Phytochemistry, 1965, Vol. 4, pp. 57 to 66. Pergamon Press Ltd. Printed in England

PHENOLIC ACIDS AND LIGNINS IN THE LYCOPODIALES*

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(*Received* 7 *July* 1964)

Abstract—Twenty-one species and varieties of *Lycopodium* have been examined for phenolic acids and for phenolic aldehydes, ketones and acids obtained on ethanolysis or alkaline oxidation of their extracted wood-meals. *p*-Hydroxybenzoic, vanillic, *p*-coumaric and ferulic acids were identified in phenolic acid fractions and *p*-hydroxybenzaldehyde, vanillin, acetovanillone, vanilloyl methyl ketone, α-hydroxy- and α-ethoxypropio-vanillone were identified among the products of lignin degradation. Those species which are included in the genera *Lycopodium* and *Diphasium*, as proposed by Rothmaler, were found to yield syringic acid in the ethanol-soluble fraction and on degradation of lignin whereas species included in the genera *Huperzia* and *Lepidotis* did not. Syringaldehyde was not obtained on alkaline oxidation of the lignin of any species thus indicating that *Lycopodium* s. lat. has essentially a gymnospermous type of lignin. Sucrose was identified as the major carbohydrate in ethanolic extracts.

INTRODUCTION

CHEMICAL investigations of the lycopods have centred around the alkaloids first discovered in them by Bödeker[1] in 1881. Although these botanically interesting plants, of which there are possibly 483 species,[2] are common elements of the flora of many parts of the world, including Europe and North America, they have not been examined extensively from either a biochemical or a chemical standpoint. Even the sugars of *Lycopodium* do not appear to have been identified (see Hegnauer[3]).

A few studies have been made of the phenolic constituents. The spores of *L. clavatum* were shown to contain dihydrocaffeic acid[4] and ferulic and vanillic acids have been isolated from *L. clavatum*, *L. annotinum* and *L. selago*.[5] Eight species examined by Ibrahim *et al.*,[6] were found to contain vanillic, *p*-coumaric and ferulic acids and some, in addition, contained syringic acid. Vanillin and *p*-hydroxybenzaldehyde have been identified as products of alkaline nitrobenzene oxidation of lignin from *L. complanatum*.[7]

A correlation has been demonstrated in plants between the presence of syringic and/or sinapic acids in ethanolic extracts and the ability to synthesize syringyl lignin.[6] Angiosperms, for example, contain derivatives of syringic and sinapic acids and give syringaldehyde as well as vanillin on alkaline nitrobenzene oxidation of their lignins. They are distinguished from gymnosperms which, with few exceptions, do not contain these acids and which give largely vanillin on similar treatment of their lignin. The restricted distribution of syringic acid within the lycopods as reported by Ibrahim *et al.*,[6] is therefore of interest and has prompted us to study, in more detail, the phenolic constituents of *Lycopodium*. We have been

*Issued as NRC No. 8239.

[1] K. BÖDEKER, *Ann.* **208**, 363 (1881).
[2] W. HERTER, *Index Lycopodiorum*, Montevideo, Uruguay (1949).
[3] R. HEGNAUER, in *Chemotaxonomie der Pflanzen*, Band 1, Birkhäuser Verlag, Basel und Stuttgart (1962).
[4] F. ZETSCHE and K. HUGGLER, *Helv. Chim. Acta* **10**, 472 (1927).
[5] O. ACHMATOWICS and F. WERNER-ZAMOJSKA, *Roczniki Chem.* **32**, 1127 (1958).
[6] R. K. IBRAHIM, G. H. N. TOWERS and R. D. GIBBS, *J. Linn. Soc.* **58**, 223 (1962).
[7] G. H. N. TOWERS and R. D. GIBBS, *Nature* **172**, 25 (1953).

