

Spectrochemical Estimation of Phenylcoumaran Elements in Lignin

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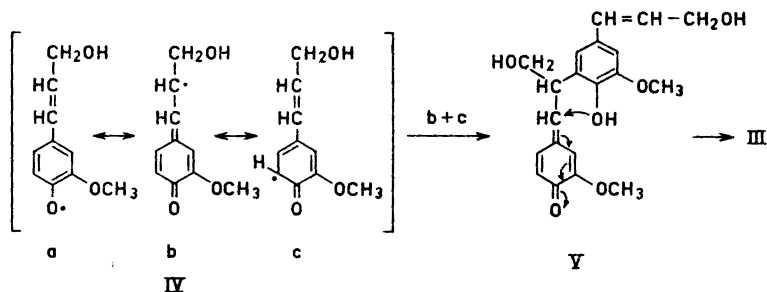
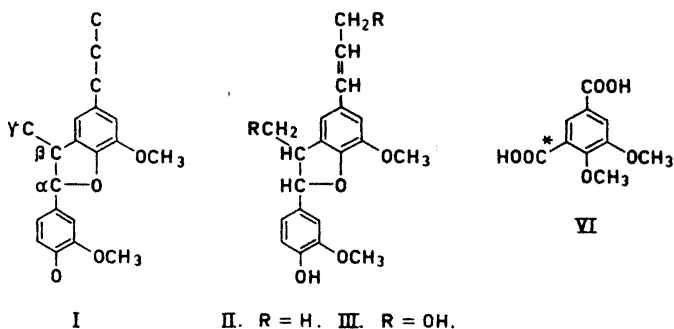
On heating in acid solution, the phenylcoumaran IX (dihydrodehydrodi-coniferyl alcohol) and its monomethyl ether X suffer dehydration, the phenylcoumarone XI and its monomethyl ether XII, respectively, being formed in good yields. Similar acid treatment of spruce Björkman lignin followed by sodium borohydride reduction results in an overall change of the ultra-violet absorption clearly demonstrating the formation of phenylcoumarone residues.

Comparison of the $\Delta\epsilon$ values of the difference curves obtained in the experiments with Björkman lignin and its diazomethane methylation product with those obtained in the model experiments indicated that about one fifth of the phenylpropane building stones of lignin is located in dimeric elements which on acid heating are converted into the phenylcoumarone structure XIX. The majority of the phenylcoumarone precursors possesses the phenylcoumaran structure XVII. Of a total of 100 phenylpropane units of the lignin, 18 units are located in 9 dimeric elements of type XVII. In addition, there seem to be 2-3 "non-cyclized condensed" dimeric elements (type XVIIIa) per 100 phenylpropane units.

As early as 1922, the coumaran system was considered by Klason¹ to be present in lignin. The possibility that lignin may be made up of condensed chains of building units involving coumaran systems was discussed by Freudenberg^{2a}, who later^{2b} proposed the occurrence of phenylcoumaran structures of type I. Erdtman³ showed that dehydrodi-iso Eugenol, a product of the enzymatic or mild chemical dehydrogenation of iso Eugenol, is a phenylcoumaran derivative (II) and he suggested that lignin might similarly be synthesized in plants by dehydrogenation of 4-propenylphenols.

The dehydrogenation concept of lignin formation has been substantiated mainly by Freudenberg's extensive work on the polymerization of coniferyl alcohol (IV a, OH instead of O) by the action of phenol dehydrogenating enzymes (for recent reviews, see Ref.⁴). Dehydrodi-coniferyl alcohol (III) was isolated, in addition to further dimeric and oligomeric products, as an intermediate in the polymerization of coniferyl alcohol by laccase as well as by manganese dioxide⁴. In analogy to Erdtman's view regarding the formation of II³, the formation of the phenylcoumaran III from coniferyl alcohol is

believed ⁴ to involve dehydrogenation to the aroxyl radical IV followed by combination of the mesomeric radical structures IVb and IVc. The resulting quinone methide V then undergoes spontaneous stabilization by the addition of its phenolic hydroxyl group to the quinonoid system.

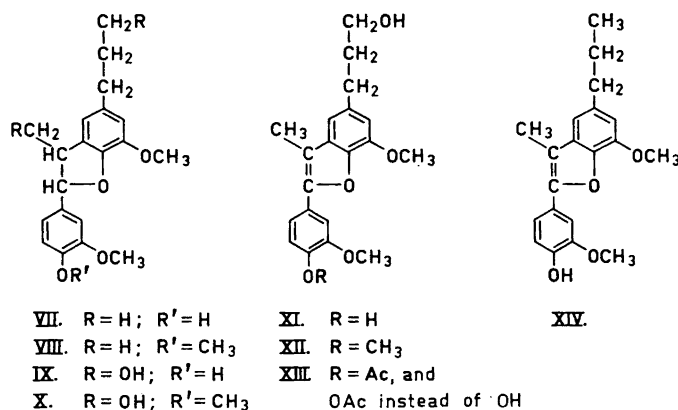


Recent evidence for the presence of the phenylcoumaran system in lignin is based on tracer studies. Freudenberg and Niedercorn ⁵ prepared a "dehydrogenation polymer" from β -¹⁴C-marked coniferyl alcohol; when subjected to hot alkali treatment (cleavage of ether linkages) followed by methylation and permanganate oxidation, the polymer gave 2–3 % radioactive isohemipinic acid (VI, C* = ¹⁴C). This result indicated that linkages between the β -C atom of one molecule and the 5-position of another molecule of coniferyl alcohol were present in the polymer (for the numbering of the phenylpropane units, see XVII). If the polymer was methylated and oxidized without having been heated with alkali, radioactive isohemipinic acid was obtained again, although in a lower yield (0.9 %). Obviously, the polymer contained phenylcoumaran units (I) as well as C_{β} - C_5 condensed dimers with a free phenolic hydroxyl group instead of the ether bridge ("non-cyclic condensed units") ^{5,6}. After the completion of the present studies (preliminary communication, see Ref.⁷), it was reported ⁸ that lignin isolated from a spruce plant after the incorporation of β -¹⁴C-phenylalanine also yielded radioactive isohemipinic acid. The conversion, in spruce, of (marked) phenylalanine into (marked) coniferin had been demonstrated earlier ⁹.

