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Acid Degradation of Lignin

II.* Separation and Identification of Low Molecular Weight Phenols

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Refluxing of lignin with hydrogen chloride in dioxane-water solution effects a degradation of the lignin with liberation of phenols of low molecular weight. The separation and identification of a number of such phenols are described, and their relationships to structural elements in lignin are discussed.

As reported earlier [1,2,3a,4] a number of low molecular weight phenols have been detected in the reaction mixture obtained on degradation of Björkman lignin [5] from Norway spruce (*Picea abies*) by refluxing with 0.2 M hydrogen chloride in dioxane-water (9:1) ("acidolysis") for 4 h. In this paper the separation and identification of these and some more recently detected degradation products are described. The total amount of low molecular weight phenolic material (compounds with one or two aromatic rings) formed was estimated to be 17 % by weight of the original lignin. The identified phenols constitute the major part of this fraction.

Concomitantly with the work on separation and identification of degradation products, studies of the reactions of lignin model compounds on acidolysis have been carried out. On the basis of these studies and of earlier work on the acidolysis of lignin and lignin model compounds see (Ref. 6) it has been possible to relate almost all of the identified compounds to structural elements in the lignin.

The degradation of the lignin effected by the acidolytic treatment could be visualized readily by gel filtration (Fig. 1). From earlier analytical studies [7,6] it was concluded that the degradation of lignin caused by acidolysis is due primarily to cleavage of the β-ether bonds in arylglycerol-β-aryl ether structures (XX) (see Fig. 2). The finding that ketol I was the most abundant of the low molecular weight phenols formed is in harmony with this view (see Fig. 2).

It has recently been reported [8] that a considerable decrease in average molecular weight occurs when Björkman lignin from spruce is treated with

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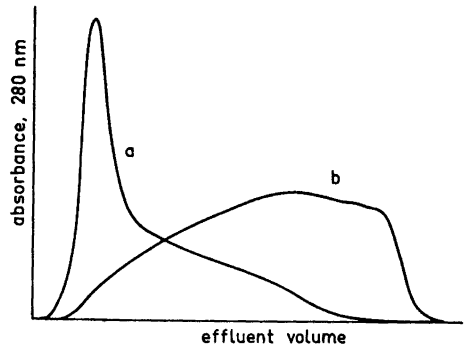
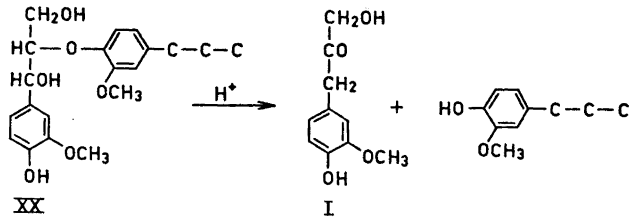


Fig. 1. Gel filtration of Björkman lignin from spruce (a) and the reaction product obtained on 4 h acidolysis of the lignin (b). Gel material: Sephadex G-50. Eluting solvent: dimethyl sulfoxide.



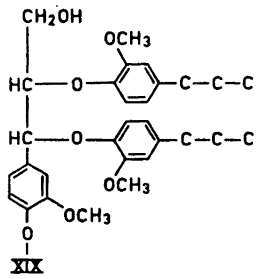
XX I

Fig. 2. The cleavage of the β-ether bond in guaiacylglycerol-β-aryl ethers on acidolysis.

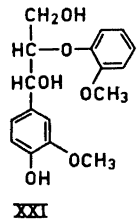
the acidolysis reagent for 24 h at 50°. Experiments with lignin model compounds indicated that under these conditions the observed degradation should be due to hydrolysis of benzyl aryl ethers of type XIX, while the β-ether bonds in structural elements of type XX (or XIX) should remain unaffected.[8] Furthermore, these findings make it reasonable to believe that benzyl aryl ether linkages in structural elements of type XIX in lignin, under the more drastic conditions of acidolysis (*i.e.* refluxing instead of heating at 50° with the acidolysis reagent), are rapidly hydrolyzed and that this reaction is of importance for the degradation of the lignin. It should be noted, however, that when the lignin was treated with the acidolysis mixture at 50° only about 3 % of phenols of low molecular weight (compounds with one or two aromatic rings) were formed.[9] This suggests that the cleavage of such bonds *per se* gives rise to only a minor portion of the low molecular weight phenols which arise on 4 h acidolysis.

A further reaction which may be of some importance for the formation of phenolic products on acidolysis of lignin is the hydrolysis of glyceraldehyde-2-aryl ethers,[10] a type of structural element which recently has been proposed to be present in lignin.[10]

In addition to the phenolic degradation products described in this paper pyruvaldehyde,[10] formaldehyde,[11] and methanol[11] also have been shown to arise on acidolysis of spruce lignin. Studies of the formation of formaldehyde showed that the yield increased when the concentration of lignin in the reac-



XIX



XXI

tion mixture was decreased.[11] A probable explanation of this finding is that liberated formaldehyde is consumed to a larger extent by reactions with the lignin when the concentration of this material is increased. A plausible reaction is condensation with phenolic units to yield diphenylmethane structures. While the reactions discussed above (acidolytic cleavage of ether bonds) degrade the lignin, such condensation reactions would act in the opposite direction. A further conceivable condensation reaction has been considered, *viz.* the reaction of benzyl alcohol groups in structural elements of type XX — which constitute a quantitatively important part of the lignin molecule — with phenolic units to form diphenylmethane structures. To check this possibility a lignin model compound of type XX, guaiacylglycerol-β-(2-methoxyphenyl) ether[12] (XXI), was subjected to 4 h acidolysis and the resulting reaction mixture, consisting mainly of guaiacol and ketols I, II, and III,[13] examined by gel filtration. The fraction of the reaction mixture consisting of compounds with two or more aromatic rings, which was about 17 % of the starting material, contained XXI (3 %) and unidentified constituents (14 %).[13] Because this latter material, which would include the condensation products searched for, constitutes a relatively small portion of the reaction mixture, this experiment indicates that the reaction considered, if it occurs, only plays a minor role on acidolysis of compound XXI. Therefore, this type of condensation reaction could be expected to be of minor importance also in the acidolysis of lignin.