TABLE 1. SOURCES OF PLANT MATERIALS[a]

Plant	Source	Collector
Lycopodium s. lat.		
L. alopecuroides L.	Alabama, U.S.A.	R. L. Alston
L. annotinum L.	Nova Scotia, Can.	W. S. G. Maass
	Quebec, Can.	G. H. N. Towers
	Wisconsin, U.S.A.	S. Kawano
	Akershus, Norway	W. S. G. Maass
	Helsinki, Finland	W. S. G. Maass
L. annotinum L. var. *acrifolium* Fern.	Nova Scotia, Can.	W. S. G. Maass
	Vermont, U.S.A.	S. Kawano
L. annotinum L. var. *pungens* Desv.	Nova Scotia, Can.	W. S. G. Maass
	Nova Scotia, Can.	W. S. G. Maass
L. carolinianum L. var *meridonale* (Underw. & Lloyd) Nessel	Trinidad, B.W.I.	F. W. Cope
L. cernuum L.	Trinidad, B.W.I.	F. W. Cope
	Sierra Leone	J. K. Morton
	Malaya	E. A. Turnau
L. cernuum L. var. *crassifolium*	Hawaii, U.S.A.	O. Degener
L. clavatum L.	Nova Scotia, Can.	W. S. G. Maass
	Quebec, Can.	G. H. N. Towers
	Brit. Columbia, Can.	A. G. O. Bahr
	Wisconsin, U.S.A.	S. Kawano
	Vermont, U.S.A.	S. Kawano
	Akershus, Norway	W. S. G. Maass
L. clavatum L. var. *divaricatum* (Wall. ex Grev. & Hk.) Racib.	Malaya	E. A. Turnau
L. complanatum L.	Nova Scotia, Can.	W. S. G. Maass
	Quebec, Can.	G. H. N. Towers
	Malaya	E. A. Turnau
L. flabelliforme (Fern.) Blanchard	Nova Scotia, Can.	W. S. G. Maass
L. inundatum L.	Vermont, U.S.A.	S. Kawano
L. inundatum L. var. *bigelovii* Tuckerm.	Nova Scotia, Can.	W. S. G. Maass
L. lucidulum Michx.	Nova Scotia, Can.	W. S. G. Maass
	Quebec, Can.	G. H. N. Towers
	Vermont, U.S.A.	S. Kawano
L. obscurum L.	Nova Scotia, Can.	W. S. G. Maass
	Quebec, Can.	G. H. N. Towers
	Vermont, U.S.A.	S. Kawano
L. obscurum L. var. *dendroideum* (Michx.) D.C.	Quebec, Can.	J. Kucymak
L. phlegmaria L.	Malaya	E. A. Turnau
L. sabinaefolium Willd.	Nova Scotia, Can.	W. S. G. Maass
	Quebec, Can.	J. Kucymak
L. saururus Lamarck	Mt. Kenya, Kenya	B. Verdcourt
L. selago (L.) Bernh.	Nova Scotia, Can.	W. S. G. Maass
	Vermont, U.S.A.	S. Kawano
L. tristachyum Pursh	Nova Scotia, Can.	W. S. G. Maass
Selaginella		
S. kraussiana A. Braun	Received from McGill University greenhouse under this name	

[a] Voucher specimens of these species have been retained

particularly interested in discovering whether some species do, in fact, synthesize syringyl lignin and whether the distribution of syringyl constituents in this group is of taxonomic significance. The species examined and the sources from which they were obtained are shown in Table 1.

RESULTS AND DISCUSSION

Before proceeding to discuss the phenolics in *Lycopodium* it is worth pointing out that a major component of the ethanol-soluble fraction in many species is sucrose and that it may be obtained easily in crystalline form. In *L. clavatum* it accounted for 11·4% of the fresh weight whereas in *L. obscurum* and *L. lucidulum* it accounted for 6·4% and 5·9% of the fresh weights respectively. Sucrose was identified by chromatography and by its i.r. spectrum which was identical with that of an authentic sample.

TABLE 2. R_f VALUES OF AROMATIC ALDEHYDES AND KETONES ON SILICA GEL G thin-layer plates

Compound	R_f solvent			Colour with 2,4-dinitro-phenylhydrazine
	A	B	C	
p-Hydroxybenzaldehyde	0·21	0·62	0·85	Brown-red
Vanillin	0·40	0·44	0·76	Brick red
Syringaldehyde	0·28	0·28	0·49	Burnt sienna
Acetovanillone	0·32	0·54	0·76	Brick red
Acetosyringone	0·25	0·35	0·52	Brown-red
Vanilloyl methyl ketone	0·40	0·46	0·74	Yellow
α-Hydroxypropiovanillone	0·20	0·42	0·69	Yellow
α-Ethoxypropiovanillone	0·39	0·56	0·78	Yellow
Coniferaldehyde	0·32	0·59	0·78	Brown

