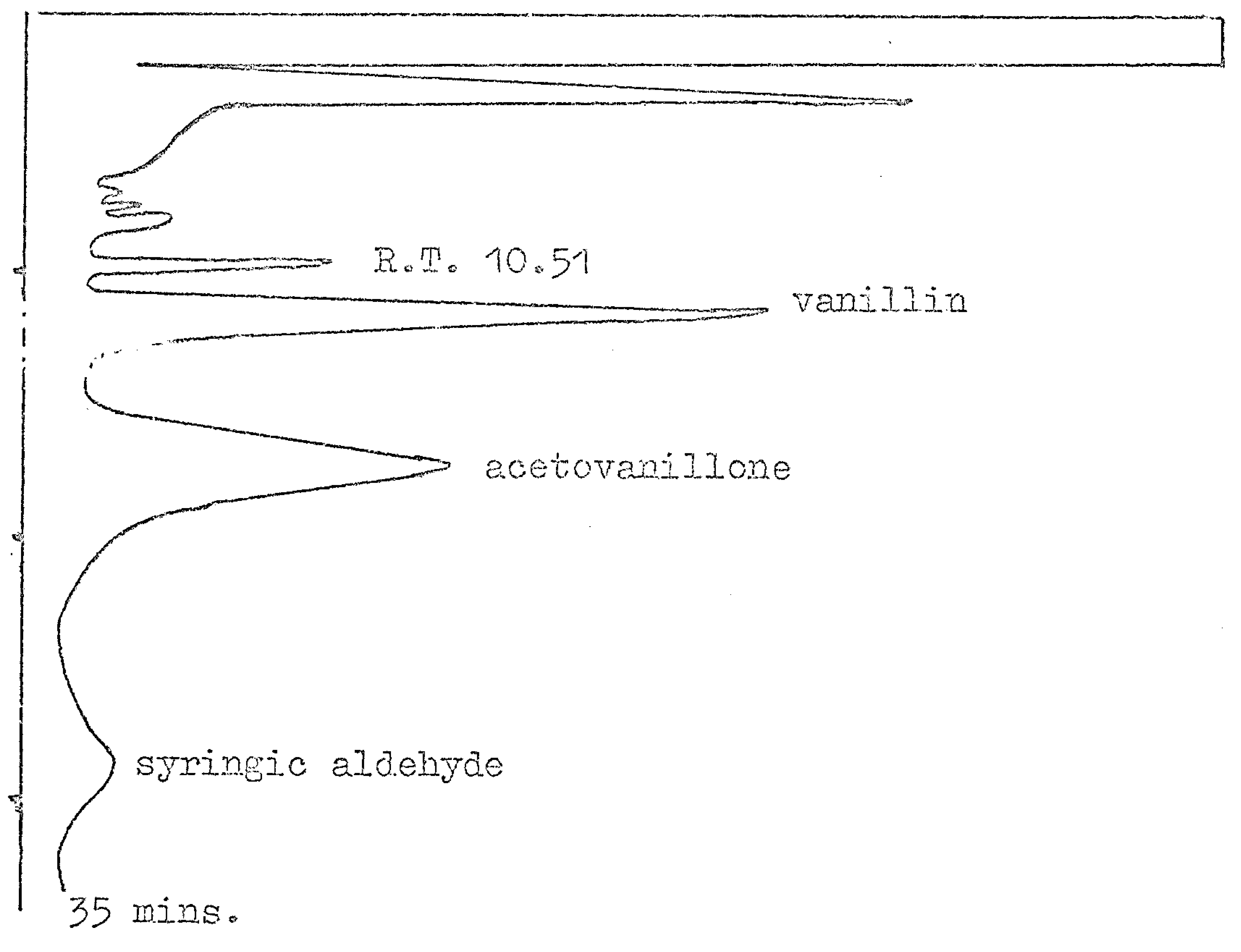
The lignin from the younger angiosperm, Sample 10, Laurinoxylon endiandroides from the Miocene (7 to 26 million years old) was found to obtain p-hydroxybenzaldehyde (1.7% of the total aldehyde content) but p-hydroxybenzaldehyde was not observed from lignin of the older wood, Sample 14, from the Palaeocene (63 to 65 million years old).

Manskaya (1951) found that the number of methoxyl groups isolated from lignin decreased with increasing geological age. It seems however that it is not only the time period of burial that is important in the processess of demethoxylation but other factors such as the type of wood, the environment and the type of microorganisms present.

One compound with a retention time of 10.51 minutes was found to be present in the products obtained from the oxidation of lignin from contemporary and fossil angiosperm-ous woods. This compound was only detected when the 3% OV 101 column was used as shown in figure (70) and was not detected when a 5% Pdegs column was employed. This compound was not syringic acid which was found to have a different retention time. Infact the chromatograms of the coalified coniferous and angiospermous woods from the Tertiary are remarkably similar to the chromatograms of their contemporary woods.

Figure (70).
A chromatogram of an angiospermous wood from the Tertiary.

Sample 11, *Chara*-shell Bed, 10 μl injection, OV 101 column.



Coal and Fossil Plants.

It was considered of interest to determine whether chemical fossils related to lignin could be found in coals using the cupric oxide method. This would possibly permit speculation on the types of vegetation contributing to the coal. Lam and Pedersen (1978) attempted to do this with aromatics from bituminous coals. One of their coal samples came from the Lower Cretaceous Korme Formation and its vegetational history had been composed of ferns, conifers and cycads. The other sample from the Upper Cretaceous Atane Formation of Nugssuaq contained fragments of tracheids and angiosperms. Unfortunately they found no real differences in the aromatics they obtained from their coal samples.

Table 17 lists the major lignin oxidation products obtained from different types of coals ranging from the brown coal stage to anthracite. The major lignin oxidation products obtained from bituminous coals of the Middle Coal Measures of the British Carboniferous are tabulated in table 19.

The main oxidation product obtained from the brown coal of Hengistbury was p-hydroxybenzaldehyde (78.8% of the total aldehyde content). The syringic aldehyde obtained from the oxidation of this brown coal was one of the lowest values obtained for syringic aldehyde from the brown coals examined here (1.3% of the total aldehyde content). There are three possible explanations for this:

1) Demethoxylation is occurring more rapidly in this coal than in the other brown coals examined in this work.
2) Syringic aldehyde and vanillin are preferentially destroyed. p-Hydroxybenzaldehyde is the least reactive of the three phenolic aldehydes because it has less oxygen groups.
3) This coal was composed largely of lower plants and grasses. These plants contain more p-hydroxybenzaldehyde in their lignin than other plant species.

The oxidation products from the German coal and the Turkish coal (Canakkale) were found to possess p-hydroxybenzaldehyde as 54.7% and 54.8% of the total aldehyde content respectively. Both coals were also found to contain similar amounts of vanillin and syringic aldehyde as oxidation products (30%and 27.8% of the total aldehyde content and 17.3% and 17.4% of the total aldehyde content respectively). These proportions of phenolic aldehydes suggest that both these coals are made up of amounts of guaiacyl-syringyl type residues such as those found in deciduous woods. As the p-hydroxybenzaldehyde proportions are large the vegetation suggested may have also consisted of lower plants and grasses (which yield p-hydroxybenzaldehyde in higher proportions).

