[CONTRIBUTION FROM THE FOREST PRODUCTS LABORATORY,[1] FOREST SERVICE, U. S. DEPARTMENT OF AGRICULTURE]

Nitrobenzene Oxidation of Lignin Model Compounds, Spruce Wood and Spruce "Native Lignin"

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Comparison of the products of alkaline nitrobenzene oxidation of spruce wood with lignin model compounds indicates that a majority of the guaiacylpropane units are not linked by carbon substitution in the aromatic nucleus. The similarity in yield of products obtained with the lignin of spruce wood and Brauns' spruce "native lignin" suggests a close relationship between the two substances. An appreciable amount of diphenyl-type linkages appears to exist in coniferous lignin.

The view that the lignin of coniferous wood is a polymer composed largely of monomers of the guaiacylpropane type has gained wide acceptance, but there is lack of agreement as to their mode of linkage. Some investigators believe the ring of one unit is combined with the side chain of a second unit to form a pyran or furan ring. This involves a carbon–carbon bond *ortho* to the phenolic hydroxyl and is often called a "condensed structure." Others hold that the linkage is essentially of the ether type between phenolic hydroxyls and the α- or β-carbon atoms in the side chains. Most lignin chemists concede that both types may be present.

One method of attacking the problem is through a study of the behavior of model compounds, especially toward the important alkaline nitrobenzene oxidation reaction. In this reaction a lignin unit in which the ring is linked by a carbon atom *ortho* to the phenolic hydroxyl would not be expected to yield vanillin unless this carbon atom was first oxidized to a carboxyl group and then eliminated by decarboxylation. Freudenberg[2] subjected 5-carboxyvanillin to alkaline nitrobenzene oxidation and found only 2% vanillin. Pearl[3] obtained no vanillin by similar treatment of 5-propenylorthovanillic acid. Leopold[4] has made extensive experiments in this field and has come to the conclusion that such *ortho* C-to-C connected units yield insignificant amounts of vanillin.

In our work the oxidation conditions described by Leopold[4] were used but the isolation procedures modified, as described in the Experimental part. The 5-carboxyvanillin (I, Table I) gave little vanillin as previously reported.[2] The ease of decarboxylation seems, however, to depend on the nature of the substituent groups in the ring. For example, eugetic acid (II) yielded a substantial amount of vanillin. On the other hand, it yielded a large amount of 5-carboxyvanillin, which is produced only in small amount in the case of the lignin in spruce wood.

Guaiacylpropane units in which the carbon atom attached *ortho* to the phenolic-hydroxyl group bears an hydroxyl group might be considered to be formed by the condensation of an aldehyde or ketone function at that point. It is conceivable that, under the conditions of nitrobenzene oxidation, a reverse condensation might take place, thus cleaving the side chain from the ring. The simple compounds of this type (5-allyl-2-hydroxy-3-methoxybenzyl alcohol (III), 5-allyl-2-hydroxy-3-methoxy-α-methylbenzyl alcohol (IV), 5-allyl-2-hydroxy-α,α-dimethoxybenzyl alcohol (V)) gave only small quantities of vanillin and large amounts of 5-formylvanillin and 5-carboxyvanillin. The compound with the flavan ring, 6-allyl-4-hydroxy-3′,4′,8-trimethoxyflavin (VI) did give considerable vanillin. However it has been reported recently[5,6] that, contrary to previous assumptions and experience, veratraldehyde is not stable under the conditions of nitrobenzene oxidation but yields vanillin. The 6-allyl-4-hydroxy-8-methoxyflavan (VII), without the veratryl unit present gave no vanillin.

Spruce wood (VIII) produced about 26% vanillin based on the lignin content. However, when the wood was first digested in sodium hydroxide to extract the lignin, the yield of vanillin dropped by nearly half. Evidently the vanillin yielding groups are unstable in hot alkali. The results obtained with Brauns' spruce "native lignin" (IX) suggest a very close relationship with the bulk of the lignin in spruce wood. The formation of vanillic acid is probably a secondary reaction and might be expected to be higher with the "native lignin," since it goes into solution immediately in the oxidation mixture and is, therefore, available for oxidation sooner than is the case with the lignin of the wood. Noteworthy is absence of appreciable quantities of 5-carboxyvanillin and the content of dehydrodivanillin. The latter is considerably higher than the 0.8% previously reported,[7] due probably to the method used for its isolation.