Solvents: A. Upper phase 4:1 BuOH:3% NH_4OH
B. 9:1 Benzene:Acetic acid
C. 3:1 Water-saturated isoamyl alcohol:*n*-butanol[8]

Phenolic acids could be detected in the ethanol-soluble fraction only after alkaline or acid hydrolysis. The derivatives of these acids appeared to be labile compounds, probably esters, which were readily hydrolysed when subjected to chromatography on silicic acid using the benzene:acetic acid:solvent system (Table 2). Every species examined yielded ferulic acid as the major phenolic acid and lesser amounts of *p*-coumaric, caffeic, vanillic and *p*-hydroxybenzoic acids. Quantitative estimations of ferulic and *p*-coumaric acids were made with some species in order to discover the range in concentration of these acids. The results are shown in Table 3. Syringic acid was obtained as a minor acid in some species (see Table 7), although it was not always detected in any given species. Thus *L. clavatum* collected from Lac Megantic, Quebec, contained this acid whereas specimens collected elsewhere did not. Similarly, *L. sabinaefolium* from Knowlton, Quebec, yielded syringic acid whereas a sample from Nova Scotia did not. On the other hand all material of *L. obscurum* gave a positive test for this acid. None of the species examined, including those which contained syringic acid, yielded sinapic acid.

[8] K. KRATZL, *Holz als Roh- u. Werkstoff* **19**, 219 (1961).

TABLE 3. YIELDS OF FERULIC AND *p*-COUMARIC ACIDS OBTAINED BY ALKALINE HYDROLYSIS OF THE ETHANOL-SOLUBLE FRACTION AND ETHANOL INSOLUBLE RESIDUES OF VARIOUS SPECIES OF *Lycopodium*

Species	μg/gram			
	Ferulic acid from		*p*-Coumaric acid from	
	sol. fraction*	insol. fraction†	sol. fraction†	insol. fraction†
L. selago	12·5	12·0	22·9	50·0
L. sauruus	5·0	45·0	1·1	5·0
L. cernuum (Trinidad)	16·0	N.D.‡	8·0	N.D.
L. cernuum (Africa)	25·0	N.D.	75·0	N.D.
L. carolinianum var. *meridionale*	93·0	N.D.	0·0	N.D.
L. annotinum var. *acrifolium*	1·71	40·0	N.D.	44·6
L. annotinum	N.D.	0·0	N.D.	2·0
L. inundatum	N.D.	20·0	N.D.	210·0
L. obscuum	N.D.	11·0	N.D.	139·0
L. clavatum	46·6	10·5	33·1	188·0
L. complanatum	74·7	15·0	trace	175·0
L. sabinaefolium	N.D.	3·0	N.D.	52·0

* Fresh wt. basis.
† Extracted dry wt. basis.
‡ N.D.—not determined.

TABLE 4. ALKALINE CUPRIC HYDROXIDE OXIDATION PRODUCTS OF EXTRACTED WOOD-MEAL OF VARIOUS SPECIES OF *Lycopodium*

Species	Compound found				
	p-Hydroxy-benzaldehyde	*p*-Hydroxy-benzoic acid	Syringic acid	*p*-Coumaric acid	Ferulic acid
L. lucidulum	±	±	−	−	+
L. selago	?	−	−	−	+
L. sauruus	?	−	−	−	+
L. phlegmaria	?	±	−	−	+
L. inundatum	±	±	−	−	−
L. inundatum var. *bigelovii*	?	±	−	−	−
L. cernuum	±	−	−	±	±
L. cernuum var. *crassifolium*	±	±	−		±
L. carolinianum var. *meridionale*	?	±	−	−	−
L. annotinum	?	−	+	−	±
L. annotinum var. *acrifolium*	?	−	+	−	±
L. annotinum var. *pungens*	±	+	±	−	+
L. clavatum	−	+	+	−	+
L. clavatum var. *divaricatum*	−	+	−	−	+
L. obscuum	−	+	−	−	+
L. complanatum	?	−	±	−	+
L. flabelliforme	−	+	−	−	±
L. tristachyum	±	+	+	−	±
L. sabinaefolium	−	±	−	±	±

* Vanillin, acetovanillone and vanillic acid were found in all these species.
± = Detected on chromatograms, − = not detected on chromatograms.
? = Doubtfully present on chromatograms.