In the course of our analytical studies of lignin structure (for recent reviews, *cf.* Ref.¹⁰) we have now been able to demonstrate the presence of phenylcoumaran units in spruce Björkman lignin and to estimate their number.

Several years ago, the behaviour of a variety of guaiacyl and veratryl carbinols and carbinol ethers towards methanol containing 0.5 % hydrogen chloride — at reflux temperature as well as at 20° — was investigated ¹¹. Whereas

primary and secondary benzyl alcohols such as vanillyl alcohol, veratryl alcohol, guaiacyl ethyl carbinol, and veratryl ethyl carbinol were converted to the corresponding benzyl methyl ethers, dihydro-dehydrodi-isoegenol (VII) and its methyl ether (VIII), which are cyclic benzyl aryl ethers, remained unaffected.



However, the analogous dihydro derivatives of dehydrodi-coniferyl alcohol, *i.e.* the phenol IX¹² and its monomethyl ether X¹², when refluxed with methanolic hydrochloric acid, were slowly converted into new products. In the course of these reactions the initial ultra-violet absorption of the solutions of IX and X, λ_{\max} 281 m μ (ϵ 5900), gradually changed, a new, considerably stronger absorption band, λ_{\max} 310 m μ , replacing the former one. This spectral change was complete after a reaction time of about 24 h. A crystalline reaction product, m.p. 173°, was obtained from the phenylcoumaran IX. Its analysis indicated that it had been formed from IX by the loss of one molecule of water. Treatment with diazomethane gave an alkali-insoluble monomethyl ether, m.p. 94°, and acetylation with acetic anhydride-pyridine yielded a diacetate, m.p. 114°. Kuhn-Roth analysis indicated the presence of one CH₃-C grouping (*cf.* also p. 24). The UV absorption curves of the product of m.p. 173°, in a neutral solvent (Fig. 1, curve 1; λ_{\max} 310 m μ , ϵ 26 400, log ϵ 4.42) and in the same solvent containing 0.1 N NaOH (Fig. 1, curve 2, λ_{\max} 330 m μ , ϵ 31 800), were found to be very similar to the corresponding curves¹³ for the coumarone derivative XIV obtained by Spets¹⁴ on dehydrogenation of dihydro-dehydrodi-isoegenol (VII) with sulphur. These results establish the phenylcoumarone structure XI for the product obtained from phenylcoumaran IX. Structures XII and XIII have then to be ascribed to the methyl ether and the diacetate of XI, respectively.

The proton-catalyzed conversion of the 3-hydroxymethyl coumarans IX and X into the 3-methyl coumarones XI and XII is easily understood as a sequence of ring-opening, allylic rearrangement and recyclization, terminated by (proton-catalyzed) migration of the *exocyclic* styrenoid double bond into the more favoured stilbenoid position, as indicated by Chart 1.

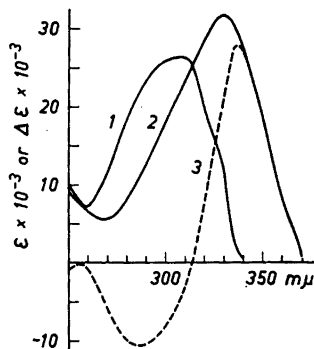


Fig. 1. Ultraviolet absorption of 2-(3-methoxy-4-hydroxyphenyl)-3-methyl-5-(γ -hydroxy-propyl)-7-methoxycoumarone (XI), (1) in neutral solution, (2) in alkaline solution; (3) ionization- $\Delta\epsilon$ curve ($\Delta\epsilon_i$ curve, curve 2 minus curve 1). Neutral solvent, dioxane-water-methanol (7:8:10); alkaline solvent, the same, containing 0.1 N NaOH.

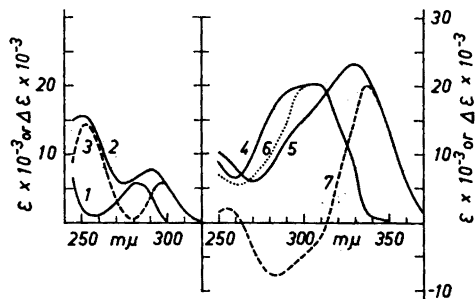
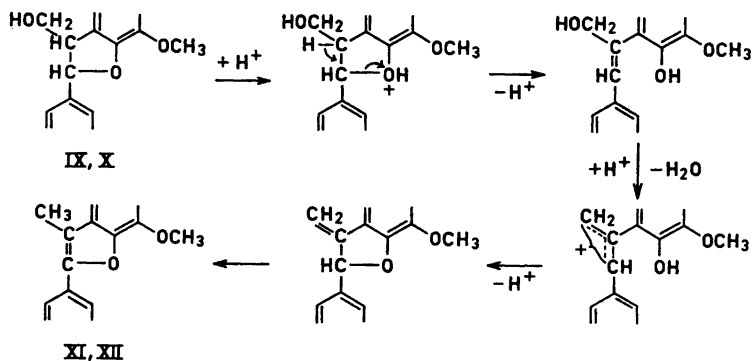


Fig. 2. Acidolysis of dihydro-dehydrodiconiferyl alcohol (IX), ultraviolet absorption. (1) IX in acid solution, (2) in alkaline solution, (3) $\Delta\epsilon_i$ curve of IX; (4) acidolyzed and borohydride-reduced reaction mixture, acid, (5) alkaline; (6) $\Delta\epsilon_a$ curve (= 4 minus 1); (7) $\Delta\epsilon_i'$ curve (= 5 minus 4). Composition of the solutions, see Exptl.

Chart 1.



Examination of the absorption spectrum of the reaction mixture obtained after 24 h refluxing of IX with $\text{CH}_3\text{OH}-\text{HCl}$ indicated that 60 % of the coumaran had been converted into the coumarone XI. The same reaction proceeded more rapidly, if it was carried out in a boiling dioxane-water (9:1) solution containing 0.2 N hydrogen chloride, the gradual spectral change of the reaction mixture being complete after a refluxing period of 10–12 h.