Dehydrodieugenol (X), dehydrodiisoeugenol (XI), dihydrodehydrodiisoeugenol (XII) and eugenol (XIII) were oxidized for further comparison. The dehydrodieugenol, as to be expected, gave a high (though not quantitative) yield of dehydrodivanillin. With none of the other model compounds could any trace of this product be detected.[8] Pearl[9] obtained 1% of dehydrodivanillin by cupric oxide oxidation of hydrovanilloin and 3% with a similar oxidation of desoxyvanilloin. Well purified samples of these compound (m.p. 234–236° and 157–158.5°, respectively) were prepared and subjected to the nitrobenzene oxidation method described. The chief product in both cases was vanillil (80 and 49%, respectively) and neither compound yielded any trace of dehydrodivanillin.

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[1] Maintained at Madison, Wis., in coöperation with the University of Wisconsin.

[2] K. Freudenberg and E. Plankenhorn, *Ber.*, **80**, 155 (1947).

[3] I. A. Pearl and D. L. Beyer, THIS JOURNAL, **74**, 4263 (1952).

[4] B. Leopold, *Acta Chem. Scand.*, **4**, 1523 (1950).

[5] J. B. Hlava and F. E. Brauns, *Holzforschung*, **7**, 62 (1953).

[6] W. J. Brickman and C. B. Purves, THIS JOURNAL **75**, 4336 (1953).

[7] B. Leopold, *Acta Chem. Scand.*, **6**, 38 (1952).

[8] Supposedly pure dehydrodiisoeugenol gave a small amount, but on further purification gave none.

[9] I. A. Pearl and D. L. Beyer, THIS JOURNAL, **76**, 2226 (1954).

TABLE I

PRODUCTS OF THE ALKALINE NITROBENZENE OXIDATION OF LIGNIN MODEL COMPOUNDS, SPRUCE WOOD AND SPRUCE "NATIVE LIGNIN"

No.	Substance	Percentage Vanillin	Vanillic acid	5-Formyl vanillin	5-Carboxy vanillin	Dehydro-divanillin
I	5-Carboxyvanillin	1.9	0	1.9	85	0
II	Eugetic acid	9.7	...	0	65	0
III	5-Allyl-2-hydroxy-3-methoxybenzyl alcohol	5.6	0	10.0	25	0
IV	5-Allyl-2-hydroxy-3-methoxy-α-methylbenzyl alcohol	3.3	0	7.2	24	0
V	5-Allyl-2-hydroxy-3-methoxy-α,α-dimethylbenzyl alcohol	3.2	0	7.9	17	0
VI[a,b]	6-Allyl-4-hydroxy-3′,4′,8-trimethoxyflavan	14.6[c]	...	0.6	14	0
VII[d]	6-Allyl-4-hydroxy-8-methoxyflavan	Trace	0	0.6	21	0
VIII[e]	Spruce wood	25.8	1.3	..	0.6	2.2
	Spruce wood[f]	14.4	.5	..	0	0.5
IX	Brauns' spruce "native lignin"	25.1	1.9	..	0	2.2
X	Dehydrodieugenol	0	0	0	0.4	69
XI	Dehydrodiisoeugenol	22.6	1.4	0	7.5	0
XII	Dihydrodehydrodiisoeugenol	11.9	Trace	0	Trace	0
XIII	Eugenol	73.1	2.0	0	0.3	0

[a] Also formed were 13.0% veratraldehyde and 2.5% veratric acid. [b] Unreacted original substance in amount of 9.2% was recovered. [c] Probably produced by demethylation of the veratryl group. [d] Also formed were 7.8% benzaldehyde and 8.8% benzoic acid. [e] Based on lignin content. No attempt was made to determine traces of other constituents which have been reported in the literature. [f] In order to dissolve the lignin digestion was first carried out under the conditions described, except that the nitrobenzene was omitted. The nitrobenzene then was added and the cooking cycle repeated.

Therefore the yield of dehydrodivanillin from spruce lignin appears to indicate the presence of an appreciable amount of diphenyl-type linkages. From spectrographic studies, Aulin-Erdtman[10] predicted about 5% of such units (containing free phenolic hydroxyl groups) for coniferous Brauns' "native lignin" and less for other coniferous lignin preparations.