In addition to the well-known phenolic acids a number of unidentified phenolic compounds were present on chromatograms, species such as *L. clavatum* and *L. sabinaefolium* being particularly rich in these.

Ferulic, *p*-coumaric and vanillic acids were also obtained on alkaline hydrolysis of ethanol-insoluble residues from all species and yields of the first two acids, for several species,

TABLE 5. YIELDS OF VANILLIN, VANILLIC ACID AND SYRINGIC ACID FROM ALKALINE CUPRIC HYDROXIDE OXIDATIONS OF PRE-EXTRACTED WOOD-MEALS

Species	Yield in mg/g dry weight*		
	Vanillin	Vanillic acid	Syringic acid
L. lucidulum	1·0	0·54	0
L. cernuum†	trace	8·20	0
L. carolinianum var. *meridionale*†	0·033	8·70	0
L. annotinum var. *acrifolium*	10·66	0·233	0·138
L. annotinum var. *pungens*	17·50	5·83	0·667
L. sabinaefolium	17·99	9·36	2·08

* Extracted dry weight.
† Dried specimens from Trinidad.

are also given in Table 3. Syringic acid was obtained in this fraction only with *L. complanatum*. There are thus obvious resemblances between *Lycopodium* and spermatophytes in that not only ethanol-soluble esters but also ethanol-insoluble esters of phenolic acids occur in substantial quantities.

TABLE 6. PRODUCTS* OF ETHANOLYSIS OF EXTRACTED WOOD-MEALS OF VARIOUS SPECIES OF *Lycopodium*

Species	α-Ethoxypropiovanillone	*p*-Coumaric acid
L. saururus	−	−
L. lucidulum	−	−
L. inundatum	−	+
L. cernuum	+	+
L. carolinianum var. *meridionale*	+	+
L. annotinum var. *acrifolium*	+	+
L. annotinum var. *pungens*	+	−
L. clavatum	+	+
L. complanatum	+	−
L. flabelliforme	−	−
L. sabinaefolium	−	+

* Vanillin, vanillic acid and vanilloyl methyl ketone were found in each case.
+ = Detected on chromatograms; − = not detected on chromatograms.

Although alkaline nitrobenzene oxidations gave higher yields of vanillin than were obtained with alkaline $Cu(OH)_2$ oxidations (10 mg/g wood-meal as compared with 8 mg/g wood-meal in the case of *L. annotinum* and 11·6 mg/g wood-meal as compared with 6·7 mg/g wood-meal in the case of *L. complanatum*) the latter method was considered preferable

because the reaction mixture contains fewer compounds interfering with chromatography. Alkaline $Cu(OH)_2$ oxidation gives relatively larger amounts of vanillic and syringic acids. Table 4 shows the compounds obtained by this method of oxidation, and in Table 5 the yields of vanillin, vanillic and syringic acids for a few species are presented.

The products of ethanolysis of various species were identified chromatographically and are listed in Table 6. Ethanolysis mixtures were found to be particularly unstable, even after partial purification, so that only those chromatograms prepared shortly after the ethanolysis reaction were useful. Considerable variation was noticed in the relative yields of vanillin, vanilloyl methyl ketone (1*a*) and α-ethoxypropiovanillone (1*c*) between species and even between samples of the same species.

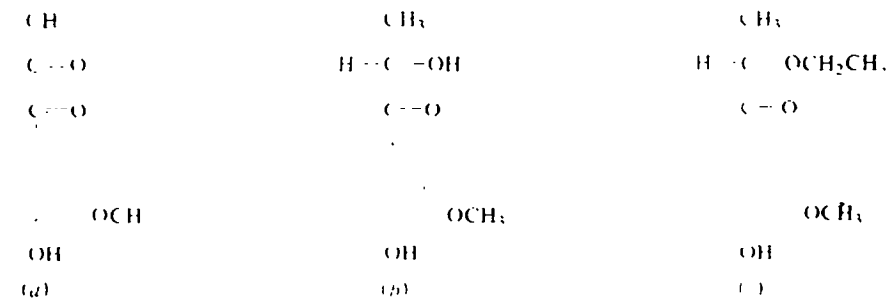

FIG. 1. (*a*) Vanilloyl methyl ketone, (*b*) α-hydroxypropiovanillone, (*c*) α-ethoxypropiovanillone.