It was expected, therefore, that phenylcoumaran units of the dehydrodiconiferyl alcohol type present in lignin might similarly be convertible into phenylcoumarone units and thus become accessible to spectrometric detection and quantitative estimation. In this case, however, spectrochemically interfering reactions had to be taken into consideration. Thus, it was known from previous work¹⁵ that heating of lignin with 0.2 N HCl in dioxane-water

("acidolysis"*) produces groupings of the "Hibbert ketone" type¹⁰. These include aryl- α -ketone groupings, which have their long-wave absorption maxima at about 310 m μ and therefore would obscure the absorption of the phenylcoumarone residues expected to be formed. This difficulty could be overcome by treating the acidolysis mixture with sodium borohydride, which reduces the ketones (*cf.* Ref.¹⁶) but does not effect the phenylcoumarone system. Comparison of the spectra of the borohydride-reduced acidolysis mixture and the similarly reduced but non-acidolyzed lignin solution could be expected to disclose any formation of phenylcoumarone systems during the acidolysis. Before describing the results obtained with lignin, we want to report the spectrochemical behaviour of the model substance, dihydro-dehydrodi-coniferyl alcohol (IX), on acidolysis under the standard conditions used in this work.

The dihydro compound IX rather than the unsaturated dehydrodi-coniferyl alcohol (III) was chosen as a model substance, since the number of α,β -unsaturated arylpropane units in lignin is known to be very low. Examination of the spectrochemical effects of catalytic hydrogenation of Björkman lignin (= "milled wood lignin") from spruce indicated the presence of only about 3 units of the coniferyl alcohol type in addition to about 3 units of the coniferyl aldehyde type per 100 phenylpropane units¹⁷.

SPECTROPHOTOMETRIC EXAMINATION OF THE ACIDOLYSIS OF MODEL COMPOUNDS IX AND X

In order to make the absorbance values obtained in the model experiments fully comparable with those obtained in the lignin experiments, borohydride was added to both the untreated and the acidolyzed solutions of the model substance. The absorption of the former solution was not affected by the borohydride, but in the acidolyzed solution, borohydride brought about a very slight reduction of the long-wave absorption; obviously side-reactions had produced small amounts of aryl conjugated carbonyl groups.

In Fig. 2, curve 1 shows the molar absorbance of the phenylcoumaran IX. Curve 4 gives the absorbance of the reaction mixture after 20 h of acidolysis of IX, followed by alkaline borohydride reduction and reacidification. The ϵ values are based on the molecular weight of the phenylcoumaran (IX). Comparison of curve 4, ϵ_{\max} 20 200, with the absorption curve of the pure coumarone XI (Fig. 1, curve 1, ϵ_{\max} 26 400) indicates that 76.5 % of the calculated amount of coumarone XI was formed in the acidolysis of coumaran IX.

As shown by Aulin-Erdtman¹⁸, the consideration of absorption changes rather than the absorption spectra as such may be very helpful in spectrochemical studies of lignin structure. In the present work, two types of difference curves ($\Delta\epsilon$ curves) proved to be of value. One of them, the "acidolysis $\Delta\epsilon$ "-curve,

$$\Delta\epsilon_a = \epsilon' - \epsilon$$

* The term "acidolysis" has been used¹⁵ for the dioxane-water-HCl treatment of lignin in order to point out the analogy with the well-known "alcoholysis" of lignin studied by Hibbert. Although, in the present connection, the term refers to a complex sum of reactions rather than an acidolysis in the proper sense, it will be maintained throughout this paper.

represents the difference between the absorbance values of the acidolyzed solution (ϵ') and those of the non-acidolyzed solution (ϵ), both solutions having undergone alkaline borohydride reduction followed by re-acidification.

As a basis for the interpretation of the lignin experiments, the $\Delta\epsilon_a$ curve for the model experiment, *i.e.* the difference between curves 4 (ϵ' curve) and 1 (ϵ curve) (Fig. 2), has been constructed. It is given by curve 6, Fig. 2.

Furthermore, the "ionization- $\Delta\epsilon$ " curves (" $\Delta\epsilon_i$ curves") for the starting solution as well as for the acidolyzed solution were constructed according to the method of Aulin-Erdtman, *viz.*

$$\Delta\epsilon_i = \epsilon_B - \epsilon \text{ and } \Delta\epsilon_i' = \epsilon_B' - \epsilon'$$

where ϵ_B and ϵ_B' represent the absorbance values of the non-acidolyzed and the acidolyzed solutions, respectively, both after borohydride reduction and in the presence of alkali; ϵ and ϵ' (see above) are the absorbance values of the corresponding re-acidified solutions (see also Experimental).

In the case of the acidolysis of coumaran IX (Fig. 2), curve 2 is the alkaline absorption curve (ϵ_B) of the untreated coumaran and curve 3 its $\Delta\epsilon_i$ curve. Curve 5 gives the alkaline absorption curve (ϵ_B') and curve 7 the ionization- $\Delta\epsilon$ curve ($\Delta\epsilon_i'$, *i.e.* curve 5 minus curve 4) of the acidolyzed solution. The positions of the long-wave maxima of curves 5 and 7, *viz.* 330 and 338 $m\mu$, respectively, agree with those of the corresponding maxima obtained with the pure coumarone XI (*cf.* Fig. 1, curves 2 and 3). When comparing the $\Delta\epsilon_{\max}$ value of curve 7 (20 200) with the corresponding value for the pure coumarone XI (see Fig. 1, curve 3, $\Delta\epsilon_{\max}$ 27 900), it is found that the 20 h acidolysis of coumaran IX produced 72.4 % of the calculated amount of coumarone XI. This is in satisfactory agreement with the value of 76.5 % derived from comparison of the acid absorbance values (see above).