The dehydrodiisoeugenol gave vanillin yields in the range of spruce lignin but a much higher amount of 5-carboxyvanillin. Hydrogenation of the side chain nearly eliminated the 5-carboxyvanillin but also cut in half the yield of vanillin. The results suggest that a majority of the units in spruce lignin are of the uncondensed type.

Experimental[11]

5-Allyl-2-hydroxy-3-methoxy-α-methylbenzyl Alcohol (IV).—Ten grams of 5-allyl-2-hydroxy-3-methoxyacetophenone[12] dissolved in 50 ml. of ethanol was cautiously mixed, with cooling, with 50 ml. of ethanol containing 1 g. of sodium borohydride. After standing 10 min., 25 ml. of water was added and the mixture allowed to stand 4 hr. at −10° for crystals of the borate intermediate to separate. The crystals, after being washed with cold water, were suspended in 10 ml. of water and 10 ml. of 6 *N* hydrochloric acid was added. After 2 hr. the oil was collected with ether, the ether solution washed with dil. sodium bicarbonate solution, then with water, and dried with anhydrous sodium sulfate. The ether was removed by evaporation at reduced pressure, and a stream of nitrogen was led through the liquid at the close of the operation. Vacuum distillation of the residual oil caused decomposition. All attempts to crystallize the oil obtained by this or by modified procedures failed.

Anal. Calcd. for $C_{12}H_{18}O_3$: C, 69.20; H, 7.75. Found: C, 69.40; H, 7.68.

5-Allyl-2-hydroxy-3-methoxy-α,α-dimethylbenzyl Alcohol (V).—To 7.3 g. (0.3 mole) of magnesium in 50 ml. of absolute ether was added dropwise 19 ml. (0.3 mole) of methyl iodide in 25 ml. of absolute ether. When solution was complete, 20.6 g. (0.1 mole) of 5-allyl-2-hydroxy-3-methoxy acetophenone[12] was slowly added. After standing overnight, the mixture was decomposed with ice and sulfuric acid and the oil extracted with ether. The ether solution was dried, the ether removed by evaporation under reduced pressure, and the residue dissolved in *n*-hexane. Crystallization was initiated by cooling in solid carbon dioxide and rubbing. Several recrystallizations from *n*-hexane gave 19.7 g. of colorless product melting at 67.5–69°.

Anal. Calcd. for $C_{13}H_{18}O_3$: C, 70.24; H, 8.16. Found: C, 70.32; H, 8.05.

6-Allyl-3′,4′,8-trimethoxyflavanone.—To a flask equipped with a powerful stirrer and immersed in a bath maintained at 55° were added 20.6 g. (0.10 mole) of 5-allyl-2-hydroxy-3-methoxyacetophenone[12] and 16.6 g. (0.10 mole) of veratraldehyde. After melting, 100 ml. of a solution, consisting of equal weights of potassium hydroxide pellets and water, was added and the mixture was stirred 3 hr. The heavy paste was diluted to 1,500 ml. with water, and the mixture was stirred at 60° for 2 hr. The crude flavanone was removed by filtration and recrystallized twice from absolute ethanol to give 19 g. of colorless crystals, m.p. 127.5–128.5°.

Anal. Calcd. for $C_{21}H_{22}O_5$: C, 71.17; H, 6.26. Found: C, 71.49; H, 6.28.

The mother liquors yielded a small amount of the corresponding 5′-allyl-2′-hydroxy-3,3′,4-trimethoxychalcone as orange crystals, m.p. 143–145°.

Anal. Calcd. for $C_{21}H_{22}O_5$: C, 71.17; H, 6.26. Found: C, 71.33; H, 6.24.

6-Allyl-4-hydroxy-3′,4′,8-trimethoxyflavan (VI).—To a warm solution of 20 g. of the above flavanone and 2 g. of boric acid in 500 ml. of ethanol, a solution of 2 g. of sodium borohydride in 100 ml. of ethanol was added cautiously. After standing overnight in a refrigerator, the crystals were separated, washed well with cold water, and recrystallized from ethanol. Colorless fine needles were obtained in a yield of 17.5 g., m.p. 158–159°. Unlike many compounds of similar nature, no color was produced with concd. hydrochloric acid. The corresponding chalcone, when reduced with sodium borohydride, gave a deep blue color with the acid.