The Polish brown coal (Konin Patnów) was found to possess the largest quantity of vanillin, 83.6% of the total aldehyde content. The proportions of the aldehydes in this coal suggest that the lignin of the original plant material was made up largely of guaiacyl residues such as those found in coniferous woods.

In the brown coals that have been examined in this work considerable amounts of p-hydroxybenzaldehyde have been observed. This may be due either to the original type of lignin present in the vegetation, demethoxylation, or the preferential destruction of vanillin and syringic aldehyde as mentioned previously. Somewhat surprisingly small amounts of syringic aldehyde were present in all the brown coals that were examined in this work. Kodina (1960) reported the presence of vanillin, vanillic acid and p-hydroxybenzaldehyde from three lignites using the nitrobenzene oxidation method as did Manskaya and Drozdova (1968) from several lignites : neither group reported the detection of syringyl residues. Using the alkaline cupric oxide method p-hydroxy and 3,4-dihydroxybenzoic acids were reported from a lignite by Hayatsu, Winans, McBeth, Scott, Moore and Studier (1979). The mass spectra of the oxidation products from this coal showed the complete absence of methoxyl groups.

In this work the presence of syringic aldehyde is shown in the chromatogram of the German brown coal, figure (65) and in the mass spectra obtained from the Hengistbury coal sample.

Mass Spectral Interpretations.

The mass spectra obtained from the major lignin oxidation products of the brown coal from Konin Patnów are presented in table 21 and figures (71) and (72) and from Hengistbury, table 22.

Table 21.

G.C./M.S. of Oxidation Products from Lignin of Konin Patnów Coal. A.E.I. M.S. 30 double focusing mass spectrometer integrated with a Pye Unicam model 104 G.C. (5% P.E.G.A. on Diatomite CLQ).

Retention Time(min.)	Mass* Spectrum m/e (relative abundance%)	M^+	Assigned molecular formulae.
76	344(7),285(17),271(1),258(12),218(5),217(16),187(10),174(5),169(2),156(5),155(45),120(100),113(42),111(41),99(50),91(31),86(33),40(50),32(100),28(100).		
164	217(6),174(1),173(22),155(5),99(30),55(100),44(40),40(66),32(100),28(100).		
174	343(3),342(4),218(6),187(2),174(8),153(6),152(100),151(84),143(10),126(5),124(6),123(13),115(9),109(24),108(12),100(11),93(14),86(10),81(33),80(10),77(8),65(15),63(17),62(11),53(22),52(25),51(38),50(18),39(18),32(8),28(15).	152	$C_8H_8O_3$ requires M=152 vanillin. HO⟨⟩CHO MeO **Figure 71
216	345(3),344(6),285(6),256(6),218(10),217(18),174(10),173(16),171(11),166(36),156(7),155(5),152(7) 151(100),142(3),128(12)127(13),123(20),112(16),108(11),100(17) 99(57),98(11),87(9),83(15),74(10),65(12),64(11),59(11),55(14),54(25),52(12),45(8),44(22),40(100),39(12),33(22),32(100),28(36).	166	$C_9H_{10}O_3$ requires M=166 acetovanillone. CH_3 C=O OMe OH **Figure 72
217	344(3),285(4),256(4),218(5),217(12),174(5),173(8),171(6),166(25),151(70),99(40),40(100),32(100).		

*For unidentified components only ions with a relative abundance greater than 30% are quoted for m/e less than 150.
**Structure assigned on basis of comparison with mass spectrum of authentic compound(figure 38 and 39).

Figure (71) acetovanillone from Konin Patnów.

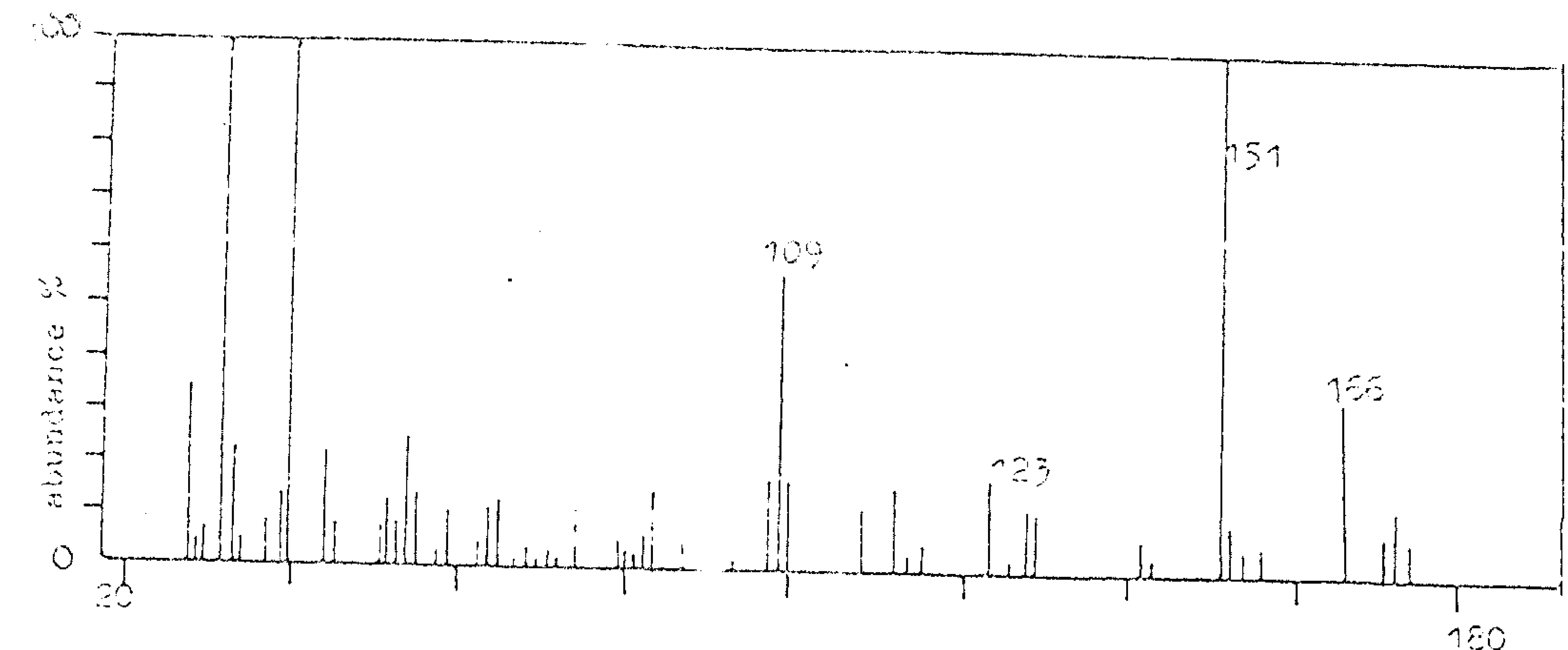


Figure (72) vanillin from Konin Patnów.