As a model experiment for the behaviour of non-phenolic phenylcoumaran units in lignin, the acidolysis of the coumaran methyl ether X¹² was also studied (Fig. 3). The absorption curve of the coumaran X (curve 1) was replaced, after 20 h of acidolysis, by curve 2, which in its general shape is similar to the absorption curve of the pure coumarone methyl ether XII. [The latter (λ_{\max} 310 $m\mu$, $\log \epsilon$ 4.45) was found to be almost identical with that of the phenolic analogue XI (Fig. 1)]. The extinction of the acidolysis curve indicates a yield of 72.6 % of coumarone methyl ether XII, *i.e.*, the degree of conversion was very similar to that observed with the phenolic model IX.

The acidolyzed solution of the coumaran methyl ether X, after being made alkaline, exhibited a distinct green fluorescence in daylight. Alkali treatment also caused a slight shift of the absorption curve, the $\Delta\epsilon_i'$ curve showing comparatively weak maxima around

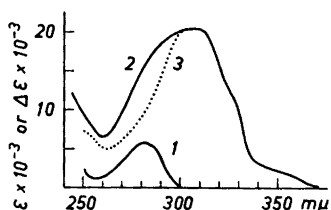
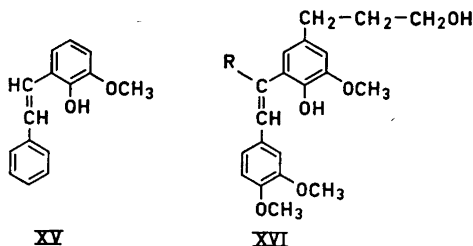


Fig. 3. Acidolysis of dihydro-dehydrodiconiferyl alcohol monomethyl ether (X), ultraviolet absorption. (1) X in acid solution, (2) acidolyzed and borohydride-reduced reaction mixture, acid; (3) $\Delta\epsilon_a$ curve (= 2 minus 1). Composition of the solutions, see Exptl.

255 $m\mu$ ($\Delta\epsilon_i'$ 1500) and 375 $m\mu$ ($\Delta\epsilon_i'$ 2450) indicating the presence of a strongly conjugated phenolic system. For comparison, 2-hydroxy-3-methoxystilbene (XV) was synthesized (see Experimental); it was found to be similar to the unknown by-product of the acidolysis mixture both with regard to the green fluorescence in alkaline solution and the general appearance of the $\Delta\epsilon_i'$ curve. The acidolysis by-product therefore may be a stilbene of type XVI, stabilized in the "open" form possibly by the loss of the $-\text{CH}_2\text{OH}$ substituent (as formaldehyde?) ($\text{R} = \text{H}$).



It was found recently in this laboratory¹⁹ that similar 2-hydroxystilbenes are formed when dihydro-dehydrodi-coniferyl alcohol (IX) is heated to 160° with aqueous sodium hydroxide in the presence of sodium sulphide ("kraft cooking" conditions).

SPECTROPHOTOMETRIC EXAMINATION OF THE ACIDOLYSIS OF BJÖRKMANN LIGNIN

In order to make the lignin curves readily comparable with the molar absorbance curves of the model compounds IX—XII, ϵ in the former curves has been based on a 2 OCH_3 equivalent weight, which approximates to the average weight of a dimeric phenylpropane unit in the lignin.

An example of the experiments carried out with Björkman lignin is given in Fig. 4. An untreated spruce lignin preparation (15.41 % OCH_3), after reduction with NaBH_4 , gave the absorption curves 1 and 2 in acid and alkaline solution, respectively. The resulting $\Delta\epsilon_i$ curve (3) exhibits the typical maximum near 300 $m\mu$ due to the ionization of non-conjugated phenols and the gradually descending slope above 315 $m\mu$ caused by the ionization of phenolic units containing a β, γ -unsaturated side-chain as well as of phenolic diphenyl units^{18,17}. (The $\Delta\epsilon_i$ maximum near 350 $m\mu$, characteristic of 4-ketoguaiacol residues¹⁸, is missing, indicating that the NaBH_4 reduction has been effective.)

After 20 h acidolysis followed by NaBH_4 reduction, the markedly increased absorption curves 4 and 5 (in acid and alkaline medium, respectively) were obtained. By subtracting curve 1 (ϵ) from curve 4 (ϵ') the "acidolysis- $\Delta\epsilon$ " curve 6 ($\Delta\epsilon_a$) was constructed. It represents the spectral change caused by the acidolysis reactions except for the absence of carbonyl chromophores (eliminated by the NaBH_4 treatment). It is obviously very similar to the corresponding $\Delta\epsilon_a$ curves obtained in the model experiments (Fig. 2, curve 6, and Fig. 3, curve 3), exhibiting the broad maximum at 305–310 $m\mu$ and the characteristic weak shoulder at about 325 $m\mu$, thus indicating the presence of (phenolic and non-phenolic) phenylcoumarone residues (type XIX, $\text{R} = \text{H}$ or C) in the acidolysis mixture.

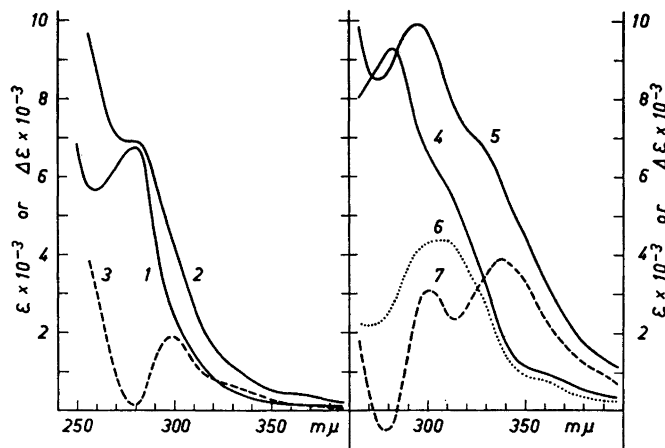


Fig. 4. Acidolysis of Björkman lignin, ultraviolet absorption. (1) NaBH_4 -reduced lignin, in acid solution, (2) in alkaline solution, (3) $\Delta\epsilon_i$ curve (= 2 minus 1); (4) acidolyzed and NaBH_4 -reduced reaction mixture, acid, (5) alkaline, (6) $\Delta\epsilon_a$ curve (= 4 minus 1), (7) $\Delta\epsilon_i'$ curve (= 5 minus 4). Composition of the solutions, see Exptl.