The nature of the products of alkaline nitrobenzene or alkaline $Cu(OH)_2$ oxidation as well as those of ethanolysis indicate that *Lycopodium* has a gymnospermous type of lignin. No traces of syringaldehyde or acetosyringone could be detected in oxidation mixtures nor was there any indication of the presence of syringoyl methyl ketone in ethanolysis mixtures. *Selaginella kraussiana*, which belongs to an allied group of plants, on the other hand, was found to give readily detectable amounts of syringaldehyde and acetosyringone on alkaline $Cu(OH)_2$ oxidation. The evolution of chemically different lignins similar to those found in higher plants is remarkable in these cryptogams with their lower level of morphological organization. The presence of gymnospermous-like lignin in *Lycopodium* and of angiospermous-like lignin in *Selaginella* may perhaps have been of significance in the tree-like forms of ancestral species.

Whilst it is true that only a relatively few species of *Lycopodium* have been included in this study, a chemical distinction, between the groups which have been segregated on biological grounds, can be made. According to more recent ideas on the taxonomy of *Lycopodium*,[9] the group is placed in two families, the Urostachyaceae and the Lycopodiaceae. *Lycopodium* is divided into four genera, *Huperzia* in the Urostachyaceae and *Lepidotis*, *Lycopodium* and *Diphasium* in the Lycopodiaceae. This arrangement is supported on cytogenetical grounds by Löve and Löve.[10] If the species which we have examined are arranged in accordance with this scheme (Table 7) it can be seen that the two genera *Huperzia* and *Lepidotis* differ from *Lycopodium* and *Diphasium* in not yielding syringic acid either on alkaline hydrolysis of ethanolic extracts or by copper oxidation of the wood-meal.

Angiosperms are distinguished from ferns and most gymnosperms by their ability to synthesize syringyl compounds. In view of this distinction between major groups of plants,

[9] W. ROTHMALER, *Feddes Repert* **54**, 55 (1944).
[10] A. LÖVE and D. LÖVE, *The Nucleus* **I**, 1 (1958).

the division within the lycopods would appear to be a fundamental chemical difference and therefore probably useful as an aid in their taxonomy. If the ability to synthesize syringyl compounds is an advanced characteristic then it would appear that *Lycopodium* and *Diphasium* are more advanced chemically than are *Huperzia* and *Lepidotis*. *Selaginella*, in turn, may be even more advanced chemically since the ability to synthesize a lignin yielding syringaldehyde as well as syringic acid, a feature characteristic of angiosperms, has appeared within this group. It would be of interest to extend this survey to other species in order to see whether this distinction holds. It would also be interesting to survey related plants such as *Phylloglossum* and *Isoëtes*.

TABLE 7. DISTRIBUTION OF SYRINGIC ACID IN RELATION TO TAXONOMY* OF LYCOPODS

Family	Genus	Species	Syringic acid: in ethanol-soluble fraction	Syringic acid: from $Cu(OH)_2$ oxidation of wood-meal
Urostachyaceae	*Huperzia*	*selago*	−	−
		lucidula	−	−
		saururus	−	−
		phlegmaria	−	−
Lycopodiaceae	*Lepidotis*	*inundata*	−	−
		inundata var. *bigelovii*	−	−
		alopecuroides	−	−
		cernua	−	−
		cernua var. *crassifolium*	−	−
		caroliniana var. *meridionale*	−	−
	Lycopodium	*annotinum*	±	+
		annotinum var. *acrifolium*	−	+
		annotinum var. *pungens*	−	+
		clavatum	±	+
		clavatum var. *divaricatum*	+	+
		obscurum	+	+
		obscurum var. *dendroideum*	+	+
	Diphasium	*complanatum*	+	+
		flabelliforme	+	+
		tristachyum	+	+
		sabinaefolium	±	+

* Division of groups according to Rothmaler.[8]
+ Indicates detected in all specimens; ± indicates detected in some specimens only; − indicates not detected.

Further work is also necessary to discover the source of the syringic acid obtained in alkaline oxidations of *Lycopodium* extracted wood-meal. It cannot be due to the complete oxidation of syringaldehyde obtained under the reaction conditions employed because syringaldehyde itself, under these conditions, gives only a 12 per cent yield of the acid.

