The Properties of Syringyl, Guaiacyl and *p*-Hydroxyphenyl Artificial Lignins

By D. E. BLAND AND A. F. LOGAN

Division of Forest Products, C.S.I.R.O., Melbourne, Victoria, Australia

(Received 27 July 1964)

1. Artificial lignins have been produced on potato parenchyma. 2. The methoxyl-free lignin and 4-hydroxy-3-methoxy (guaiacyl) lignins could be estimated by the sulphuric acid method but the 4-hydroxy-3,5-dimethoxy (syringyl) lignins could not. 3. Permanganate oxidation of isolated *p*-coumaric lignin gave 4-hydroxybenzoic acid, 4-hydroxyisophthalic acid and small amounts of hydroxytrimesic acid and 4-hydroxyphthalic acid. Ferulic lignin gave vanillic acid and 5-carboxyvanillic acid and also small amounts of 4-hydroxybenzoic acid and dehydrodivanillic acid. The sinapic lignin gave traces of syringic acid and of 4-hydroxybenzoic acid. 4. The *p*-coumaric lignin is a highly condensed polymer. The ferulic lignin is partly uncondensed and partly condensed through the 5-position like gymnosperm lignin. The sinapic lignin shows no evidence of condensation and is probably an ether-linked polymer.

The production of an artificial lignin from eugenol on potato parenchyma was studied by Bland (1961*a*). This substance was definitely a lignin-like material, but it differed from natural lignin in certain ways and so deductions about natural lignification from such experiments must be made with caution.

All naturally occurring lignins are believed to be phenylpropane polymers. They fall into three classes according to the substitution of the aromatic nucleus. Moss lignins are predominantly polymers of methoxyl-free units, gymnosperm lignins consist mainly of guaiacyl (4-hydroxy-3-methoxyphenyl) units, whereas angiosperm lignins contain both guaiacyl and syringyl (4-hydroxy-3,5-dimethoxyphenyl) units. The lignins of Gramineae are believed to be polymers of all three types of unit.

Only small amounts of 4-hydroxybenzaldehyde

have been observed by Bland, Ho & Cohen (1950) and Leopold (1952) as an oxidation product of different natural lignins. Freudenberg & Chen (1960) obtained 4-methoxyisophthalic acid, methoxytrimesic acid and small amounts of anisic acid from methylated spruce wood. Kyogoku & Hachihama (1961) noted that no 4-hydroxybenzaldehyde was obtained from beech lignin but the first two acids were obtained on methylation and oxidation. No anisic acid was detected. These results all indicate that most methoxyl-free units in natural lignin are of the condensed type, i.e. at least one carbon atom of the aromatic nucleus is linked directly to another outside the nucleus.

In the present work different types of artificial lignins have been produced on potato parenchyma to investigate the properties of methoxyl-free lignin, guaiacyl lignin and syringyl artificial lignin.

R = H where unspecified

R₁ = CH:CH·CO₂H

R₁ = CH:CH·CO₂H; R₃ = O·CH₃

R₁ = CH:CH·CO₂H; R₃ = R₅ = O·CH₃

R₁ = CH₂·CH:CH₂; R₃ = R₅ = O·CH₃

R₁ = CO₂H

R₁ = CO₂H; R₃ = O·CH₃

R₁ = CO₂H; R₃ = R₅ = O·CH₃

R₁ = R₅ = CO₂H

R₁ = R₆ = CO₂H

R₁ = R₅ = CO₂H; R₃ = O·CH₃

R₁ = R₆ = CO₂H; R₃ = R₅ = O·CH₃

R₁ = R₃ = R₅ = CO₂H

p-Coumaric acid

Ferulic acid

Sinapic acid

1-Allyl-4-hydroxy-3,5-dimethoxybenzene

4-Hydroxybenzoic acid

Vanillic acid

Syringic acid

4-Hydroxyisophthalic acid

4-Hydroxyphthalic acid

5-Carboxyvanillic acid

4-Hydroxy-3,5-dimethoxyphthalic acid

Hydroxytrimesic acid

Although syringyl lignin is not known in Nature, an artificial specimen has been prepared and its properties were investigated. The study of lignin-like materials built up from one type of aromatic nucleus only should be of assistance in indicating types of linkage in natural lignins.