Conjugated chromophores of the coniferyl alcohol type, if formed in the acidolysis reaction, would make a contribution to the $\Delta\epsilon_a$ curve. (In ethanol, coniferyl alcohol has λ_{max} 266 $m\mu$, ϵ 15 800 and a shoulder at about 295 $m\mu$, ϵ 6700.) It was found, however, that on acid treatment of coniferyl alcohol the absorption spectrum is converted to that characteristic of non-conjugated phenols (λ_{max} 281 $m\mu$, ϵ 4000), this change being due to the well-known acid polymerization of coniferyl alcohol. It was also found that (+)-pinosresinol, when treated according to the standard "acidolysis" procedure, did not give rise to any appreciable number of (non-ketonic) conjugated phenolic chromophores.

Subtraction of the ϵ' values (Fig. 4, curve 4) from the ϵ_B' values (curve 5) gave the "ionization- $\Delta\epsilon$ " curve (7) of the acidolyzed and NaBH_4 -treated lignin product ($\Delta\epsilon_i'$). It differs from the $\Delta\epsilon_i$ curve 3 (non-acidolyzed lignin after NaBH_4 -treatment) in exhibiting a strong maximum at 337–338 $m\mu$, identical with that of the $\Delta\epsilon_i$ curve of the phenolic coumarone XI (Fig. 1, curve 3, and Fig. 2, curve 7).

The appearance of the $\Delta\epsilon_a$ and the $\Delta\epsilon_i'$ curves (6 and 7, Fig. 4), in our opinion, constitutes clear evidence for the presence of phenylcoumarone structures (XIX) in the acidolyzed lignin.

The close resemblance between the difference curves of the acidolyzed lignin and the model coumarones (XI and XII) permitted the quantitative estimation of the coumarone residues present in the acidolyzed lignin. The $\Delta\epsilon_a$ value found at 310 $m\mu$ (Fig. 4, curve 6) was 4360; by comparison with the mean value of ϵ_{310} for the pure phenylcoumarones XI and XII, *i.e.* 27 250, it follows that $4360 \times 100/27\,250 = 16\%$ of all the dimeric units of the acidolyzed lignin possessed the phenylcoumarone structure (XIX). Since, in the model experiments, the average yield of phenylcoumarone from the coumarans IX and X was 74%, it may further be concluded that in the original Björkman lignin $16/0.74 = 22\%$ of the dimeric units possessed the phenylcoumaran

structure (XVII) or similar structures which, on acidolysis, yielded the phenylcoumarone structure (XIX).

A similar calculation could be based on the ionization- $\Delta\epsilon$ curves. The $\Delta\epsilon_i'$ value at 338 $m\mu$ (Fig. 4, curve 7), *i.e.* 3900, when compared with the $\Delta\epsilon_i$ value (27 900) of the phenylcoumarone XI at the same wavelength (Fig. 1, curve 3), would indicate the presence of 14 % phenolic phenylcoumarone residues in the acidolyzed material. This figure, however, probably is slightly too high, since phenolic 6,6'-diguaiacyl elements may contribute to the $\Delta\epsilon_i'$ value at 338 $m\mu$ (*cf.* the $\Delta\epsilon_i$ curve of a 6,6'-diguaiacyl model substance reported by Aulin-Erdtman¹⁵).

The acidolysis experiment reported above was repeated with good reproducibility with the same lignin preparation, the two figures obtained being 22 and 21 % of dimeric units of the phenylcoumaran type. An experiment with another Björkman lignin preparation (OCH₃ 15.0 %) gave a value of 24 % for the proportion of phenylcoumaran type dimers.

It may also be mentioned that after an acidolysis period of 3 h, the $\Delta\epsilon_a$ values were about 60 % of the final ones (obtained after 20 h), both with the model coumarans and the lignin. This finding constitutes further support for the view that the phenylcoumarone residues formed in the acidolysis of lignin originate from phenylcoumaran or similar units.

In addition to the phenylcoumaran units (XVII), non-cyclic condensed dimeric systems (XVIII) may be precursors of the phenylcoumarone systems (XIX) formed in the acidolysis of lignin. The occurrence, in lignin, of systems of the type XVIII *a* (R'' = H) has been postulated (see p. 14). In such systems, OR''' probably cannot be a free OH group, as this almost certainly would favour spontaneous ring closure²⁰; a benzyl ether (R''' = C), however, would readily be hydrolyzed under the acidolysis conditions, and phenylcoumarone (XIX) would be formed.

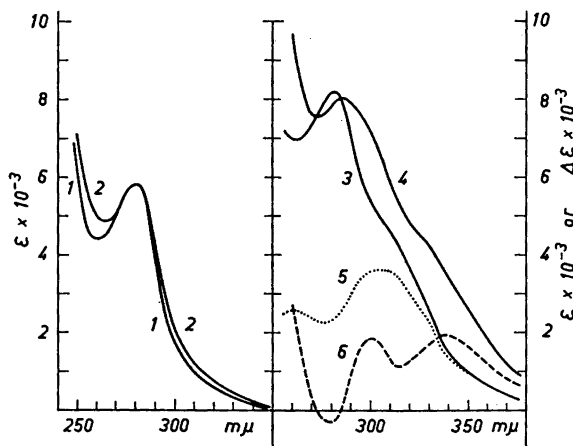
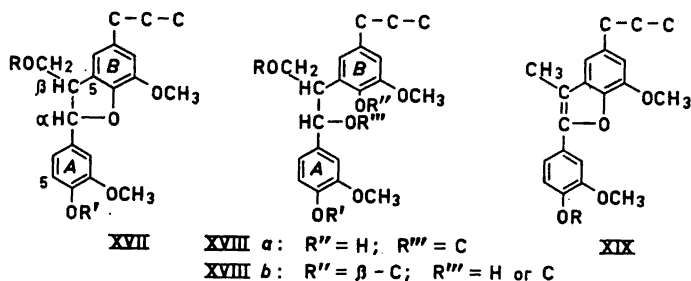


Fig. 5. Acidolysis of diazomethane-methylated Björkman lignin, ultraviolet absorption. (1) NaBH₄-reduced methylated lignin in acid solution, (2) in alkaline solution; (3) acidolyzed and NaBH₄-reduced reaction mixture, acid, (4) alkaline; (5) $\Delta\epsilon_a$ curve (= 3 minus 1), (6) $\Delta\epsilon_i'$ curve (= 4 minus 3). Composition of the solutions, see Exptl.