PROCEDURES FOR SEPARATION

From the product obtained on 4 h acidolysis of lignin the major part of the polymeric material was removed by adsorption onto silica gel. This was accomplished by chromatography on a silica gel column using benzene-dioxane (3:1) as eluent. Elution was continued until no more ketol I — the most abundant of the low molecular weight compounds formed — could be detected on examination of the eluate by thin layer chromatography. The eluate fraction, which was 26 % of the weight of the original lignin, was found to consist mainly of low molecular weight compounds. Comparison of the results of gel filtration experiments with this fraction and the total acidolysis product indicated that the losses of low molecular weight products (compounds with one or two aromatic rings) on the silica gel column were negligible;[13] thus the eluate fraction contained the major part of such constituents.

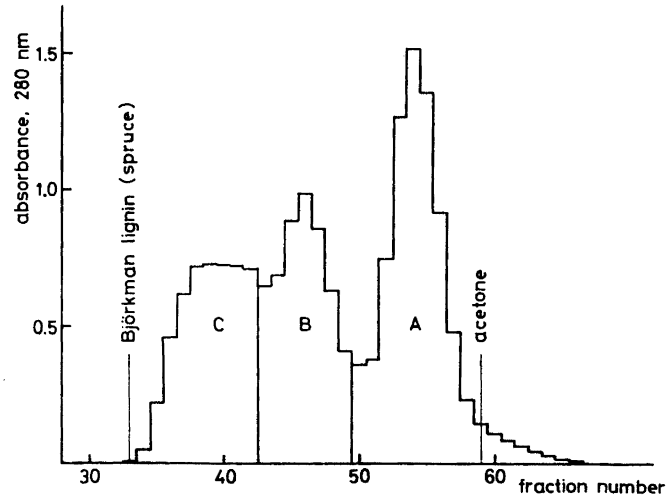


Fig. 3. Gel filtration of an essentially low molecular weight fraction separated by column chromatography from the reaction mixture obtained on 4 h acidolysis of Björkman lignin from spruce (Sephadex G-25, eluting solvent: dioxane-water (1:1)). The location of the elution peaks of Björkman lignin (spruce) and acetone are also given.

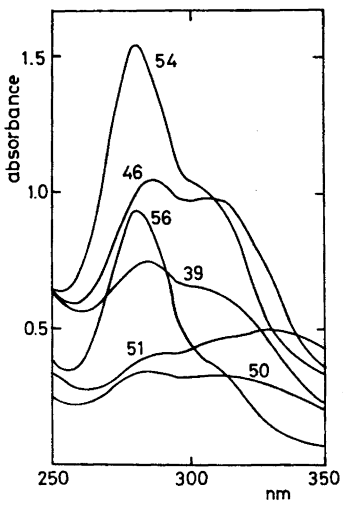


Fig. 4. UV absorption curves of some of the fractions indicated in Fig. 3.

The material eluted from the above-mentioned silica gel column was subjected to gel filtration on Sephadex G-25, using dioxane-water (1:1) as eluent. Aliquots from the fractions thus obtained were diluted with ethanol and the UV spectra in the range 250—350 nm recorded (see Figs. 3 and 4).

It was found that the UV curves of the fractions had their main maximum at 280 nm or slightly above, with the exception of fractions 51 (λ_{max} 328 nm, see Fig. 4) and 52 (λ_{max} 310 nm). The location of the maxima at a higher wavelength in these fractions could at least in part be explained by the presence of coniferaldehyde (IX) (λ_{max} 342–343 nm (ethanol) [14]), which was detected in fractions 50–56 by thin layer chromatography. Further investigations by thin layer chromatography revealed the presence of stilbene XII in fractions 45–50. In Fig. 4 the UV absorption of fraction 46, which is typical for these fractions, is shown. However, the presence of XII (λ_{max} 333 nm (ethanol) [15]) does not to any appreciable extent influence the appearance of the absorption curve. Instead the curve indicates the presence of phenylcoumarones such as compound XVI (see Refs. 14 and 16), which has been obtained from fraction B (the material present in fractions 43–49, see below).

The effluent fractions containing UV absorbing material were divided into fraction groups A, B, and C (see Fig. 3). The material comprising fraction group A ("fraction A") and fraction group B ("fraction B") was investigated to identify individual components. Fraction A and fraction B were 11 % and 6 % of the weight of the original lignin, respectively. Fraction group C, containing comparatively high molecular weight material (see Fig. 3), was not further investigated. It should be mentioned that a certain amount of dark colored constituents, which were present in the sample subjected to gel filtration, accumulated in fraction group C.