EXPERIMENTAL

Precursors. Eugenol (W. J. Bush and Co., Mentone, Victoria, Australia), coniferyl alcohol (given by Professor Kratzl, Vienna, Austria), 1-allyl-4-hydroxy-3,5-dimethoxybenzene prepared by the method of Mauthner (1918), dehydrodieugenol prepared by adding 5g. of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (British Drug Houses Ltd., Poole, Dorset) to 5.0g. of eugenol vigorously stirred in 4l. of water at 20° and crystallizing the product from ethanol (yield 1.5g., m.p. 105°), 4-hydroxycinnamic acid (*p*-coumaric acid), 4-hydroxy-3-methoxycinnamic acid (ferulic acid) and 4-hydroxy-3,5-dimethoxycinnamic acid (sinapic acid) (last three compounds from Aldrich Chemical Co. Inc., Milwaukee, Wis., U.S.A.) were tested as lignin precursors individually and in some cases in pairs.

Preparation of reference compounds. 4-Hydroxyphthalic acid was prepared by sulphonation of phthalic anhydride according to the method of Bentley & Weizmann (1907) and alkali fusion of the sulphonation mixture. 4-Hydroxy-3,5-dimethoxyphthalic acid was prepared by the method of Alimchandani & Meldrum (1920).

Preparation of artificially lignified potato parenchyma. This was carried out by macerating peeled potatoes, allowing the starch to settle, recovering the parenchyma by filtration and stirring it into a buffered solution containing H_2O_2 and the lignin precursor, as described in detail by Bland (1961a). In the attempted lignification by dehydrodieugenol the standard procedure was varied as this substance was too insoluble to disperse in the buffer-peroxide solution. In this case the parenchyma was stirred in the buffer-peroxide solution and an ethanolic solution of dehydrodieugenol added dropwise over 5 days.

Determination of lignin in the artificially lignified parenchyma. Experiments with the unignified parenchyma showed that to be sure of obtaining a zero lignin value it was necessary to treat with acid for longer times than those usually employed. The following procedure was adopted. About 1g. of parenchyma was stirred for 6hr. with 15ml. of 72% (w/v) H_2SO_4 ; the mixture was then diluted to 3% (w/v), kept overnight at room temperature and boiled under reflux for 6hr. The residue was collected by filtration on a Norton alundum crucible R.A. 360, washed, dried, weighed and analysed for nitrogen and methoxyl. Where possible an estimate of the lignin content was obtained from the total methoxyl content of the lignified parenchyma.

Identification of nitrobenzene oxidation products of the lignins. Specimens of the lignified parenchyma (1g.) were oxidized with nitrobenzene (0.3ml.) in 2*N*-NaOH (10ml.) for 3hr. at 160° in stainless-steel bombs. Aromatic aldehydes produced were identified and estimated by separation on borate paper by the method of Bland & Stamp (1955). Acids present were identified by the chromatographic method of Billek & Kindl (1962), where separation is effected on paper with benzene-acetic acid-water (4:2:1, by vol.) solvent and location is by a diazotized sulphanilic

acid spray [1 ml. of 0.5% sulphanilic acid in 2*N*-HCl + 3 ml. of 5% (w/v) NaNO_2 + 3 ml. of 20% (w/v) Na_2CO_3]. Ferric chloride spray [5% (w/v) $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$] was also used.

Determination of *p*-hydroxybenzyl alcohol groups. These were estimated by the indophenol reaction with *N*-chloro-*p*-quinoneimine (Fisher) according to Gierer (1954), with vanillyl alcohol and syringyl alcohol as standards. The indophenol colours were measured at 640m μ on a Unicam SP.600 spectrophotometer against a blank solution prepared from an equal weight of unignified parenchyma tissue. This procedure was necessary to correct for interference by the parenchyma. The results are only roughly quantitative because of this interference and the tendency of traces of starch to adsorb the colour.

Isolation of artificial lignins. This was carried out by fine grinding in toluene followed by extraction with 90% (v/v) dioxan, as used by Björkman (1956) in the isolation of milled-wood lignin.

Infrared-absorption spectra of isolated lignins. These were determined in KCl disks according to the method of Higgins (1957).

Identification of permanganate oxidation products of the isolated lignins. Specimens of the lignins isolated by the Björkman (1956) procedure (0.1g.) were oxidized by heating at 160° for 3hr. with KMnO_4 (0.5g.) in 8% (w/v) NaOH solution (50ml.) in stainless-steel bombs. The reaction mixture was filtered and the filtrate acidified with HCl and extracted with ether. The ether was distilled off and the residue taken up in ethanol (10ml.). The acids were identified by paper chromatography by using diazotized sulphanilic acid spray reagent (Billek & Kindl, 1962). Ferric chloride spray reagent was used to detect 4-hydroxyisophthalic acid, and inspection in ultraviolet light to detect hydroxytrimesic acid. Yields of these acids were estimated by visual comparison of the spots on the chromatograms with spots containing known amounts of the compound in question.