The possible contribution made by non-cyclic structures of type XVIII *a* to the formation of phenylcoumarone residues was examined by studying the acidolysis of a diazomethane-methylated Björkman lignin (OCH₃ 19.64 %, obtained from a lignin preparation containing 15.0 % OCH₃). In this lignin derivative, the phenolic hydroxyl groups (OR'' = OH) of units of type XVIII *a* would be expected to be replaced by OCH₃ groups, which would prevent such units from being converted into coumarone systems. Spectrochemical analysis of the acidolysis mixture (Fig. 5, ϵ -values based on a weight equivalent corresponding to 2 OCH₃ present before the diazomethane methylation) indicated that, in the methylated lignin, 18 % of the dimeric units were phenylcoumarone precursors. Since, in untreated lignin, the number of such precursors was found to be 21–24 % (see p. 21), the majority is of the phenylcoumaran type (XVII), only a minor number (about 4 %) possibly being of the non-cyclic type XVIII *a*.

DISCUSSION

The general phenylcoumaran structure XVII may be ascribed to the majority of the dimeric units in Björkman lignin which yield phenylcoumarones. The substituent ROCH₂— may be a free hydroxymethyl group (R = H) or it may represent an ether, for instance a benzyl ether grouping (R = α -C of



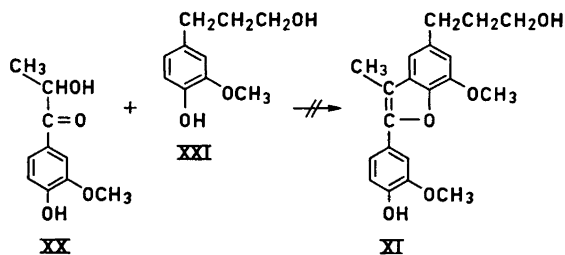
another unit). In the latter case, the initial ring opening steps (see Chart 1) would produce a cinnamyl (-benzyl) ether grouping which would be readily hydrolyzed; conversion into the phenylcoumarone system XIX would then be possible. R' may be a hydrogen atom or — in a considerable portion of the phenylcoumaran dimers — the β -C atom of an arylglycerol unit; this particular type of ether structure is cleaved on acidolysis^{15,10} and therefore would account for the high percentage of phenolic phenylcoumarone residues present in the acidolyzed lignin (p. 21).

The 5-position of ring A may be free or "condensed" with a further unit forming, for example, a diphenyl linkage. In the latter case, the phenylcoumarone formed would contain a more highly conjugated chromophore. However, the $\Delta\epsilon_a$ and $\Delta\epsilon_i'$ curves of the acidolyzed lignin were very similar to the corresponding curves of the simple phenylcoumarone (XI, XII). This indicates either that diphenyl condensed phenylcoumaran systems are rare in lignin, or that the influence of diphenyl conjugation on the absorption of the phenyl-

coumarone systems formed is small due to lack of coplanarity in the diphenyl grouping.

In addition to the non-cyclic dimeric systems of type XVIII *a* (p. 21–22), those of type XVIII *b*, with an ether bridge (OR'') between ring B and the β -position of an arylglycerol unit, might also give phenylcoumarone, since this type of aryl alkyl ether has been shown to be hydrolyzed by acids^{15,10}. So far, there is no way of examining the possible participation of such units in the formation of phenylcoumarone. Finally, OR'' in XVIII might be an aryl benzyl ether grouping, which would be easily hydrolyzed; the occurrence of such groupings in lignin, however, is less probable^{10a,21}, although intermediates involving aryl benzyl ether groupings have been obtained on dehydrogenation of coniferyl alcohol *in vitro*^{4,22}. If structures of type XVIII *b* are present at all in lignin, their number would probably be rather low, since ring closure of the quinone methide V would be expected to be favoured. Until further work has been done it seems justified to ascribe the phenylcoumaran structure (XVII) to the majority of the phenylcoumarone precursors.

The reaction of lignin with phenols in the presence of acids was suggested by Wacek and co-workers²³ to involve the formation of phenylcoumarone systems (XIX) by condensation between primarily formed Hibbert ketone groupings and the added phenol. Similar reactions might be considered to take place in the acidolysis process, Hibbert ketone groupings reacting with phenolic nuclei present in the lignin. If actually occurring, such reactions would interfere with the interpretations given above. However,



spectrophotometric examination showed that no phenylcoumarone (XI) was formed when a mixture of α -hydroxypropioguaiacone (XX) and dihydroconiferyl alcohol (XXI) was subjected to the acidolysis procedure.

To summarize, the present investigation has demonstrated the occurrence of phenylcoumaran elements (XVII) and, possibly, that of "open" condensed systems (XVIII*a*) in Björkman lignin, both types of dimeric units giving rise to phenylcoumarone systems (XIX) on acidolysis. Furthermore, it seems justified to draw the following quantitative conclusions. Per 100 phenylpropane units (approximately equal to 100 OCH₃ groups) of the lignin, about 9 units (A in formula XVII) are linked to an adjacent unit (B) by a C _{α} -aryl ether as well as a C _{β} -C₅ bond, giving rise to about 9 dimeric units of a phenylcoumaran structure (XVII). In addition, 2–3 units (A in formula XVIII *a*) seem to be linked to a unit B only by the C _{β} -C₅ bond, the phenolic hydroxyl group of unit B being free. The 11–12 dimeric units thus formed then account for 22–24 of a total of 100 phenylpropane units.