It has been reported [17] that aromatic compounds on gel filtration with Sephadex are delayed and are not eluted in the order of molecular size. This was attributed to adsorption effects. However, the phenolic lignin degradation products investigated in this study required for complete elution a volume of eluting solvent which was less than the bed volume. Furthermore, they were eluted almost entirely within the elution volumes of a polymer, Björkman lignin from spruce (or Blue Dextran), and a small molecule, acetone (Fig. 3). This indicated that the molecular sieving action of the gel is not obscured by adsorption effects under the conditions used in spite of the aromatic nature of the sample investigated (*cf.* Ref. 2). In separate studies [18] it could be demonstrated that such adsorption effects were largely supressed by the use of a suitable eluting solvent, *e.g.* dioxane-water (1:1) and dimethyl sulfoxide, which were used in the present studies.

On the basis of the findings reported in this section as well as the composition of fractions A and B (see below) it seems justified to conclude that the amounts of phenols with one or two aromatic rings formed on 4 h acidolysis of Björkman lignin from spruce were about 17 % (fraction A+fraction B) by weight of the original lignin. It may be noted that yields given are based on one single experiment, which is described in detail in this paper. The corresponding yields obtained in other similar experiments showed only slight divergences.

To identify individual components, fractions A and B were each chromatographed on silica gel columns by a gradient elution method (see Exptl.). The effluent fractions were collected into pools on the basis of thin layer chromatographic examination. The residues remaining after removal of solvents were investigated for the detection and isolation of individual compounds.

COMPOSITION OF FRACTION A

Compounds I–XI were found to be constituents of fraction A (Fig. 5). The subfractions of fraction A which contained the identified components comprised 9.4 % of the weight of the original lignin or 89 % of fraction A.

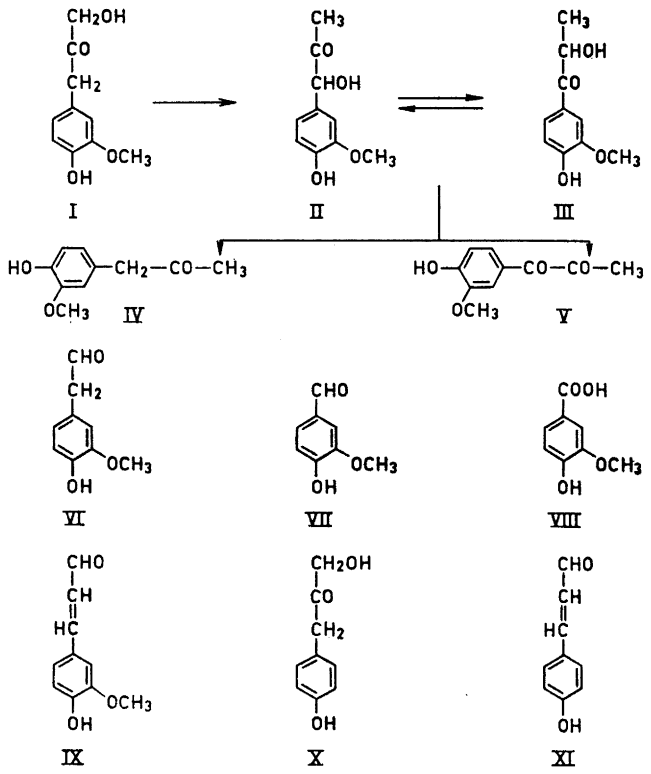


Fig. 5. Compounds detected in fraction A. The interrelationships of ketones I—V under the conditions of acidolysis are shown.

All of the compounds with the exception of X — the synthesis of which is described in the present paper — were known previously.

Compounds I, III, VII, VIII, and X were obtained in a crystalline state and identified with authentic samples by IR and mixed m.p. For the detection of compounds II, IV, and V paper chromatography was used. (It has been found in previous studies [19] that the lignin acidolysis products I—V, under the conditions of acidolysis, are interrelated as shown in Fig. 5.) The cinnamaldehydes IX and XI were also detected by paper chromatography, using a selective spraying agent (see Exptl.). The presence of homovanillin (VI) was demonstrated by paper chromatography and confirmed by its conversion to a derivative, the diacetate of 2-(4-hydroxy-3-methoxyphenyl)-ethanol and identification of this compound by gas chromatography-mass spectrometry.[11]

The predominating single constituent of fraction A was ketol I. This compound was obtained in a relatively pure fraction, which was 5.6 % of the weight of the original lignin. In a similar degradation experiment the weight of the corresponding fraction was 6 % of the starting material. Purification

of this material by recrystallisation from benzene gave ketol I with m.p. 81° (Lit.[20] 81—82°) in an amount corresponding to 5 % of the original lignin. It thus seems reasonable to conclude that ketol I is formed in 5—6 % yield on 4 h acidolysis of spruce lignin.

The weight of the fraction containing ketols II and III, which were not completely separated from each other when fraction A was chromatographed on the silica gel column, was 1.7 % of the original lignin. In agreement with the earlier finding [19] that these two compounds on acidolysis form an equilibrium mixture in which the ratio III:II is 4:1, III was found to be the major constituent.

The yield of coniferaldehyde, as determined by colorimetry, was 0.35 % of the original lignin.

Among the remaining identified constituents, comprising <1.85 % of the original lignin, vanillin was relatively abundant (about 0.5 % of the lignin).

COMPOSITION OF FRACTION B

The compounds which were detected in fraction B are shown in Fig. 6. The total weight of the material in the subfractions of fraction B which were examined for individual components comprised 4.1 % of the weight of the original lignin, or 68 % of fraction B.