EXPERIMENTAL

Sources of plant material are listed in Table 1. Phenolic acids and aldehydes were purchased from Light and Co., England, or from Fluka and Co., Switzerland. Vanilloyl methyl ketone and acetosyringone were gifts from Dr. J. M. Pepper, Chemistry Department, University of Saskatchewan. α-Hydroxypropiovanillone was a gift from Dr. A. C. Neish of this

laboratory. α-Ethoxypropiovanillone was prepared from α-hydroxypropiovanillone by the method of West *et al.*[11]

Chromatography

Methods for the identification, by chromatography, of phenolic acids have been described.[12] R_f values of phenolic aldehydes and ketones chromatographed on silica gel in various solvent systems are given in Table 2.

Analyses for Phenolic Acids

Plant material was washed carefully and homogenized with hot absolute ethanol in a Waring Blendor. The insoluble material (I) was removed by filtration and repeatedly extracted with 80% ethanol under reflux on the steam bath until the extracts were almost colourless. The combined filtered extracts were evaporated to dryness in a rotary evaporator and the residue, thus obtained, was treated with boiling water and filtered hot through a bed of Celite (Analytical Filter-Aid). To the cooled filtrate was added sufficient 10 N NaOH to make a twice normal solution. After standing for 14 hr in the refrigerator, the solution was acidified with HCl to pH 4 and continuously extracted with ether for 20 hr. The ether extract was analysed by two-directional paper chromatography for phenolic acids. Phenolic acids were eluted from chromatograms and determined quantitatively by spectrophotometry.[12]

A portion of the ethanol-insoluble residue (I) was subjected to hydrolysis in 2 N NaOH at 30° for 4 hr after which the solution was acidified with conc. HCl and continuously extracted with ether for 20 hr. The ether extract was analysed for phenolic acids by chromatography.

Lignin Oxidations

The insoluble residue (I) was air-dried and ground to a powder (40 mesh) in a Wiley Mill. The powder was extracted for 48 hr in a soxhlet with water followed by similar extractions with 1:1 ethanol:benzene and with benzene. The powder, which was dried in air, was used as a source of lignin and is referred to as extracted wood-meal in the text.

Alkaline nitrobenzene oxidations were carried out using the micro-method of Stone and Blundell[13] as modified as Basyouni and Towers.[12]

Alkaline copper hydroxide oxidations were carried out as follows: 300 mg extracted wood-meal, 1·3 g $Cu(OH)_2$ and 10 ml 2 N NaOH were placed in a stainless-steel bomb, sealed and placed in an oil-bath at 175° for 3 hr with occasional shaking. The bomb was cooled and the reaction mixture filtered, the insoluble material being washed with hot water. The filtrate and washings were acidified with HCl to pH 2–3 and extracted with chloroform in a separatory funnel. The chloroform extract was evaporated to dryness, the residue redissolved in a small volume of ethanol and analysed by chromatography for phenolic aldehydes and acids.

A large-scale oxidation was carried out with 15 g extracted wood-meal of *L. clavatum*. The reaction mixture was acidified and extracted with ether and the ethereal solution extracted sucessively with 5% Na_2CO_3 and 10% $NaHSO_3$. The fraction containing the acids was chromatographed on paper using the benzene–acetic acid solvent and the bands corresponding to vanillic and syringic acids were eluted and rechromatographed using 2% HCOOH. The appropriate bands were eluted and the eluates chromatographed on silica gel plates using

[11] K. A. West, L. Hawkins and H. Hibbert, *J. Am. Chem. Soc.* **63**, 3038 (1941).
[12] S. Z. El-Basyouni and G. H. N. Towers, *Can. J. Biochem.* **42**, 203 (1964).
[13] J. E. Stone and M. J. Blundell, *Analyt. Chem.* **23**, 771 (1951).

toluene:ethyl formate:formic acid (8:4:1) as a solvent. The eluted bands were extracted into ether and the vanillic acid (8 mg) crystallized from water. It was identified by a mixed m.p. and by its i.r. spectrum. The very small amount of syringic acid isolated precluded a m.p. determination but the i.r. spectrum obtained was essentially similar to that of syringic acid. The aldehyde fraction of the oxidation mixture was banded on silica gel plates and chromatographed in benzene–acetic acid and the eluted vanillin (45 mg) and *p*-hydroxybenzaldehyde (3·1 mg) purified by sublimation. They were identified by mixed m.p.'s and by their i.r. spectra. Acetovanillone, also present in this fraction, was identified by chromatography.