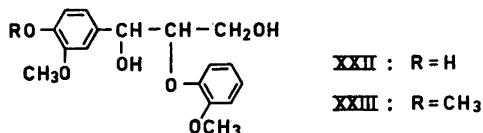
ADDENDUM: A NOTE ON THE OCCURRENCE OF $\text{CH}_3\text{-C}$ GROUPS IN LIGNIN

According to Hibbert and co-workers²⁴, 2.2–2.4 % acetic acid is formed, if pre-extracted spruce wood is oxidized with chromic acid. Since the determination of ester acetyl groups in the wood gave a similar value, it was concluded that wood contains no appreciable quantity of $\text{CH}_3\text{-C}$ groups.

If Björkman lignin (spruce) was subjected to the Kuhn-Roth analysis, 3 % of acetic acid (average of three determinations) was formed, indicating a content of 0.1 C-bound CH_3 group per OCH_3 . For the interpretation of this result, however, the following considerations are of importance.

When the chromic-sulfuric acid mixture used in the Kuhn-Roth analysis is acting upon the lignin, some conversion of the 3-hydroxymethyl-phenylcoumaran and similar residues (XVII, XVIII) into 3-methyl-phenylcoumarone residues (XIX) may take place, in competition with the direct oxidation of the 3-hydroxymethyl substituent. Subsequently, acetic acid would originate from the 3-methyl-phenylcoumarone residues formed. In fact, dehydrodi-coniferyl alcohol (III) was found to give, in the Kuhn-Roth analysis, an apparent content of 0.17 C-bound CH_3 groups per mole. On the basis of the results reported in the present paper, one therefore might expect that the residues XVII + XVIII present in lignin (22 % of all dimeric elements, or 0.11 per OCH_3) can give rise to $0.11 \times 0.17 = 0.02 \text{ CH}_3\text{-C/OCH}_3$ and thus account for about 20 % of the $\text{CH}_3\text{-C}$ value found for the lignin.

In addition, acetic acid could be assumed to originate from "Hibbert ketone" structures arising from arylglycerol(- β -aryl ether) units, if these undergo the acid-catalyzed Hibbert rearrangement in competition with their direct oxidation. In fact, the model



compounds XXII²⁵ and XXIII²⁶, when subjected to the Kuhn-Roth analysis, gave an apparent content of 0.21 and 0.16 $\text{CH}_3\text{-C}$ per mole, respectively. If 35–40 % of all the phenylpropane units in lignin were arylglycerol units, these, together with the phenylcoumaran elements, might well account for the amount of acetic acid obtained from Björkman lignin.

On the basis of these results, the assumption of any appreciable number of C-bound CH_3 groups in the phenylpropane units of lignin seems to be unjustified.

EXPERIMENTAL

The "acidolysis" reaction and its spectrochemical evaluation. The sample (15–17 mg of the models IX or X or about 100 mg of the Björkman lignin preparations) was dissolved in 50 ml of a dioxane-water (9:1) mixture containing 0.2 N HCl. (The dioxane was carefully purified^{27a} and distilled through an efficient column in order to remove spectrophotometrically interfering traces of benzene frequently found in commercial dioxane^{27b}; if peroxides could be detected, they were removed by redistilling the solvent over sodium.) The solution was heated under reflux, nitrogen being introduced into the solution during the first hour, and in the upper part of the condenser during the remaining reaction period. Spectrophotometric examination had shown that the conversion of the model compounds IX and X was complete after 10–12 h; standard acidolysis of the models and of the lignin preparation was carried on for a period of 20 h.

Five ml samples of the untreated solution or of the reaction mixture after 20 h acidolysis were mixed with 5 ml of 0.2 N aqueous NaOH, 5 ml of dioxane and 10 ml of aqueous NaBH_4 solution (2 g NaBH_4 in 250 ml of 0.03 N aqueous NaOH). The mixtures were kept at room temperature for 6 days.

Samples of the reduced solutions for the determination of the acid and alkaline absorption spectra were prepared as follows.

Acid sample: 5 ml of the NaBH_4 -reduced solution were mixed with 5 ml of a 19:1 dioxane-water mixture containing 0.1 N HCl, followed by 5 ml of water and 10 ml of methanol.

Alkaline sample: As the acid sample, 5 ml of 0.5 N aqueous NaOH, however, being added instead of water.

The ultraviolet spectra were recorded on a Perkin-Elmer Spectracord 4000-A instrument. Blank solutions of the same composition as the acid or alkaline samples were used.

Dehydrodi-coniferyl alcohol (III) was prepared by enzymatic^{12,28} or manganese dioxide²² dehydrogenation of coniferyl alcohol. The separation of III was accomplished by chromatography on a silica gel column. The solvent, benzene-acetone 4:1, was chosen on the basis of thin layer chromatography experiments.

*Dihydro-dehydrodi-coniferyl alcohol (IX)*¹² was obtained by catalytic hydrogenation of III according to Adler and Marton²⁹.

2-(3-Methoxy-4-hydroxyphenyl)-3-methyl-5-(γ -hydroxypropyl)-7-methoxycoumarone (XI). A solution of 1 g of dihydro-dehydrodi-coniferyl alcohol (IX) in 250 ml of a 9:1 mixture of dioxane-water, containing 0.2 N HCl, was refluxed under nitrogen for 16 h. The reaction mixture was neutralized with solid NaHCO_3 and the solvent evaporated under vacuum. The residue was dissolved in a mixture of chloroform and water, and the chloroform layer separated and evaporated to dryness. The oily residue was dissolved in methanol. Cooling of the methanol solution gave prisms of XI, m.p. 171–172° (yield, 33 %), after recrystallization from methanol, m.p. 173°. (Found: C 69.80; H 6.62; OCH_3 18.23; CH_3 bound to C (Kuhn-Roth) 4.17. Calc. for $\text{C}_{26}\text{H}_{22}\text{O}_5$: C 70.16; H 6.48; OCH_3 18.13; $\text{CH}_3(-\text{C})$ 4.39.)

In a similar way, the coumarone XI is obtained after refluxing a $\text{CH}_3\text{OH}-\text{HCl}$ solution of IX^{7,11}. UV absorption spectrum, Fig. 1.