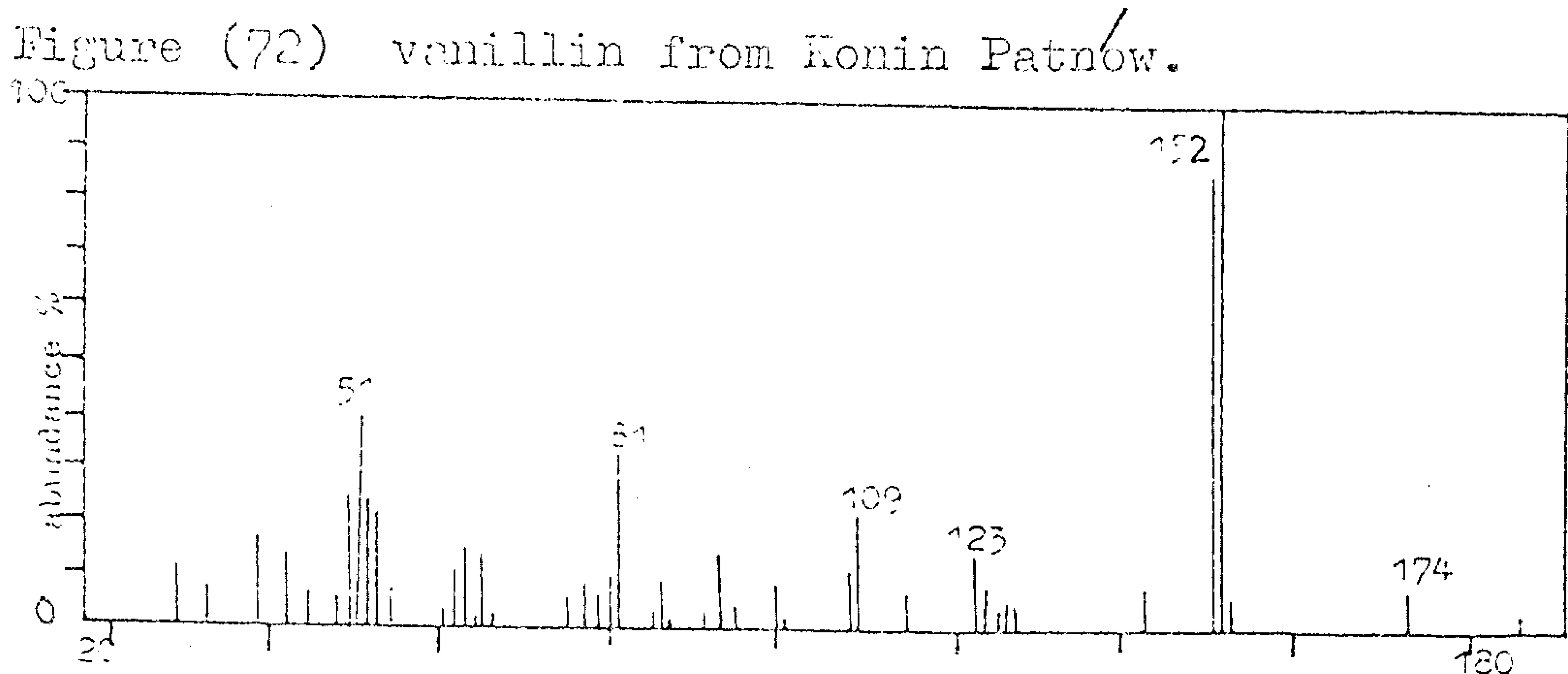


Table 22.

G.C./M.S. of Oxidation Products from Lignin of Hengistbury Coal.

A.E.I. M.S. 30 double focusing mass spectrometer integrated with a Pye Unicam model 104 G.C. (5% P.E.G.A. on Diatomite CLQ).

Retention Time(min.)	Mass* Spectrum m/e (relative abundance%)	M^+	Assigned molecular formulae.
25	83(68),70(34),55(100),41(45), 28(4[illegible]).		
32	97(50),73(62),71(85),57(36), 55(100),43(60),28(35).		
36	113(32),97(44),73(74),71(76), 58(42),55(96),43(100),41(58).		
47	98(46),57(100),41(35).		
54	145(30),112(36),111(38),90(30), 82(36),57(100),56(33),54(31).		
88	122(31),121(38),83(32),73(78), 69(72),65(30),55(100),37(43).		
95	123(11),122(88),121(100),105(16), 97(8),94(6),93(55),92(4),83(16), 81(3),79(3),77(14),76(2),75(42), 73(20),69(30),66(14),65(73),64 (12),63(23),62(11),61(6),55(33), 53(9),51(16),50(15),49(5),47(7), 40(1),39(40),38(11),37(7),32(30).	122	$C_7H_6O_2$ requires M=122 p-hydroxybenz-aldehyde HO–C6H4–CHO **

*For unidentified components only ions with a relative abundance greater than 30% are quoted for m/e less than 150.
**Structure assigned on basis of comparison with mass spectrum of authentic compound(figure 37).

Table 22 cont.

Retention Time(min.)	Mass* Spectrum m/e (relative abundance%)	M+	Assigned molecular formulae.
125	153(8),152(98),151(93),148(10),147(6),140(4),137(11),133(15),132(12),131(100),124(3),120(7),115(2),111(1),110(8),109(30),108(15),104(29),103(65),102(21),99(3),96(5),91(6),89(11),83(18),81(57),77(30),72(4),71(6[illegible]),70(11),63(6),57(2),53(12),52(33),51(44),50(16),49(3),36(6).	152	$C_8H_8O_3$ requires M=152 vanillin HO-⟨ring⟩-CHO MeO **
128	167(8),166(56),152(23),151(36),136(8),123(13),121(5),108(11),95(3),92(6),80(2),77(12),66(9),65(12),55(2),52(11),51(3),50(1),43(24),41(6),32(6).	166	$C_9H_{10}O_3$ requires M=166 acetovanillone CH_3 C=O ⟨ring⟩ OMe OH **
282	183(7),182(26),181(12),167(15),153(2), 139(13),135(3),123(1),111(10),96(8),81(5),79(6),69(4),65(5),54(7),53(4),52(3),52(6),51(2),40(7),39(10),38(4),32(12),28(40).	182	$C_9H_{10}O_4$ requires M=182 syringic aldehyde MeO HO-⟨ring⟩-CHO MeO **
296	85(40),71(66),57(100),43(58),41(38).		

*For unidentified components only ions with a relative abundance greater than 30% are quoted for m/e less than 150.
**Structures assigned on basis of comparison with mass spectrum of authentic compound(figures 38,39 and 40).