Pure vanillin and syringaldehyde were subjected to alkaline $Cu(OH)_2$ oxidation. With vanillin a 9 per cent yield of vanillic acid and with syringaldehyde a 12 per cent yield of syringic acid was obtained.

Ethanolysis of Lignin

Ethanolyses were carried out on 300 mg samples of extracted wood-meal using the method of Kratzl.[14] The ethereal extract of the ethanolysis mixture was reduced in volume and the oily residue dissolved in chloroform. The chloroform extract was passed through a small bed of silicic acid in a sintered glass funnel and 100 ml of eluate collected. This eluate, after removal of solvent, was chromatographed for aldehydes and ketones.

One hundred grams of extracted wood-meal of *L. clavatum* was subjected to ethanolysis. The weight of residual wood-meal was 81·0 g and of ethanol lignin, 3·8 g. The filtrate obtained after removal of the ethanol lignin was extracted continuously with ether to give 7 g of a dark-brown oil. This oil was mixed with silicic acid and transferred to a column of silicic acid (4 × 40 cm) in $CHCl_3$. A number of yellow and red bands moved with the solvent front or close behind it. These were collected as one fraction in almost 4 l. of eluate. This fraction yielded 1·4 g of an orange-brown oil. Chromatography on silica gel plates using the benzene: acetic acid solvent indicated that there were at least fifteen compounds giving colour reactions with 2,4-dinitrophenylhydrazine. One of the most conspicuous, because of its dark purple fluorescence in u.v. light, was isolated in the following way: (1) Chromatography on silica gel using *n*-butyl ether, elution and rechromatography on silica gel in benzene:acetic acid, 9:1; (2) chromatography on Whatman No. 1 using 2% HCOOH; (3) sublimation; (4) chromatography of the sublimate on silica gel plates in toluene:ethylformate:formic acid (5:4:1) followed by sublimation; (5) chromatography in 4:1 butanol:3% NH_4OH on silica gel plates followed by sublimation. In this way 73 mg of a chromatographically pure colourless oil was obtained. The compound was identified by its NMR spectrum as α-ethoxypropiovanillone.

The NMR spectrum was kindly interpreted by Dr. A. G. McInnes of this laboratory as follows: A triplet centred at a τ value of 8·76, with an intensity corresponding to 3 protons and with a spacing of 7 c/s could be assigned to the methyl group of the ethoxy moiety since the corresponding signal for the methylene protons occurred at an average τ value of 6·44, also with a common spacing of 7 c/s. A doublet, due to 3 protons, at an average τ value of 8·47 with a spacing of 7 c/s was assigned to the methyl group on the carbon bearing a single hydrogen since the corresponding quartet for the single hydrogen appeared with the same spacing at an average τ value of 5·34. A single signal due to 3 protons at a τ value of 6·05 could be unambiguously assigned to the methoxyl group on the aromatic ring. The three aromatic protons appeared as a multiplet centred at a τ value of 2·63, and the signal due to

[14] K. KRATZL, *Mikrochim. Acta* **1**, 159 (1956).

one phenolic hydroxyl group was temperature-dependent appearing at an approximate τ value of 3·3. Consequently the NMR spectrum was in agreement with the structure given in Fig. 1*c*.

The isolated compound was chromatographically identical with a sample prepared from α-hydroxypropiovanillone. Methylation with diazomethane gave α-ethoxypropioveratrone, m.p. 79·5–81·5° uncorrected (reported 81–82°), which was obtained by sublimation and crystallization from dilute acetone.

Bands on chromatograms, corresponding to vanillin, were pooled, and after sublimation, 40 mg of this aldehyde were obtained. α-Hydroxypropiovanillone was also identified as a minor constituent of the ethanolysis mixture by co-chromatography with an authentic sample, by its characteristic dark-blue fluorescence in u.v. light and by its colour reaction with 2,4-dinitrophenylhydrazine. The band corresponding to vanilloyl methyl ketone was purified by chromatography on silica gel using the benzene:acetic acid and butanol–NH_4OH solvent systems. It was finally identified by co-chromatography with an authentic sample in three solvent systems.

Acknowledgements—We wish to thank the many people who provided us with plant material and especially Dr. S. Kawano, University of Wisconsin, U.S.A., who introduced one of us (G. H. N. T.) to problems of *Lycopodium* taxonomy. The excellent technical assistance of C. W Glennie is gratefully acknowledged.