Substances XII and XIII, which were obtained in a crystalline state, were found to be identical to synthetic samples (Ref. 21 and Ref. 3a, respectively)

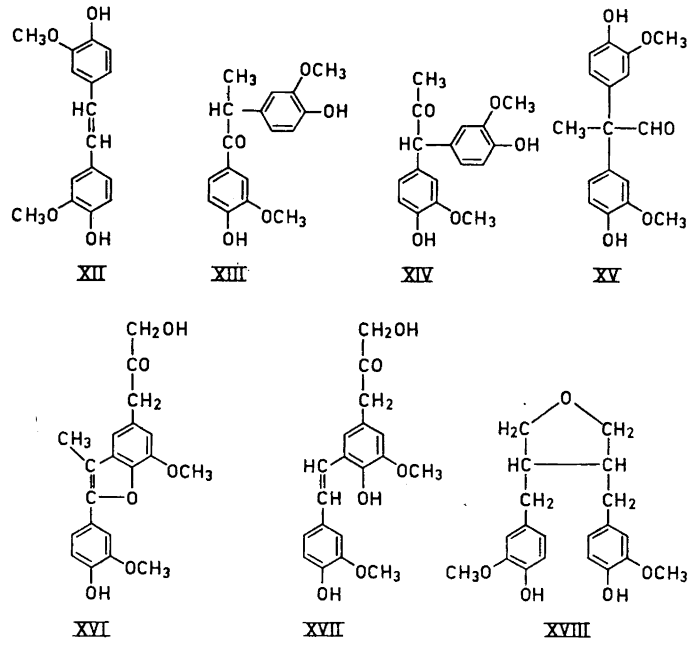
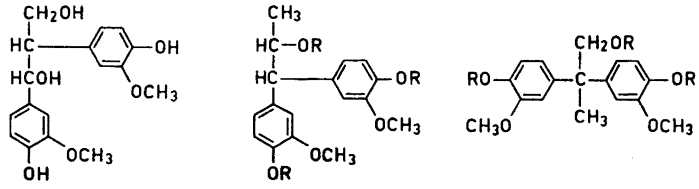


Fig. 6. Compounds detected in fraction B.

by IR and mixed m.p. These two compounds as well as XIV and a compound which, based mainly on spectral and chromatographic data, tentatively has been assigned structure XV, also were formed on acidolysis of 1,2-bis(4-hydroxy-3-methoxyphenyl)-1,3-propanediol (XXII).[3] Conclusive evidence for the presence of XIV in fraction B was obtained by its conversion to the triacetate XXIV, which was identified with a synthetic sample [3b] by gas chromatography-mass spectrometry. By the same method the lignin product assigned structure XV was identified *via* a derivative (presumed to have structure XXVI) with one of the compounds formed on acidolysis of XXII

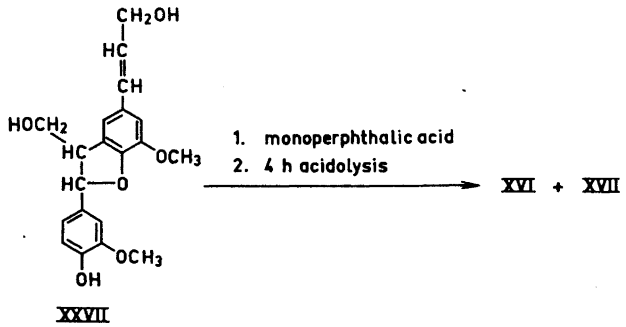


XXII

XXIII R = H
XXIV R = $COCH_3$

XXV R = H
XXVI R = $COCH_3$

The ketols XVI and XVII were obtained in a crystalline state and shown to be identical to the corresponding synthetic materials (IR and mixed m.p.). Synthesis was accomplished by oxidation of dehydrodiconiferyl alcohol [22] (XXVII) with monoperphthalic acid followed by 4 h acidolysis of the oxidation product (Fig. 7).[23]



XXVII

Fig. 7. Preparation of XVI and XVII from dehydrodiconiferyl alcohol (XXVII).

(±)-3,4-Divanillyltetrahydrofuran (XVIII), which also was obtained in a crystalline state, was identified with a synthetic sample (description of synthesis, see Exptl.) by means of IR and mixed m.p.

Most of the identified compounds appeared to be present in amounts which were in the order of a few tenths of a per cent of the original lignin; the possibility to use gas chromatography to estimate quantitatively certain of the products is being investigated.

DISCUSSION

The lignin acidolysis products I—VII, which constitute the major part of fraction A also are formed (in addition to guaiacol), on 4 h acidolysis of guaiacylglycerol-β-(2-methoxyphenyl) ether (XXI),[13,11] a representative model compound [12] for the guaiacylglycerol-β-aryl ether structures (XX) in lignin. The relative abundance of compounds I—VII was essentially the same in the reaction mixtures obtained from lignin and XXI; in both cases ketol I, the major primary reaction product formed on the acidolytic cleavage of guaiacylglycerol-β-aryl ethers (XX),[6,24,25,13] was the predominating compound.