The oxidation products from a bituminous coal (Daw Mill Colliery, S. Midland Coalfield) were found to contain trace amounts of syringic aldehyde. Other bituminous coals which were examined were also found to contain syringic aldehyde (table 19) and will be discussed subsequently. In the Daw Mill coal, vanillin made up the largest proportion of the aldehydes (65.4% of the total aldehyde content) suggesting that this coal was derived from plants containing largely a guaiacyl type of lignin. Hayatsu, Winans, McBeth, Scott, Moore and Sadier (1979) found no methoxyl groups in the oxidation products from the bituminous coals that they examined using the cupric oxide method. However syringyl units have been reported by Bimer, Given and Raj (1978) from the oxidation of vitrinite coals with performic acid. To our knowledge no syringic aldehyde has been previously reported from bituminous coals using the cupric oxide method.

No syringic aldehyde was detected from the oxidation products of the anthracite (Bettws, S. Wales Coalfield) that was examined in this work. Vanillin and p-hydroxybenzaldehyde were detected, vanillin being present as the major aldehyde (88.3% of the total aldehyde content). Again this may be indicative of plants with a guaiacyl type lignin originally being present as the major type of vegetation.

Table 19 lists the lignin oxidation products obtained from the bituminous coals of the Middle Coal Measures of the British Carboniferous. No syringic aldehyde was found to be present in the bright coals which contained a high proportion of the Lycospora - type miospores, table 18. Infact p-hydroxybenzaldehyde was found to be the major phenolic aldehyde in the bright coals (an average of 86.3% of the total aldehyde content).

The dull coals containing a high proportion of the Densosporites - type miospores (table 18) shows rather different proportions of lignin derivatives. The three dull coals examined, contained considerable amounts of syringic aldehyde, an average of 10.1 µg/gm, especially as these

coals are ~ 300 million years old. Infact the ratio of vanillin to syringic aldehyde approaches 1:1 in the Peckfield coal sample.

The coal from Flockton Orgreave contained the largest proportion of vanillin (54.4% of the total aldehyde content) from the samples examined from the Middle Coal Measures.

In all three dull coals examined (where the vegetation was thought to be a raised bog) the p-hydroxybenzaldehyde content was much smaller than that obtained from the bright coals (where the vegetation was submerged). An average of 21.2% of the total aldehyde content was observed in the dull coals compared to an average of 86.3% of the total aldehyde content in the bright coals.

The bright coals containing the Lycospora - type miospores are thought to represent the spores from arborescent lycopods such as Lepidodendron. Chaloner (1953) found miospores from three species of Lepidostrobus, The cone bearing structure of Lepidodendron. All the miospores belonged to the genus Lycospora of Schopf, Wilson and Bentall (1944). In 1954 Felix also reported the presence of Lycospora - type miospores in certain American arborescent lepidodendrons.

Densosporites - type miospores are thought to be produced by herbaceous lycopods such as Selaginellites. Chaloner (1958) reported the presence of the Densosporites - type miospores from a cone Selaginellites canonbiensis belonging to a herbaceous lycopod and suggested that it may have been the dominant component of the vegetation which gave rise to the Densosporites of the rich coals of the durain type. Densosporites have also been reported from Porostrobus canonbiensis, a cone belonging to a minute herbaceous lycopod (Chaloner and Muir, 1968).

These coals were oxidized to see if lignin oxidation products could be detected and if they could be used as additional evidence to test the earlier suggestions of Chaloner (1953, 1958) and Felix (1954).

Four arborescent lycopods were examined for lignin oxidation products from extremely compressed material (plates (68) to (70)) and included two specimens of Sigillaria ovata, one specimen of Lepidodendron and one specimen of Lepidophlois. All the specimens were found to contain p-hydroxybenzaldehyde, vanillin and acetovanillone in small, often trace amounts, as recorded in table 20. Syringic aldehyde was detected in both specimens of Sigillaria ovata only. The miospores of Sigillarian affinity are however believed to be of the Crassispora - type (Smith, 1962). Since both Lepidodendron and Lepidophlois have little secondary xylem development, the lignin derivatives obtained are likely to have come from the extensive development of the periderm. Within the periderm are fibres and sclerotic cells (Taylor, 1981) and cells of the cortex possess anastromising bands of fibres. In the present work (Chapter 3) fibres were found on oxidation to give lignin residues.

As the arborescent lycopods Lepidodendron and Lepidophlois did not give detectable amounts of syringic aldehyde it can be suggested that the bright coals, which also contain no syringic aldehyde, are made up of these candidates. Unfortunately compression material of Selaginellites, the herbaceous lycopods, were not available in sufficient quantities to oxidize their coalified remains. However their direct descendents the present day Selaginellas do give syringic aldehyde in considerable amounts as shown in table 12.

As syringic aldehyde units were present in coals which are ~300 million years old it seems possible that the Selaginellites, ancestors of the Selaginellas would contain this unit and therefore one can suggest that they contributed to its presence in the dull coals.

Mass Spectral Interpretations.

The mass spectra obtained from the oxidation of Lepidodendron are presented in table 23 and figures (73) to (75).

Table 23.

G.C./M.S. of Oxidation Products from Lignin of *Lepidodendron*.
A.E.I. M.S. 30 double focusing mass spectrometer integrated with a Pye Unicam model 104 G.C. (5% P.E.G.A. on Diatomite CLQ).

Retention Time(min.)	Mass* Spectrum m/e (relative abundance%)	M^+	Assigned molecular formulae.
136	207(9),181(6),169(4),120(100), 91(97),77(30),73(70),65(50),59 (32),29(32).		
158	207(4),169(5),153(7),152(67), 151(68),137(7),136(19),135(4), 123(26),122(25),121(100),120 (5),110(2),109(28),108(12),107 (7),95(9),93(77),92(6),83(5), 82(9),81(53),79(15),77(17),75 (8),70(8),67(8),66(13),65(77), 63(38),62(12),61(8),55(22),53 (32),52(28),51(34),50(28),49(7), 42(4),39(29),38(6),33(4),31(2), 29(19).	152	$C_8H_8O_3$ requires M=152 vanillin. HO⟨ring⟩CHO, MeO **Figure 74
164	167(4),166(42),153(3),152(4), 151(100),124(11),123(42),120 (5),108(20),95(5),93(4),80(13), 79(13),77(22),67(16),66(11), 65(21),63(16),56(20),54(12), 53(37),52(35),51(13),43(37), 41(10),29(51).	166	$C_9H_{10}O_3$ requires M=166 acetovanillone. CH_3–C=O on ring with OMe, OH **Figure 75
268	149(100),76(32),57(50),56(49), 41(42).		

*For unidentified components only ions with a relative abundance greater than 30% are quoted for m/e less than 150.
**Structure assigned on basis of comparison with mass spectrum of authentic compound(figure 38 and 39).