RESULTS

An estimate of the amount of lignification in experiments with the different precursors may be made from the results shown in Table 1. The lignin contents estimated by the sulphuric acid method showed wide variation between different precursors and seem to indicate that dehydrodieugenol and 1-allyl-4-hydroxy-3,5-dimethoxybenzene were relatively ineffective as lignin precursors. Sinapic acid also appears from the sulphuric acid result to be almost ineffective as a lignin precursor in the system being tested.

The methoxyl content of the unignified parenchyma was determined as 0.5%. That of the parenchyma lignified with *p*-coumaric acid was also 0.5% (Table 1). The methoxyl contents of the lignified parenchyma and the percentage of lignin calculated from the corrected methoxyl content are also shown in Table 1.

All the sulphuric acid lignins isolated in sufficient quantity for analysis contained nitrogen (Table 1). The sulphuric acid lignin values, corrected on the assumption that this nitrogen was in the form of protein containing 6.25% of N, are shown in Table 1.

Table 1. *Lignins from different precursors*

Artificial lignins were produced by treating potato parenchyma with buffered solutions of precursor and H_2O_2 . Lignin was determined by the H_2SO_4 method and correction applied for protein contamination. Where possible lignin content was also estimated from the methoxyl content of the lignified parenchyma.

Precursor	Amount of precursor (g./100 g. of original potato)	O·CH ₃ content of lignified parenchyma (%)	H ₂ SO ₄ lignin			Yield of lignin (%)	
			Yield (%)	N content (%)	O·CH ₃ content (%)	H ₂ SO ₄ lignin corrected for protein	Calc. from total O·CH ₃ content
<i>p</i> -Coumaric acid	0.5	0.52	6.06	3.18		4.85	
Coniferyl alcohol	0.5	1.68	9.12	3.67	11.09	7.03	6.85
Ferulic acid	0.5	1.34	4.48	2.68	12.47	3.73	5.13
Sinapic acid	0.5	1.80	1.24		approx. 13		4.62
Ferulic acid + sinapic acid	0.25 + 0.25	1.91	4.10	2.85	16.17	3.37	6.34
1-Allyl-4-hydroxy-3,5-dimethoxybenzene	0.5	1.05	0.45				1.88
Eugenol + 1-allyl-4-hydroxy-3,5-dimethoxybenzene	0.25 + 0.25	1.58	7.33	4.39	14.69	5.32	4.25
Dehydrodieugenol	0.2	0.41	0.11				

Table 2. *Nitrobenzene oxidation products of lignified parenchyma*

Yields of aldehydes and ketones were determined by quantitative paper chromatography. Tests for acids were qualitative only.

Precursor	Yield of lignin calc. from total O·CH ₃ content (%)					Acids present
	Vanillin	Syring-aldehyde	4-Hydroxy-benzaldehyde	Aceto-vanillone	Aceto-syringone	
<i>p</i> -Coumaric acid	0	0	Trace	0	0	4-Hydroxybenzoic acid, <i>p</i> -coumaric acid
Coniferyl alcohol	3.5	0	0	Trace	0	Vanillic acid
Ferulic acid	5.8	0	0	1.3	0	Vanillic acid, ferulic acid
Sinapic acid	0	8.8	0	0	2.7	Syringic acid, sinapic acid
Ferulic acid + sinapic acid	4.4	0	0	0.6	1.2	Vanillic acid, ferulic acid, syringic acid, sinapic acid
1-Allyl-4-hydroxy-3,5-dimethoxybenzene	0	3.6	0	0	0.8	Syringic acid
Eugenol + 1-allyl-4-hydroxy-3,5-dimethoxybenzene	2.9	2.2	0	Trace	Trace	Vanillic acid, syringic acid
Dehydrodieugenol	0	0	0	0	0	

The corrected lignin values are also shown. The discrepancies apparent between different estimates of lignin content are discussed below.