An observed dissimilarity was that a relatively greater amount of vanillin is obtained from lignin than from XXI. However, this difference was not observed when borohydride-reduced lignin [26] was used as starting material.[13] Since the borohydride reduction eliminates the carbonyl groups in the lignin it seems that the vanillin formed on acidolysis of non-reduced lignin arises in part from lignin units containing carbonyl groups; *e.g.* vanillin linked to the lignin by ether bonds which are cleaved on the acidolysis. With the exceptions that less vanillin and only traces of coniferaldehyde were obtained the composition of the fraction corresponding to fraction A obtained in the experiment with borohydride-reduced lignin was essentially the same as that with fraction A.[13]

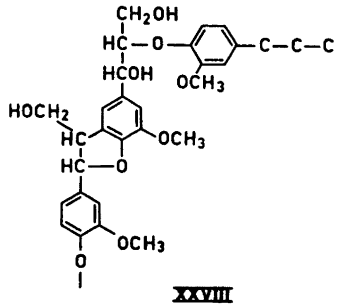
In earlier work on the acidolysis of lignin and lignin model compounds of type XX, it was concluded that structural elements of type XX are present in lignin and constitute a quantitatively important portion of the lignin molecule.[7,6] The above findings confirm this conclusion. The relevance of the degradation studies presented in this paper with respect to the arylglycerol-β-aryl ether structure in lignin will be further discussed in a later paper concerning the acidolysis of lignin model compounds of type XX.

Besides compounds I—VII two further compounds of the guaiacyl type have been found in fraction A, *viz.* coniferaldehyde (IX) and vanillic acid (VIII). Since only traces of coniferaldehyde were obtained on acidolysis of borohydride-reduced lignin one would explain, by reasoning in a similar way as above concerning vanillin, the formation of coniferaldehyde from non-reduced-lignin as being due to the liberation of IX from coniferaldehyde end groups (see Ref. 27) by hydrolysis of ether bonds. Recently, small amounts of coniferaldehyde and vanillin linked to guaiacylglycerol molecules by β-ether bonds have been obtained on "mild hydrolysis" of spruce wood.[28] This provides direct evidence for the presence in spruce lignin of coniferaldehyde and vanillin attached to the lignin by ether bonds which would be expected to be cleaved on acidolysis. The formation of vanillic acid on acidolysis of lignin is probably due to liberation of vanillic acid linked to the lignin by bonds which are hydrolyzed on acidolysis (ester or ether linkages, *cf.* Ref. 29).

It has been shown by several workers (see Ref. 30) that the units in spruce lignin are mainly of the guaiacyl type but that minor amounts of *p*-hydroxyphenyl and syringyl units also are present. The compounds X and XI thus arise from *p*-hydroxyphenylpropane moieties in the lignin. They obviously are related to structural elements in lignin in the same way as the analogous guaiacyl compounds (I and IX), whose origin has been discussed above.

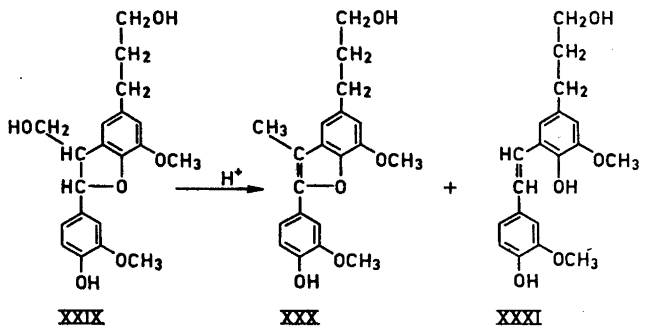
Constituents XII—XV of fraction B also were obtained on acidolysis of 1,2-bis(4-hydroxy-3-methoxyphenyl)-1,3-propanediol (XXII).[3] Based on the

finding that lignin and XXII give common reaction products on acidolysis it was concluded [3a] that compound XXII and/or a precursor is incorporated in the lignin. At about the same time, XXII and related compounds were obtained by Nimz [31] on "mild hydrolysis" of spruce and beech woods.



XXVIII

The degradation products XVI and XVII, which also were constituents of fraction B, originate, according to results of acidolysis experiments with lignin model compounds, from lignin structures of type XXVIII. Thus, the phenylcoumaran XXIX on acidolysis gives the phenylcoumarone XXX [16] and the stilbene XXXI [23] (Fig. 8, *cf.* also Fig. 7), and, as mentioned above,

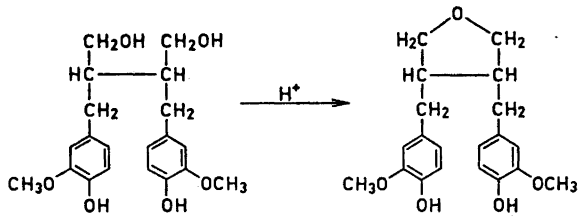


XXIX XXX XXXI

Fig. 8. Acidolysis of dihydrodehydrodiconiferyl alcohol (XXIX).

arylglycerol-β-aryl ethers are cleaved with formation of arylpropane units with ketol side chains of the type present in compounds XVI and XVII (see Fig. 2). The stilbene XXXI, which is formed in low yield on acidolysis, is obtained in high yield on kraft cooking of XXIX, being the only detected product.[32]

Finally, (±)-3,4-divanillyltetrahydrofuran (XVIII) was obtained from fraction B. As yet no satisfactory explanation for the presence of this compound among the degradation products has been achieved. However, it has been clarified that XVIII could have arisen *via* (±)-2,3-divanillyl-1,4-butanediol (XXXII), because it was found that XVIII can be prepared by acidolysis of XXXII (Fig. 9).