Figure (73)

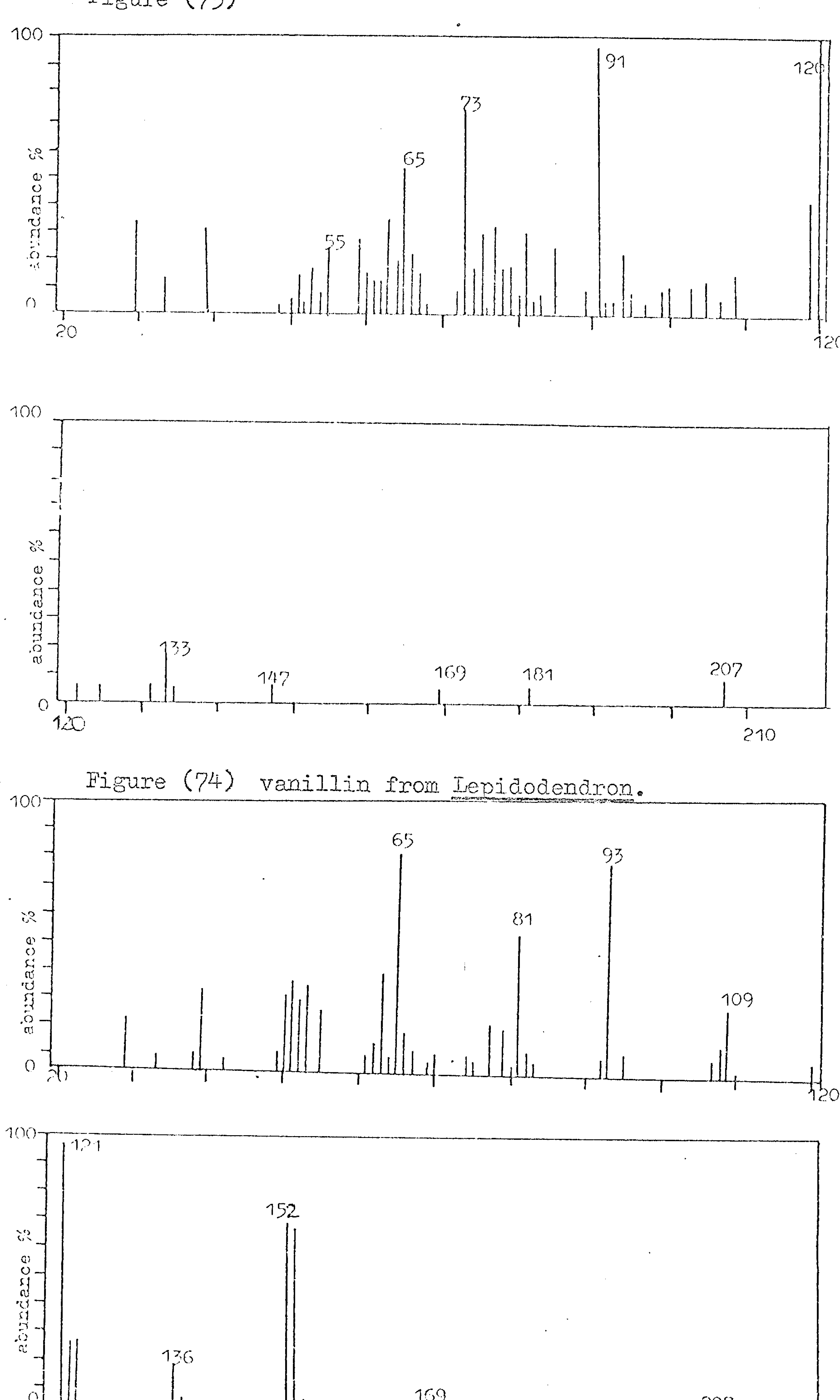
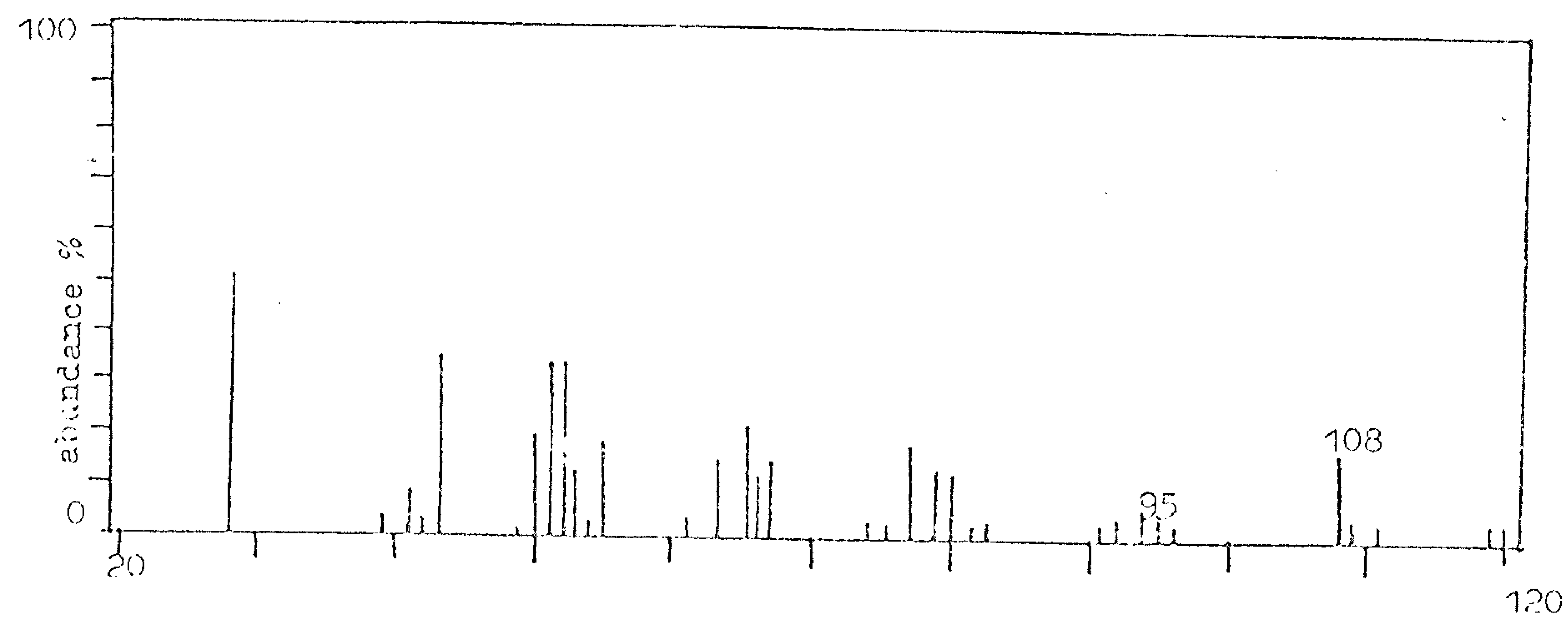
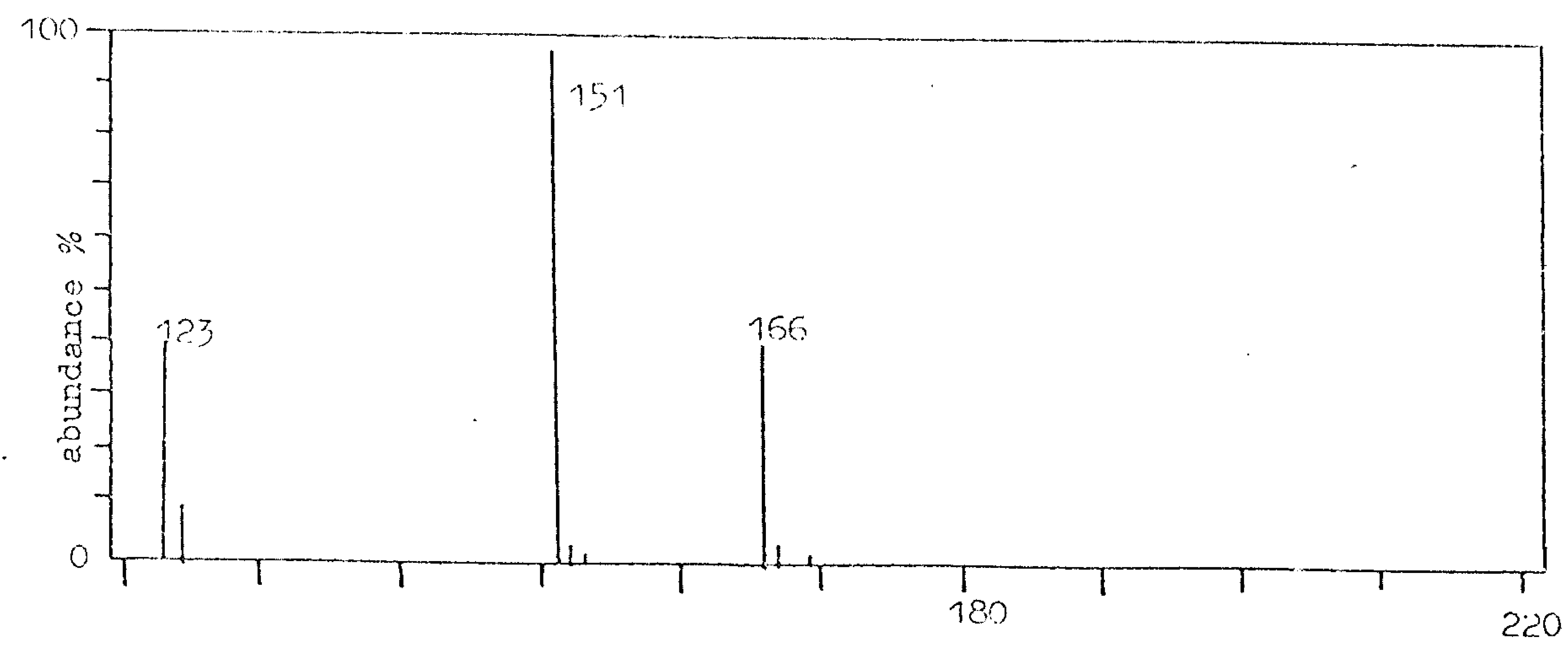