Anal. Calcd. for $C_{21}H_{24}O_5$: C, 70.77; H, 6.79. Found: C, 70.99; H, 6.73.

5′-Allyl-2′-hydroxy-3′-methoxychalcone.—A mixture of 16.5 g. (0.08 mole) of 5-allyl-2-hydroxy-3-methoxyacetophenone,[12] 8.1 g. (0.08 mole) of benzaldehyde and 80 ml. of a solution of equal weights of potassium hydroxide pellets and water was heated 4 hr. at 60° with vigorous stirring. The crude chalcone which separated on dilution with water and acidification was recrystallized from ethanol to give 9 g. orange-colored crystals, m.p. 93–95°.

Anal. Calcd. for $C_{19}H_{18}O_3$: C, 77.53; H, 6.16. Found: C, 77.66; H, 6.11.

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[10] G. Aulin-Erdtman, *Svensk. Papperstidn.*, **55**, 745 (1952).

[11] All melting points are corrected.

[12] J. C. Pew, THIS JOURNAL, **74**, 2855 (1952).

6-Allyl-8-methoxyflavanone.—A solution of 12 g. of the above chalcone in 600 ml. of ethanol and 360 ml. of 3% aqueous hydrochloric acid was refluxed 24 hr., the solution cooled and seeded with the flavanone. After crystallization the mixture was filtered and the filtrate which contained unchanged chalcone was reworked. The flavanone was recrystallized from ethanol to give a white crystal powder, m.p. 98.5–99.5°, total yield 10 g.

Anal. Calcd. for $C_{19}H_{18}O_3$: C, 77.53; H, 6.16. Found: C, 77.49; H, 6.07.

6-Allyl-4-hydroxy-8-methoxyflavan (VII).—To a warm solution containing 10 g. of the above flavanone and 1.0 g. of boric acid in 200 ml. of ethanol, a solution of 1.0 g. of sodium borohydride in 40 ml. of ethanol was added slowly. After this mixture stood 2 hr., 240 ml. of ethanol and 360 ml. of water were added. The mixture was then boiled until the precipitate dissolved, and the solution was allowed to stand 2 hr. The crystals which formed were recrystallized from aqueous alcohol to give 9.2 g. of a white crystal powder, m.p. 126–127°.

Anal. Calcd. for $C_{19}H_{20}O_3$: C, 77.00; H, 6.80. Found: C, 77.27; H, 6.62.

Nitrobenzene Oxidation.—Two grams of model compound or isolated lignin or air-dry extracted spruce wood meal equivalent to 10 g. of dry wood was enclosed in a stainless-steel bomb together with 120 ml. of 2 *N* sodium hydroxide solution and 16 ml. of nitrobenzene. The bomb was placed in an autoclave and steam admitted to bring the pressure to 131 p.s.i. (180°). The autoclave was rotated at 6 r.p.m. for 2 hr., the pressure released, and the bomb removed. The contents of the bomb were transferred to a separatory funnel with ether and a restricted quantity of water and separated into 4 fractions. The first fraction (A) was obtained by extraction with three 200-ml. portions of ether. The residual aqueous solution (after filtration in the case of the wood meal) was acidified to *p*H 4.0 with strong sulfuric acid, cooled to room temperature and allowed to stand about 3 hr. The precipitate, constituting fraction B, was removed by filtration or, if tarry, separated by decantation followed by collection with acetone. The filtrate was next adjusted to *p*H 6.4 with a solution of sodium hydroxide and extracted 24–48 hr. with benzene in an efficient continuous extractor to remove fraction C. The aqueous solution, now *p*H 7–8, was acidified with sulfuric acid to *p*H 1.0. When present in considerable quantity, 5-carboxyvanillin precipitated and was removed by filtration. The filtrate was extracted with three 150-ml. portions of ether, the ether extract was transferred to a separatory funnel, and any precipitated 5-carboxyvanillin was added to the ether extract. This mixture was processed as fraction D.