Monomethyl ether (XII). Diazomethane in ether was added to the methanolic solution of XI. After 2 h, the solvent was removed, and the oily residue, which had no phenolic properties (no shift of UV absorption on addition of alkali), crystallized on the addition of a little methanol and cooling to -20° . The crystals were collected after the addition of a little ether. M.p. 94°. (Found: OCH_3 26.13. Calc. for $\text{C}_{27}\text{H}_{24}\text{O}_5$: OCH_3 25.98.) The IR absorption spectrum shows a hydroxyl band at 3330 cm^{-1} .

Diacetate (XIII). From XI with acetic anhydride and pyridine at room temperature. M.p. 114° after recrystallization from ethanol. (Found: C 67.39; H 6.11; OCH_3 14.68; acetyl 18.2. Calc. for $\text{C}_{24}\text{H}_{26}\text{O}_7$: C 67.59; H 6.16; OCH_3 14.55; acetyl 20.2.) The IR spectrum showed no hydroxyl band; CO-bands at 1768 (aryl ester) and 1748 cm^{-1} (alkyl ester).

2-Hydroxy-3-methoxystilbene (XV). Prepared from *o*-vanillin and benzyl chloride according to the method reported³⁰ for the preparation of 2-hydroxystilbene. M.p. 87° after recrystallization from methanol. (Found: C 79.44; H 6.24; OCH_3 13.37. Calc. for $\text{C}_{15}\text{H}_{14}\text{O}_2$: C 79.62; H 6.24; OCH_3 13.72.)

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REFERENCES

1. Klason, P. *Ber.* **55** (1922) 448; **56** (1923) 300.
2. a) Freudenberg, K. *Sitz. ber. Heidelberg. Akad. Wiss., Math.-naturw. Kl.*, Abhandl. 19 1928; b) Freudenberg, K. and Sohns, F. *Ber.* **66** (1933) 262; Freudenberg, K. *Ann. Rev. Biochem.* **8** (1939) 81.
3. Erdtman, H. *Biochem. Z.* **258** (1933) 177; *Ann.* **503** (1933) 186; *Research* **3** (1950) 63; Erdtman, H. and Wachtmeister, C. A. *Festschrift Arthur Stoll*, Basel 1957, p. 144.
4. Freudenberg, K. *Chem. Ber.* **92** (1959) LXXXIX; *J. prakt. Chem.* **10** (1960) 220; *J. Polymer Sci.* **48** (1960) 371; *Holz, Roh- u. Werkstoff* **18** (1960) 282; Freudenberg, K. and Sidhu, G. S. *Holzforschung* **15** (1961) 33.
5. Freudenberg, K. and Niedercorn, F. *Chem. Ber.* **89** (1956) 2168.

6. Richtzenhain, H. *Svensk Papperstid.* **53** (1950) 644; *Acta Chem. Scand.* **4** (1950) 206.
7. Adler, E., Delin, S. and Lundquist, K. *Acta Chem. Scand.* **13** (1959) 2149.
8. Freudenberg, K. and Lehmann, B. see Ref.⁷ in Freudenberg, K., Jovanovic, V. and Topfmeier, F. *Chem. Ber.* **96** (1961) 3227.
9. Freudenberg, K. and Niedercorn, F. *Chem. Ber.* **91** (1958) 591.
10. a) Adler, E. *Paper and Timber (Finland)* **43** (1961) 634; b) *Papier* **15** (1961) 604.
11. Adler, E. and Delin, S. *Unpublished results*; for preliminary reports, see Adler, E. *Ind. Eng. Chem.* **49** (1957) 1377; *Proc. IVth Intern. Congr. Biochem.*, Vol. II, Pergamon Press, London 1959, p. 137.
12. Freudenberg, K. and Hübner, H. H. *Chem. Ber.* **85** (1952) 1181.
13. Aulin-Erdtman, G. *Svensk Papperstid.* **56** (1953) 91, 287.
14. Spets, Å. *Acta Chem. Scand.* **8** (1954) 360.
15. Adler, E., Pepper, J. M. and Eriksoo, E. *Ind. Eng. Chem.* **49** (1957) 1391.
16. Adler, E. and Marton, J. *Acta Chem. Scand.* **13** (1959) 75.
17. Marton, J. and Adler, E. *Acta Chem. Scand.* **15** (1961) 370.
18. a) Aulin-Erdtman, G. *Svensk Kem. Tidskr.* **70** (1958) 145. b) Aulin-Erdtman, G. and Hegbom, L. *Svensk Papperstid.* **61** (1958) 187.
19. Falkehag, I. *Paper and Timber (Finland)* **43** (1961) 655 (*Preliminary communication*).
20. Freudenberg, K., Wilk, H., Leuck, H.-U., Knof, L. and Fung, T. H. *Ann.* **630** (1960) 1.
21. Törnquist, J. *Paper and Timber (Finland)* **43** (1961) 671 (*Preliminary communication*).
22. Freudenberg, K. and Friedmann, M. *Chem. Ber.* **93** (1960) 2138.
23. von Wacek, A. and Däubner-Rettenbacher, H. *Monatsh.* **81** (1950) 266; von Wacek, A. and Zeisler, F. *Ibid.* **83** (1952) 5; von Wacek, A. and Limontschew, W. *Ibid.* **84** (1953) 453.
24. MacGregor, W. S., Evans, T. H. and Hibbert, H. *J. Am. Chem. Soc.* **66** (1944) 41.
25. Adler, E. and Eriksoo, E. *Acta Chem. Scand.* **9** (1957) 1391.
26. Adler, E., Lindgren, B. O. and Saedén, U. *Svensk Papperstid.* **55** (1952) 245.
27. a) Vogel, A. I. *Practical Organic Chemistry*, 2nd Ed., London 1954, p. 175. b) Tunnicliff, D. D. *Talanta* **2** (1959) 341.
28. Freudenberg, K. and Schlüter, H. *Chem. Ber.* **88** (1955) 617.
29. Adler, E. and Marton, J. *Acta Chem. Scand.* **15** (1961) 357.
30. Kaufmann, H. P. *Ann.* **433** (1923) 241.

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