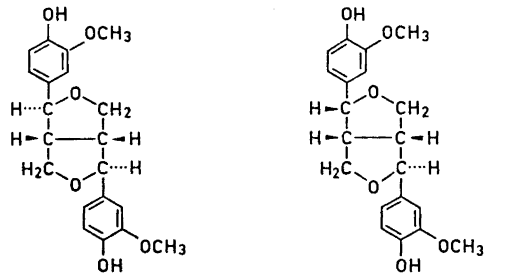


XXXII (±)-2,3-divanillyl-1,4-butanediol XVIII (±)-3,4-divanillyltetrahydrofuran
XXXIIa (-)-2,3-divanillyl-1,4-butanediol XVIIIa (-)-3,4-divanillyltetrahydrofuran

Fig. 9. Formation of compounds XVIII and XVIIIa on acidolysis of compounds XXXII and XXXIIa, respectively.

The facts that small amounts of 3,4-divanillyltetrahydrofuran have been detected in extractives from spruce [33] and that (−)-2,3-divanillyl-1,4-butanediol (XXXIIa) has been found in wood extracts [34,35] and in a resinous exudate of spruce [36] suggest that XVIII in fraction B might have originated from contaminating extractives in the lignin used as starting material. The presence of 3,4-divanillyltetrahydrofuran in extracts of spruce wood was demonstrated only by paper chromatography [33] and it was not clarified whether the product was optically active or not; thus it is not known if the detected compound is identical in this respect with the product obtained from fraction B. It also should be pointed out that considering the procedure used in the preparation of Björkman lignin [5] it seems unlikely that free XVIII would be present in this material. The finding that the optically active XXXIIa on acidolysis gave (−)-3,4-divanillyltetrahydrofuran (XVIIIa) (Fig. 9) excludes the possibility that the optically inactive lignin acidolysis product is formed from XXXIIa. Further researches into the origin of the acidolysis product XVIII are in progress.

Convincing evidence for the presence of (±)-pinoresinol (XXXIII) incorporated in lignins from conifers has been achieved.[37,38] Minor amounts of its diastereomer (±)-epipinoresinol (XXXIV) also are considered to constitute a part of the lignin structure.[39,40] The lignin acidolysis mixture was therefore examined for pinoresinol and epipinoresinol. However, the attempts to detect these two compounds failed. Thus, if pinoresinol and epipinoresinol were present in the acidolysis mixture the amounts must have been small.



XXXIII (±)-pinoresinol XXXIV (±)-epipinoresinol
XXXIIIa (+)-pinoresinol XXXIVa (+)-epipinoresinol

Acidolysis (4 h) of (+)-pinoresinol (XXXIIIa) gave a reaction product consisting of a mixture of (+)-pinoresinol and (+)-epipinoresinol (XXXIVa) in which the latter compound somewhat dominated. Thus pinoresinol undergoes the known acid-catalyzed isomerization to epipinoresinol (see Ref. 41) also under the conditions of acidolysis (*cf.* Ref. 40). It follows that if pinoresinol is liberated on acidolysis of lignin, pinoresinol and epipinoresinol can be expected to be present in the reaction mixture.

To find an upper limit of the amounts of pinoresinol and epipinoresinol which possibly could have been formed on the acidolysis of the lignin, comparative acidolysis experiments with lignin and lignin containing a small percentage of added pinoresinol were performed. Even when the amount of added pinoresinol was in the order of a tenth of a per cent of the lignin, pinoresinol and epipinoresinol could be readily detected in the resulting acidolysis mixture, while in agreement with the results mentioned above these compounds could not be detected in experiments with no pinoresinol added.[13] These results are interpreted to indicate that if pinoresinol and epipinoresinol are liberated on the acidolytic treatment of the lignin, the amount of these two compounds formed must be considerably less than 0.1 % of the original lignin.

The proportion of the pinoresinol type of structure in spruce lignin has been suggested to be rather high (Ref. 42; *cf.* also Ref. 38). If so is the case the present results indicate that the number of these structural elements accessible to acidolytic liberation of pinoresinol (or epipinoresinol) is very small. A possible explanation would be that the alkyl aryl ether linkages by which the pinoresinol type of units are attached to the lignin are especially stable. However, it is hard to find any support for this explanation. Furthermore, it has been shown that pinoresinol linked to guaiacylglycerol by a β-ether bond is liberated on acidolysis.[40] A further possibility would be that the guaiacylpropane units in the pinoresinol type of structures to an unexpectedly large extent are linked to adjacent units by carbon-carbon linkages or acid-stable diaryl ether linkages, which would preclude the liberation of pinoresinol (or epipinoresinol).

As mentioned in the introductory section of this paper, formaldehyde is liberated on acidolysis of spruce lignin. To elucidate the origin of the formaldehyde, its formation from a number of lignin model compounds on acidolysis has been studied.[11] The results in general have been in harmony with those obtained earlier in similar investigations [43,44] performed with 28 % sulphuric acid as reagent. Our acidolysis experiments with lignin model compounds indicate that the lignin products VI, XII, and XVII are formed in reactions involving the release of formaldehyde from guaiacylglycerol-β-aryl ether structures (XX), 1,2-bis(4-hydroxy-3-methoxyphenyl)-1,3-propanediol (XXII) incorporated in the lignin and phenylcoumaran structures as in XXVIII, respectively.