Figure (75) acetovanillone from Lepidodendron.





Certain other fossils were oxidized using the cupric oxide method to see if any lignin oxidation products could be detected from such compressed material. The results are tabulated in table 20. Specimens were limited because more than 2gms of fossil compression material was needed which entailed finding an abundance of that compression fossil and then destroying it.

The Cretaceous material from the Isle of Wight Pseudofrenelopsis parceromosa is a leafy shoot, plate (64). It is thought that these shoots belong to the wood of the Chierolepidaceae, both fossils being found from the same locality. They are thought to be shrubby plants rather than tall forest trees (Watson, 1977). In the oxidations from the wood and the shoots no syringic aldehyde was detected. Present day conifers (tables 2 and 3) were found to possess small amounts of syringic aldehyde as did some fossil wood from the Quaternary and Tertiary. In these compression fossils one would suggest that if only a small proportion of syringic aldehyde was present it would be demethoxylated or would be preferentially destroyed. As these fossil plants are regarded as coniferous plants one would still expect there to be a substantial amount of vanillin. Vanillin was present in the extremely compressed wood as 49.8% of the total aldehyde content. However the young shoot had only 9.7% of the total aldehyde content as vanillin, p-hydroxybenzaldehyde being the major aldehyde. This is not likely to be due to demethoxylation proceeding faster in the young shoot because both shoots and wood are from the same site. It is more likely that the shoot, because it is immature had more p-hydroxybenzaldehyde than that of the more mature wood. A similar case was encountered in Chapter 3.

The leaves of a number of Jurassic fossils were oxidized (Ginkgo huttoni a gymnosperm, Ptilophyllum pectinoides a Bennettitales and Pachypteris lanceolata

a Pteridosperm). All were leaf compressions, largely composed of cutin but some lignin did seem to be retained within the leaf compressions because trace amounts of the lignin derivatives vanillin, acetovanillone and p-hydroxybenzaldehyde were detected in the oxidation products. No syringic aldehyde was detected. This is perhaps not so surprising because when the leaves of contemporary cycads were oxidized only small amounts of syringic aldehyde were present and no syringic aldehyde was detected in the leaves of the conifer Pinus sylvestris (Chapter 3).

It is clear that lignin derivatives may be detected in fossil compressions of plants. However in my view links between the fossil residues and living plant residues are questionable because in very compressed material chemical changes have occurred to such an extent that the original proportions of the aldehydes have been altered out of all recognition. Some use of lignin composition can be made in a biochemical context to supplement morphological and anatomical studies as shown using the bright and dull coals from the Middle Coal Measures.

Palaeobiochemistry has been thought to provide evidence in the controversy surrounding the land plants and their origins. This controversy has resulted in two schools of thought. One being that land plants existed in the Precambrian (600 million years ago) and includes several reports of putative land plants (Lister, 1970; and Gray and Boucot, 1971). The opposing view is favoured by Banks (1975a and 1975b) who proposed that the land plants first appeared in the late Silurian (400 million years ago). The largest group of land vascular plants are characterized by a vascular system containing tracheids which are thought to be unique to vascular plants. In critical reviews by Banks (1975a and 1975b) evidence from microfossils such as 'tubes'

(regarded as tracheids by other authors; Gray and Boucot, 1977 and Niklas and Pratt, 1980) should not be considered as evidence for vascular plants. Isolated tracheids ('tubes') are only acceptable as evidence if some biochemical evidence, a lignin constituent, supports their assignment as tracheids. Because these 'tubes' are open ended and only their gross morphology is like tracheids Banks (1975a, 1975b) has suggested that they originate from algae, bryophytes, graptolites or crinoids. Niklas and Pratt (1980) used chemical analysis on banded 'tubes' with annular spiral ribbing from the early Silurian. They reported the presence of p-hydroxybenzaldehyde and vanillin from these 'tubes' using the alkaline nitrobenzene oxidation method. They concluded that these 'tubes' because of their lignin chemistry may have been functionally anologous to hydroids or tracheids of early land vascular plants. Niklas and Pratt (1980) discount reports of lignin or pseudolignin occurring in mosses due to the work of Erickson and Miksche (1974) and subsequently conclude that these 'tubes' are not from non-vascular land plants. However many authors (Lindberg and Theander, 1952; Farmer and Morrison, 1964; Nilsson and Tottmar, 1967 and Bland, Logan, Menshun and Sternhell, 1968) some of whom used alkaline nitrobenzene as the oxidant, all found lignin derivatives present in the mosses examined. In the present work lignin oxidation products were found to occur with the mosses and liverworts that were examined (Chapter 4) using the cupric oxide method. Hence the presence of lignin in the 'tubes' does not necessarily imply that the 'tubes' come from vascular plants. Since fibres contain lignin derivatives (Chapter 3) the determination of the lignin components do not necessarily lead to the conclusion that the 'tubes' are from the vascular system of land plants.