The nitrobenzene oxidation products of the lignified parenchyma specimens are shown in Table 2. The *p*-coumaric lignin gave only a trace of 4-hydroxybenzaldehyde. Both the coniferyl alcohol- and ferulic acid-lignified parenchyma gave yields of vanillin, but these are much lower, about one-sixth and one-quarter respectively, of the yields from natural guaiacyl (gymnosperm) lignins. The sinapic lignin gave syringaldehyde, as expected, but the yield was low.

The artificial syringyl-guaiacyl lignin gave both vanillin and syringaldehyde but, once again, the yields were low compared with similar natural (angiosperm) lignins (cf. Bland *et al.* 1950).

Small quantities of acids were present among the nitrobenzene oxidation products of all the artificial lignins. The original acids reappeared as oxidation products of the *p*-coumaric, ferulic and sinapic lignins. A trace of 4-hydroxybenzoic acid was detected as an oxidation product of the *p*-coumaric lignin, vanillic acid occurred as an oxidation product of the ferulic lignin and syringic acid as a product of the sinapic lignin. All four of the acids

Table 3. *Yields and composition of artificial lignins isolated by the Björkman (1956) procedure*

Lignified parenchyma was milled in toluene, and the lignin extracted with 90% dioxan and precipitated from acetic acid into water.

Precursor	Yield (% of parenchyma)	Elementary composition					O-CH ₃ content (%)	O-CH ₃ content (corr.) (%)	O-CH ₃ groups/C unit
		C (%)	H (%)	N (%)	O (%)	Total (%)			
<i>p</i> -Coumaric acid	2.6	59.0	5.6	2.2	32.4	99.2	2.0	2.3	0.1
Coniferyl alcohol	1.5	61.1	6.5	1.5	30.1	99.2	11.5	12.7	0.8
Ferulic acid	1.5	59.4	5.3	1.9	32.9	99.5	12.7	14.4	0.9
Sinapic acid	0.3	57.4	6.3	2.8	32.1	98.6	15.5	18.8	1.3
Ferulic acid + sinapic acid	1.3	58.3	5.7	1.8	33.7	99.5	17.3	19.5	1.3
1-Allyl-4-hydroxy-3,5-di- methoxybenzene	Trace								
Eugenol + 1-allyl-4-hydroxy- 3,5-dimethoxybenzene	1.3	59.3	6.3	2.1	31.1	98.8	15.9	18.3	1.2
Dehydrodieugenol	0								

Table 4. *p*-Hydroxybenzyl alcohol groups in artificial lignins

The *p*-hydroxybenzyl alcohol groups were determined by colour reaction of *N*-chloro-*p*-quinoneimine with lignified parenchyma.

Precursor	<i>p</i> -Hydroxybenzyl alcohol groups (mole fraction)
<i>p</i> -Coumaric acid	0.00
Coniferyl alcohol	0.01
Ferulic acid	Trace
Sinapic acid	0.04
Ferulic acid + sinapic acid	0.03
1-Allyl-4-hydroxy-3,5-dimethoxy- benzene	0.17
Eugenol + 1-allyl-4-hydroxy-3,5- dimethoxybenzene	0.03

mentioned above were present as oxidation product of the mixed ferulic-sinapic lignin, but only vanillic acid and syringic acid appeared as oxidation products of the eugenol and 1-allyl-4-hydroxy-3,5-dimethoxybenzene lignin.

The yields and compositions of lignins isolated by milling and extraction according to the Björkman (1956) procedure are shown in Table 3. The Table shows that, compared with the sulphuric acid lignins, the nitrogen content was low, indicating less contamination with protein.

The estimates of the *p*-hydroxybenzyl alcohol groups are shown in Table 4. The zero result from the *p*-coumaric lignin is as expected from the finding of Gierer (1954) that *p*-hydroxybenzyl alcohol itself gives only a faint colour with the reagent. Only those lignins containing syringyl units show any significant number of *p*-hydroxybenzyl alcohol groups. Only the lignin derived from 1-allyl-4-hydroxy-3,5-dimethoxybenzene shows a number of *p*-hydroxy-

Table 5. *Phenolic acids from permanganate oxidation*

Isolated lignins were heated with KMnO₄ in NaOH solution for 3 hr. at 160°. Yields were estimated by paper chromatography.