Fraction A.—In most cases extraction of this ether solution with 20% sodium bisulfite solution yielded no trace of aldehyde. With the flavan VI, fairly pure veratraldehyde was isolated from the bisulfite extract and determined as the 2,4-dinitrophenylhydrazone. When the ether was distilled from the bisulfite-extracted solution and the residual oil diluted with *n*-hexane, unreacted flavan separated. This amounted to over 50% when the reaction was carried out under the standard procedure, but it was reduced to less than 10% by using more vigorous agitation. The latter results are the ones recorded. The flavan VII yielded benzaldehyde but no unreacted material.

Fraction B.—This precipitate was treated with absolute ether, the ether solution filtered, and the filtrate extracted with the two 5-ml. portions of 20% sodium bisulfite solution to remove any trapped vanillin. The bisulfite solution was decomposed in the usual way, the aqueous solution being added to fraction C and any insoluble matter added to the above ether-insoluble part. The extracted ether solution was evaporated to give an orange colored resin. Extraction of this resin with *n*-hexane and cooling to 0° yielded 0.040 to 0.100 g. of crude *p*-hydroxyazobenzene (m.p. 151–152°, when purified). Treatment of the hexane-insoluble residue with alcohol sometimes gave a second orange product melting at 248–249°, probably *p*-phenylazobenzoic acid.[13]

The ether-insoluble residue was treated with a small amount of acetone. The acetone extract on evaporation gave a reddish-brown resin. The brown acetone-insoluble powder was vacuum sublimed (270°, 1 mm.) to give a sublimate of dehydrodivanillin, which was washed with a little acetone and dried (m.p. 315–316°). The unsublimed, nearly black residue was generally infusible.

Fraction C.—The benzene solution was shaken with three 10 to 30 ml. portions of 20% sodium bisulfite solution, the bisulfite extract decomposed with 2 ml. of concd. sulfuric acid per 10 ml. of extract with the aid of heat and a stream of carbon dioxide. The resulting solution was thoroughly extracted with ether, the ether extract dried with anhydrous sodium sulfate, and the ether distilled. The residue was extracted with boiling *n*-hexane until all substance soluble in this solvent was removed. The hexane was distilled to give a residue containing vanillin and sometimes 5-formylvanillin. No very satisfactory method was found to make a clean separation of these compounds. The residue was taken up in absolute ether and, when present in considerable amounts, much 5-formylvanillin remained undissolved and was removed by filtration. The ether solution was then evaporated and 100 ml. of water per gram of residue added. The mixture was allowed to stand at 25° with frequent rubbing and stirring until all vanillin was dissolved. The undissolved 5-formylvanillin was removed by filtration and recrystallized from water; m.p. when well purified, 125–126°. Vanillin in the filtrate was precipitated by adding twice as much 2,4-dinitrophenylhydrazine (in the form of a 1% solution in 6 *N* hydrochloric acid) as the probable content of vanillin. The precipitate was allowed to digest several hours on the steam-bath, filtered through a fritted-glass crucible, washed, and dried to constant weight at 105°.

The benzene solution after extraction by bisulfite, was extracted with 8% sodium bicarbonate solution, the extract acidified and extracted with ether. Evaporation of the ether extract yielded only a small amount of gummy residue. Treatment of the benzene solution in a similar fashion with 5% aqueous sodium hydroxide gave an orange-colored sticky residue smelling of guaiacol.

Fraction D.—The ether mixture was extracted with 20% sodium bisulfite solution, and the extract was decomposed as with fraction C. When a considerable amount of 5-carboxyvanillin precipitated, it was removed by filtration, washed with water, and dried. This residue was suspended in absolute ether, the suspension filtered, the residue washed with a few drops of acetone and then dried and weighed. When only a small amount of the compound was present, the aqueous solution was extracted with ether, the ether evaporated, the residue washed with a few drops of absolute ether and then sublimed at 1 mm. and 230°. The 5-carboxyvanillin melted at 256–258°.

The ether extract, after extraction by the bisulfite, was next extracted with 8% aqueous sodium bicarbonate, the bicarbonate extract acidified and extracted with ether. On evaporation of the ether a resinous residue remained, which was vacuum sublimed (1 mm., 160°) to give vanillic acid, which was recrystallized from water; m.p. 206–208°.

The ether solution was finally extracted with 5% sodium hydroxide solution, but only traces of gummy substance were recovered by this extraction.

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[13] J. E. Jayne, *TAPPI*, **36**, 571 (1953).