General Conclusions.

1. Lignin oxidation products from a variety of living and fossil plants were obtained using the alkaline cupric oxide method. The phenolic aldehydes and ketones obtained from the lignin oxidations can be successfully separated using gas chromatography. The prefered and recommended gas chromatography system is a 5% Poly-(Diethylene Glycol Succinate) on Diatomite CLQ 80-100 mesh column.

2. Gas chromatography/mass spectrometry was used to verify the structures of certain of the lignin oxidation products obtained both from living plant material and from fossil plant material.

3. Vanillin was the major phenolic aldehyde obtained from the oxidation products of the lignin from the contemporary gymnosperms, an average of 94% of the total aldehyde content. Small amounts of p-hydroxybenzaldehyde were detected ranging from 0.3% to 13% of the total aldehyde content in the gymnosperms examined in this work. Syringic aldehyde, previously reported spasmodically from certain gymnosperms was found to be present in small amounts ranging from 0.2% to 5% of the total aldehyde content in all the gymnosperms examined here. This is attributed to the sensitive techniques employed.

4. p-Hydroxybenzaldehyde was observed as a lignin oxidation product from the woodmeal of a *Bambusa* species and *Sabal palmetto*. In the monocotyledon fibres that were examined no p-hydroxybenzaldehyde was detected. More syringic aldehyde was present in the fibres of the monocotyledons than in the woodmeal of the monocotyledons examined. It appears that within the monocotyledons the fibres contain different ratios of the aldehydes to the woodmeals.

5. Small amounts of p-hydroxybenzaldehyde have been detected from the oxidation products of the twigs of the dicotyledons but it was not detected with the mature wood of dicotyledons. The suggestion is made that an enzyme such as tyrase may be preferentially used in immature cells during lignification converting tyrosine to p-coumaric acid.

6. All three types of lignin nuclei were observed from the barks that were examined. Lignification is due to the presence of sclereids in the bark tissue. p-Hydroxybenzaldehyde was found in greater amounts with the bark tissue than with woodmeal from the same species. Syringic aldehyde was found to be high (10.5% of the total aldehyde content) in the gymnospermous bark Sciadopitys verticillata.

7. In the literature a correlation has been drawn between the presence of syringic aldehyde and the occurrence of vessels in the xylem of these plants. In this thesis the shortcomings of this hypothesis have been pointed out. Monocotyledonous fibres, for instance, have given the highest yields of syringic aldehyde. Selaginella wildenovii belonging to the Heterophyllum in which no vessels have been reported had substantial amounts of syringic aldehyde (58% of the total aldehyde content) and the gymnosperms containing tracheids, not vessels, yielded small amounts of syringic aldehyde. The suggestion that fibres do contain syringic aldehyde has been made.

8. Vanillin was found to be the major lignin oxidation product of the tree ferns (an average of 97.9% of the total aldehyde content). Trace amounts of syringic aldehyde were detected. The tree fern Dennstaedtia bipinnata was found to be the exception and contained a significant proportion of the aldehyde syringic aldehyde (24.3% of the total aldehyde content).

9. Phenolic aldehydes , including syringic aldehyde, were

obtained from the lignin oxidations of mosses and liverworts examined in this work. These plants are non-vascular land plants and the suggestion that these lignin oxidation products have come from lignified fibres is made.

10. Chemical changes either demethoxylation or the preferential destruction of the phenolic aldehydes appears to occur in fossil wood when the lignin oxidation products of fossil and contemporary wood are compared. The lignin oxidation products of the Quaternary woods changed slightly in comparison to their contemporary woods. Certain samples from the Quaternary were found to give higher yields of aldehydes when their lignin was oxidized than the contemporary woods. The lignin derivatives obtained from the Tertiary woods showed a decrease in the yield of aldehydes and ketones when compared to their contemporary woods. An increase in p-hydroxybenzaldehyde was observed in both the coniferous and angiospermous woods of the Tertiary presumably due to chemical changes of the phenolic aldehydes. Surprisingly small amounts of syringic aldehyde were observed with some Tertiary coniferous woods. Both the Quaternary and Tertiary woods could be differentiated into coniferous or angiospermous woods on the basis of their lignin oxidation products. The coniferous woods retained a high proportion of vanillin whereas syringic aldehyde and vanillin were the major oxidation products obtained from the angiospermous woods.

11. All three lignin units were observed from the brown coals and certain bituminous coals examined in this work. Syringic aldehyde still remained as an oxidation product in coal of $\sim$ 300 million years old. Vanillin and p-hydroxybenzaldehyde were observed as lignin oxidation products from the anthracite coal. One can speculate on their vegetational origin using the phenolic aldehydes

obtained, but chemical change alters the original ratios and this must be taken into consideration. In my opinion this renders the speculations as dubious.

12. Lignin derivatives can be used as supporting evidence for anatomical and morphological studies as shown by the studies on bright and dull bituminous coals.

13. Compressed fossils yielded very small amounts of lignin oxidation products as shown in table 20. The proportions of these aldehydes must alter significantly due to chemical change induced by heat, pressure, time, weathering and microorganisms.

14. Lignin derivatives are often used as biological markers in geochemistry and subsequently suggestions have been made by authors (Leo and Barghoorn, 1970; Wayne, Gardiner and Menzel, 1974 and Hedges and Parker, 1976) as to the original type of vegetation usually with reference to higher plants. However the contribution that lower plants may make should not be ignored.

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APPENDIX I

External Standards.