Lignin	Acids detected	Approx. yield (% of lignin)
<i>p</i> -Coumaric lignin	4-Hydroxybenzoic acid	7
	4-Hydroxyisophthalic acid	10
	Hydroxytrimesic acid	0.4
	4-Hydroxyphthalic acid	0.1
Ferulic lignin	4-Hydroxybenzoic acid	0.3
	Vanillic acid	2.6
	5-Carboxyvanillic acid	0.8
	Dehydrodivanillic acid	Trace
Sinapic lignin	4-Hydroxybenzoic acid	Trace
	Syringic acid	Trace
Ferulic-sinapic lignin	4-Hydroxybenzoic acid	Trace
	Vanillic acid	1.3
	5-Carboxyvanillic acid	0.1
	Dehydrodivanillic acid	Trace
	Syringic acid	1.6

benzyl alcohol groups in excess of that observed in natural lignins (cf. Adler, 1958).

The phenolic acids detected in the permanganate oxidation products of the isolated lignins are shown in Table 5. 4-Hydroxybenzoic acid and 4-hydroxyisophthalic acid were the main degradation products of the *p*-coumaric lignin. The occurrence of small quantities of hydroxytrimesic acid and 4-hydroxyphthalic acid is noteworthy.

Vanillic acid and 5-carboxyvanillic acid as oxidation products of the ferulic lignin are as expected. The origin of 4-hydroxybenzoic acid from the ferulic

and sinapic lignins is obscure. None could be obtained from blank experiments with unligified parenchyma.

DISCUSSION

It is apparent from the different behaviour of the lignins in the sulphuric acid determination that there are considerable differences in the structure of the lignins. The sinapic lignin, unlike other artificial and natural lignins, appears to be acid-hydrolysable, as the yield of acid lignin was only about one-quarter of the lignin content estimated from the methoxyl content.

The fact that the *p*-coumaric lignin gave a negligible yield of 4-hydroxybenzaldehyde suggests similarity between the mode of linkage in the artificial lignin and the methoxyl-free units in natural lignins. The absence of 4-hydroxybenzaldehyde as an oxidation product of a lignin does not necessarily prove the methoxyl-free units to be of the condensed type. Wacek & Kratzl (1947) showed that the substituent on the α -carbon atom of the side chain was important in determining whether aldehyde was produced from the unit. The occurrence of 4-hydroxyisophthalic acid and hydroxytrimesic acid also shows similarity to the methoxyl-free units of natural lignins, the corresponding methoxy acids having been identified from methylated wood.

At first it would appear that the 4-hydroxybenzoic acid from our *p*-coumaric lignin and the anisic acid of Freudenberg & Chen (1960) was derived from non-condensed units without the necessary α -substituent for aldehyde production. However, condensation may occur through the 6-position of the nucleus, as indicated by the appearance of small amounts of 4-hydroxyphthalic acid. Under the conditions of the permanganate oxidation employed in these tests, approximately half of a specimen of 4-hydroxyphthalic acid was decarboxylated to 4-hydroxybenzoic acid. In view of the large excess of 4-hydroxybenzoic acid over 4-hydroxyphthalic acid it appears that only a small fraction of the former could have been derived from 6-condensed aromatic units and that most was derived from uncondensed units without an aldehyde-directing substituent on the α -carbon atom. That the *p*-coumaric lignin consisted largely of 5-condensed aromatic units is shown by the high yield of 4-hydroxyisophthalic acid. The occurrence of hydroxytrimesic acid as an oxidation product indicates the presence of some double condensed aromatic nuclei in the *p*-coumaric lignin.

The origin of the 4-hydroxybenzoic acid in the oxidation products of the ferulic and sinapic lignins is not obvious. Blank experiments with unligified parenchyma gave no trace of this acid. Neither did oxidation of the starting materials (ferulic acid and

sinapic acid) give any trace of 4-hydroxybenzoic acid. The results therefore point to demethoxylation during the formation of the lignin. Tables 2 and 5 show that vanillin, acetovanillone, vanillic acid and 5-carboxyvanillic acid were the main oxidation products detected from ferulic lignin. The vanillic acid is an expected product from uncondensed guaiacyl units and the 5-carboxyvanillic acid from 5-condensed guaiacyl units. Thus the main types of linkage occurring in the ferulic lignin appear to be the same as the main types occurring in gymnosperm lignin.

The isolation of a sinapic 'lignin' is of interest as no syringyl lignin is known in Nature. The modification of the properties of the potato parenchyma brought about by lignification with sinapic acid was similar to that brought about by other lignification treatments, i.e. after grinding it could be extracted with dioxan without clumping. Untreated parenchyma gave a rubbery mass not amenable to the extraction treatment. The isolated material, although obtained only in low yield, had a composition approximating to that of a lignin, although protein contamination was higher than in other cases and the methoxyl content was low for a syringyl-type polymer. The yields of syringaldehyde and acetosyringone confirm that a syringyl polymer had been isolated. Only traces of syringic acid were obtained from permananate oxidation of this material, but this is consistent with the instability of syringic acid under the conditions of the oxidation.