Products formed on the acidolysis of Björkman lignin of birch also have been studied using the methods described in this paper.[13] The amount of low molecular weight phenols formed was greater in the case of birch lignin than in the case of spruce lignin. The material corresponding to fractions A and B was about 30 % of the original birch lignin. The fraction corresponding to fraction A has been found to contain compounds I, II, III, V, VII, VIII, and IX and also the syringyl analogues of I, III, V, VII, and IX. Investigations of the fraction corresponding to fraction B are in progress. In addi-

tion to compounds also obtained from spruce lignin (*e.g.* XII and XV) a compound has been detected which probably is the previously unknown (±)-epi-syringaresinol (the syringyl analogue of XXXIV).

EXPERIMENTAL

Melting points are uncorrected.

Materials. Dioxane and ethyl acetate were purified according to Vogel.[45] Silica gel for column chromatography was Mallinckrodt analytical reagent, 100 mesh, dried at 110° overnight.

IR spectra were recorded using KBr pellets (unless otherwise specified), with a Beckman IR 9 instrument. *UV spectra* were taken on a Beckman DK 2A instrument.

Gas-liquid chromatography was accomplished with a Perkin-Elmer Model 900 instrument. Column dimensions: 100 × 0.3 cm o.d. stainless steel tubing. Solid support: Chromosorb G, acid-washed and treated with dimethyldichlorosilane, 80—100 mesh. Stationary phase: Silicone elastomer SE-30, General Electric (5 % by weight of the solid support). Temperatures: Injection 300°, detector 280° and column 260°. Carrier gas: N_2, 30 ml/min. Detector: Flame ionization detector.

For identification of components the mass spectra were taken with an LKB 9000 gas chromatograph-mass spectrometer unit.

Paper chromatography was performed using the solvent system employed by Kratzl and Schweers[46] (upper layer of ligroin-water-chloroform-methanol (7:5:2:1) moving phase). Diazotized sulphanilic acid in 2 % aqueous Na_2CO_3 was used as spraying agent. For R_F data of compounds II, III, IV, and V, see Ref. 19. In the present research the above solvent system also was used for the detection of vanillin (VII) (R_F 0.45, orange-red) and coniferaldehyde (IX) (R_F 0.33, magenta). Coniferaldehyde was also made visible, as a violet spot, by spraying with a mixture of equal amounts of 0.1 M phloroglucinol in 60 % ethanol and 4 M hydrochloric acid (see Ref. 27).

Thin layer chromatography was accomplished on plates coated with a 0.3 mm thick layer of silica gel (Merck G). As developing solvent benzene-ethyl acetate (1:1) was used. Spots were made visible by iodine vapour. R_F-values: XXXII (and XXXIIa), 0.06; XVII, 0.18; I, 0.21; XVI, 0.25; XXIII, 0.25; X, 0.30; XVIII, 0.40; XIV, 0.43; IX, 0.45; XIII, 0.45; XII, 0.50.

Preparative thin layer chromatography was performed with plates similar to those used for analytical purposes, except that the plates were coated instead with silica gel containing a fluorescent additive (Merck HF_{254}). The zones of silica gel containing the materials of interest, detected by UV light, were scratched off and compounds eluted with acetone.

Standard procedure for column chromatography on silica gel using gradient elution. Columns were packed with 75 g of silica gel using benzene-ethyl acetate (4:1) as solvent. The columns thus prepared were conditioned by washing with about 300 ml solvent, and had a final size of approximately 2 × 50 cm.

Mixtures of compounds to be chromatographed were dissolved in 10 ml solvent (benzene-ethyl acetate (4:1)), applied to the top of the column, and washed into the gel with a few milliliters of solvent. The top of the column was connected *via* a closed system to a mixing chamber containing initially 400 ml benzene-ethyl acetate (4:1) and fitted with a magnetic stirrer. The mixing chamber was in turn connected *via* a closed system to a reservoir containing benzene-ethyl acetate (2:3). The effluent was collected in 10 ml fractions. Collection of fractions was started when the 10 ml solvent containing the sample was applied to the column. Flow rates were 20—40 ml/h.

The fractions obtained from the silica gel column were examined individually by thin layer chromatography to provide a basis for their combination into pools. From the pooled fractions thus obtained the solvent was removed by film evaporation to yield residues which were weighed after being dried at 20 mm Hg over KOH and P_2O_5. These residues then were investigated to identify individual compounds.

Analytical gel filtration experiments * were performed on Sephadex G-50 (fine) with dimethyl sulfoxide as eluent. The UV absorption of the eluate at 280 nm was followed by the use of a LKB Uvicord.

Syntheses

p-*Acetoxyphenylacetic acid* was prepared by acetylation of *p*-hydroxyphenylacetic acid by means of the procedure used by Gardner [47] for the acetylation of homovanillic acid; the product had m.p. 108° (Lit.[48] 108°).

1-Hydroxy-3-(4-hydroxyphenyl)-2-propanone ** *(X)* was prepared from *p*-acetoxyphenylacetic acid (preparation, see above) according to the procedure described by Fischer and Hibbert [50] for the preparation of 1-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-2-propanone (I) from 4-acetoxy-3-methoxyphenylacetic acid. From 0.7 g of starting material 0.2 g of crude product was obtained. Purification on a silica gel column using benzene-ethyl acetate (1:1) as eluent gave 0.17 g of product with m.p. 71—73° (yield 28%). Recrystallisation from chloroform raised the m.p. to 74—75°. (Found: C 64.94; H 6.05; O 28.71. Calc. for $C_{10}H_{12}O_4$: C 65.04; H 6.07; O 28.89). The IR spectrum showed a band at 1725 cm^{-1} (unconjugated carbonyl). The NMR spectrum was recorded on a Varian A-60 instrument with TMS as internal standard (solvent: deuterochloroform). It was consistent with structure X. Singlets at $\delta = 3.6$ ppm (2 H) and 4.3 ppm (2 H) are assigned to the methylene group adjacent to the aromatic ring and methylene protons in the hydroxymethyl group, respectively.