3% OV 101 Column 1.

p-OH	VAN.	ACETO.	SYR.	
3 mg/ml	10 mg/ml	10 mg/ml	10 mg/ml	
53620	256100	282450	202200	
51210	247200	296200	216900	
53420	232600	266320	215300	
54690	237800	277900	196200	
51620	248600	308300	193500	units
52710	246600	302600	204600	
53600	265000	283000	225500	
52930	264200	285700	222600	
52320	267300	292500	189200	

3% OV 101 Column 2, Calibration 1.

p-OH	VAN.	ACETO.	SYR.	
2.5 mg/ml	2.5 mg/ml	2.5 mg/ml	2.5 mg/ml	
665400	992300	785600	514300	
656500	1004300	762750	536400	
643200	1108900	775400	523400	
634500	998900	768900	517800	
652300	1006700	776500	534500	
645400	1055600	783600	542310	units
634200	989950	768900	537800	
624500	1007800	775400	516500	
645300	997800	763100	552300	
663200	987800	754500	534500	
653200	1006700	745600	543200	
665600	1067820	723780	523400	

3% OV 101 Column 2,Calibration 1. (continued)

p-OH	VAN.	ACETO.	SYR.	
634500	1106700	735600	542200	
656600	1005600	756700	553280	
647800	996700	765640	524600	
658700	987860	754600	513400	
665700	1003450	768900	536500	units
635200	1006700	758900	543400	
643200	995430	763400	518900	
621300	986700	758900	524500	
654600	997860	734500	534200	
617800	995600	768900	512200	

3% OV 101 Column 2,Calibration 2.

p-OH	VAN.	ACETO.	SYR.	
1 mg/ml	1 mg/ml	1 mg/ml	1 mg/ml	
324900	604500	354500	273500	
336700	637800	336600	284600	
319800	632500	327800	267500	
306300	629500	326700	265700	units
316700	654800	316500	254600	
326800	623100	338900	268900	
337500	638900	315690	274500	
328600	627800	326700	286700	
335600	643500	364500	276800	

5% Pdegs Column 1.

p-OH	VAN.	ACETO.	SYR.	
3 mg/ml	3 mg/ml	3 mg/ml	3 mg/ml	
1604800	1556700	1564500	806700	
1639800	1467400	1548900	855400	
1614300	1584500	1532100	835600	
1627400	1564700	1523800	825600	
1605600	1539800	1536400	814200	
1635400	1574300	1526900	846500	
1598900	1578400	1575800	832800	
1616700	1546700	1564000	815800	
1618500	1538600	1554700	826400	
1608900	1506700	1549800	835600	
1595700	1497900	1544300	842200	
1576700	1514500	1538900	818900	units
1536700	1506700	1527800	798900	
1598900	1568700	1559800	826700	
1584500	1548900	1543700	815800	
1575600	1536700	1546400	809700	
1565000	1553700	1533400	806600	
1546300	1568900	1546200	836600	
1538800	1547700	1515600	825500	
1586800	1564700	1574800	816600	
1547800	1555800	1526900	843800	
1595700	1563800	1514300	836700	
1567800	1532200	1503200	794500	
1557300	1516700	1496700	815600	

5% Pdegs Column 2.

p-OH	VAN.	ACETO.	SYR.
1 mg/ml	1 mg/ml	1 mg/ml	1 mg/ml
554600	535300	528900	365800
553600	534800	525690	357500
543800	541200	512300	337800
547400	554800	516900	336700
553200	548300	527400	357500
547900	536400	516500	367300
547300	528400	506700	348800
536900	521100	499800	358900
534700	536800	513800	348200
528500	528400	498700	336400
539400	515900	495600	364500 units
524600	505600	517800	353700
564700	538700	537500	367800
554200	526400	526500	347600
534600	536700	514300	335900
536700	527500	524300	345500
527400	506700	498900	329900
567400	548300	534700	365700
554800	539800	533250	358300
546300	534500	542700	327800
536300	529800	513900	336400

5% Pdegs Column 3.

p-OH	VAN.	ACETO.	SYR.	
1 mg/ml	1 mg/ml	1 mg/ml	1 mg/ml	
.564700	524300	501900	463500	
563800	521300	500100	457800	
554200	517400	494500	463400	
537800	497800	485690	453400	
526700	487400	479900	445200	
515500	518900	503700	457800	
516400	504980	497800	432700	units
507800	517600	485500	453800	
497800	503100	496700	437500	
509400	513200	487500	426700	
498800	476300	509400	452900	
554600	523800	517300	475600	
538900	537200	503800	486500	
572200	527400	498000	457600	

APPENDIX 2.

Interpretations of fragmentation patterns.

p-Hydroxybenzaldehyde.

CHO]$^{+\cdot}$ (benzene ring) OH
M^+= 122(98%)

—$-H^\cdot$→

$\overset{+}{C}$=O (benzene ring) OH
m/e=121(100%)

↓ $-\dot{C}HO$ (from m/e 122); −CO (from m/e 121)

+ (benzene ring) OH
m/e=93(56%)

Vanillin.

CHO]$^{+\cdot}$ (benzene ring) OCH_3 OH
M^+=152(98%)

—$-\dot{C}H_3$→

CHO]$^{+\cdot}$ (benzene ring) O OH
m/e=137(8%)

↓ $-H^\cdot$

$\overset{+}{C}$=O (benzene ring) OCH_3 OH
m/e=151(100%)

↘ −CHO (from M^+)

+ (benzene ring) OCH_3 OH
m/e=123(18%)

—$-\dot{C}H_3$→ (from m/e 123); $-\dot{C}HO$ (from m/e 137)

(benzene ring) O OH]$^{+\cdot}$
m/e=108(14%)

Acetovanillone.

$M^{+} = 166 (46\%)$ —($-\dot{C}H_3$)→ $m/e = 151 (100\%)$

$M^{+} = 166 (46\%)$ —($-CH_3\dot{C}O$, $m/e = 43 (14\%)$)→ $m/e = 123 (20\%)$

$m/e = 151 (100\%)$ —($-CO$)→ $m/e = 123 (20\%)$

$m/e = 123 (20\%)$ —($-\dot{C}H_3$)→ $m/e = 108 (12\%)$

Syringic aldehyde.

$M^{+} = 182 (100\%)$ —($-\dot{C}HO$)→ $m/e = 153 (9\%)$

$M^{+} = 182 (100\%)$ —($-\dot{C}H_3$)→ $m/e = 167 (14\%)$

$M^{+} = 182 (100\%)$ —($-H^{\bullet}$)→ $m/e = 151 (57\%)$

o or p methoxyl phenol.

OH, OCH_3 (M^{+}=124) —$-CH_3$→ OH, O (m/e=109)

M^{+}=124 —$-CH_3\dot{O}$→ OH, + (m/e=93)

4 ethyl resorcinol.

C_2H_5, OH, OH (M^{+}=138) —$-\dot{C}_2H_5$→ OH, OH (m/e=109)

m/e=109 —$-OH$→ OH (m/e=92)

Acetosyringone.

$M^{+}=196$ —($-\dot{C}H_3$)→ $m/e=181$

$M^{+}=196$ —($-CH_3\dot{C}O$, $m/e=43$)→ $m/e=153$

$M^{+}=196$ —($-C_8H_9O_3$)→ CH_3CO^{+} $m/e=43$

$m/e=181$ —($-CO$)→ $m/e=153$

$m/e=153$ —($-\dot{C}H_3$)→ $m/e=138$

$m/e=138$ —($-\dot{C}H_3$)→ $m/e=123$

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