The absence of 5-carboxyvanillic acid as an oxidation product shows that no 5-linked condensed units were present. This, of course, would have required demethoxylation of a syringyl nucleus. The only alternative type of condensation would appear to be that through the 6-position of the nucleus, but as no 4-hydroxy-3,5-dimethoxyphthalic acid was detected there is no evidence of this type of condensation.

This sinapic 'lignin' must therefore differ in its mode of linkage both from natural lignins and the artificial lignins studied above. The fact that it was hydrolysed during a sulphuric acid lignin determination suggests that it is not a condensed type of lignin polymer, but is probably an ether-linked polymer. The results of ethanolysis agreed with this; the main product corresponded in R_f to α -ethoxypropiosyringone according to Kratzl & Schweers (1954). Thus the *p*-coumaric lignin and the sinapic lignin appear to be lignin-like polymers in the sense that the nuclei are linked by types of bonds thought to occur in natural lignins, but one type of bond only predominates in each. The infrared spectrum of the *p*-coumaric lignin resembled that of moss lignin (D. E. Bland, unpublished work) in that the bands at 6.85, 7.05 and 9.0 μ were very

weak. The relative strengths of these bands in the spectrum of ferulic lignin were similar to those determined by Bland (1961b) under the same conditions for milled-wood lignin of *Pinus radiata*. In the same way the relative strengths for these bands in the spectrum of sinapic lignin were similar to those found for *Eucalyptus goniocalyx* lignin.

Durie, Lynch & Sternhell (1960) showed that on demethylating lignin with hydroiodic acid the band at 7.0μ disappeared, and therefore attributed this band to a bending vibration of the methoxyl group. We found that the spectrum of *p*-coumaric acid also has a band in this region and it may be attributed to an ethylenic C–H bending vibration. It is not possible to say therefore how much the weakening of this band indicates disappearance of ethylenic groups by alkyl–aryl condensation and how much it merely reflects the methoxyl content of the lignins.

The authors thank Dr I. A. Pearl for a specimen of 5-carboxyvanillic acid and Dr E. G. Hawkins for a specimen of hydroxytrimesic acid. They are grateful to Dr A. J. Michell for determination of infrared spectra and assistance with their interpretation.

REFERENCES

- Adler, E. (1958). *Proc. 4th int. Congr. Biochem., Vienna*, vol. 2, 137.
Alimchandani, R. L. & Meldrum, A. N. (1920). *J. chem. Soc.* **117**, 964.
Bentley, W. H. & Weizmann, C. (1907). *J. chem. Soc.* **91**, 98.
Billek, G. & Kindl, H. (1962). *Mh. Chem.* **93**, 85.
Björkman, A. (1956). *Svensk PappTidn.* **59**, 477.
Bland, D. E. (1961a). *Biochem. J.* **81**, 23.
Bland, D. E. (1961b). *Holzforschung*, **15**, 102.
Bland, D. E., Ho, G. & Cohen, W. E. (1950). *Aust. J. sci. Res. A*, **3**, 642.
Bland, D. E. & Stamp, C. (1955). *Aust. J. appl. Sci.* **6**, 353.
Durie, R. A., Lynch, B. M. & Sternhell, S. (1960). *Aust. J. Chem.* **13**, 156.
Freudenberg, K. & Chen, C.-L. (1960). *Chem. Ber.* **93**, 2533.
Gierer, J. (1954). *Acta chem. scand.* **8**, 1319.
Higgins, H. G. (1957). *Aust. J. Chem.* **10**, 496.
Kratzl, K. & Schweers, W. (1954). *Chem. Ber.* **89**, 186.
Kyogoku, Y. & Hachihama, Y. (1961). *Tech. Rep. Osaka Univ.* **11**, 439.
Leopold, B. (1952). *Acta chem. scand.* **6**, 38.
Mauthner, F. (1918). *Liebigs Ann.* **414**, 251.
Wacek, A. V. & Kratzl, K. (1947). *Öst. ChemZtg*, **48**, 36.
Cited by Brauns, F. E. (1952). *Chemistry of Lignin*, p. 557. New York: Academic Press Inc.