(−)-2,3-Divanillyl-1,4-butanediol (XXXIIa) was prepared by catalytic hydrogenation of (+)-pinoresinol (XXXIIIa) (this compound was obtained from a resinous exudate of spruce according to Refs. 41 and 49, m.p. 119—120° (Lit.[41] 121—122°)) using a procedure that differed somewhat from that described by Weinges.[36] (+)-Pinoresinol (0.70 g) was hydrogenated at room temperature in ethanol using 0.22 g 10 % Pd/C (Cornbrock) as catalyst. After 90 min the consumption of hydrogen ceased. The catalyst was filtered off and solvent removed by film evaporation to yield a product which crystallized after several days. Recrystallisation from chloroform/hexane gave 0.46 g of (−)-2,3-divanillyl-1,4-butanediol (XXXIIa), m.p. 113—114° (Lit.[34] 113.5°). Yield: 65 %.

(−)-3,4-Divanillyltetrahydrofuran (XVIIIa) was prepared from (−)-2,3-divanillyl-1,4-butanediol (XXXIIa) according to Ref. 33, m.p. 116—117° (Lit.[33] 116—117°). Compound XVIIIa was also obtained by acidolysis of XXXIIa (*cf.* the preparation of XVIII, described below).

(±)-Pinoresinol (XXXIII) From the reaction product obtained by dehydrogenation of coniferyl alcohol with manganese dioxide according to Freudenberg and Friedmann [50] crude (±)-pinoresinol was obtained by chromatography on a silica gel column using ethyl acetate as eluent. Purification by column chromatography using the standard procedure, followed by recrystallisation from ethyl acetate/hexane, gave (±)-pinoresinol (XXXIII) of m.p. 111° (Lit.[51] 111°).

(±)-3,4-Divanillyltetrahydrofuran (XVIII). (±)-Pinoresinol (80 mg) was hydrogenated as described above for the hydrogenation of (+)-pinoresinol. Thin layer chromatography of the reaction mixture indicated that the major constituent was the previously unknown (±)-2,3-divanillyl-1,4-butanediol (XXXII) (as reference compound XXXIIa was used). The crude reaction product was dissolved in 10 ml 0.2 M HCl in dioxane-water (9:1) and the solution was refluxed 6 h (nitrogen atmosphere). After cooling, the reaction mixture was neutralized with 5 ml 0.4 M $NaHCO_3$ and then extracted with chloroform. The extract was dried over anhydrous Na_2SO_4 and the solvent removed by film evaporation to yield 68 mg of crude product. Purification on a silica gel column using benzene-ethyl acetate (1:1) as eluent gave 53 mg of crystals of m.p. 132—133° (69 % yield). Recrystallisation from methylene chloride/ether gave 37 mg product with the same m.p. (Found: C 69.70; H 6.96. Calc. for $C_{20}H_{24}O_5$: C 69.75; H 7.02). The IR spectrum in chloroform solution was identical with that obtained from (−)-3,4-divanillyltetrahydrofuran (XVIIIa). The IR spectrum in KBr differed slightly from that of XVIIIa (taken in KBr). The base peak in the mass spectrum * of the compound was the molecular ion: *m/e* 344.

* These experiments were kindly conducted by Mr. G. E. Miksche.

** The p reparation of this compound was carried out by B. Khanna, B.Sc.

Acidolysis of (+)-pinoresinol

A solution of 266 mg (+)-pinoresinol (this compound was obtained from a resinous exudate of spruce according to Refs. 41 and 49, m.p. 119–120° (Lit.[41] 121–122°)) in 50 ml dioxane-water (9:1) containing 0.2 M HCl was refluxed 4 h (nitrogen atmosphere). To the cooled reaction mixture 0.84 g $NaHCO_3$ was added, then the mixture was left in a refrigerator overnight. The solution was filtered and then extracted three times with 50 ml chloroform. The combined extracts were dried over anhydrous Na_2SO_4 and the solvent removed under vacuum. A residue weighing 0.28 g was obtained. This was subjected to column chromatography on silica gel according to the standard procedure. Tubes 33–41 gave 133 mg of crystals of m.p. 135°. Recrystallisation from methanol raised the m.p. 136–137°. The product was identified as (+)-epipinoresinol ** (XXXIVa) (m.p. 140.5–141.5° [53]) by IR and mixed m.p. Tubes 42–52 gave 123 mg crystals of m.p. 117°. Recrystallisation from methanol raised the m.p. to 118–120°. This compound was identified as starting material by IR and mixed m.p.

Acidolysis of lignin and investigations of the resulting reaction mixture

Acidolysis. A solution of 3.95 g of spruce lignin (methoxyl content 15.6 %), prepared from Norway spruce according to Björkman,[5] in 200 ml 0.2 M HCl in dioxane-water (9:1) was refluxed 4 h. Nitrogen was bubbled through the solution during the heating. To the cooled reaction mixture approximately 100 ml 0.4 M $NaHCO_3$ was added to rise the pH to about 3. The solution was extracted with 200 ml chloroform and then three times with 100 ml chloroform. On the first addition of chloroform some precipitate was formed which was not further investigated. The combined extracts were dried over anhydrous Na_2SO_4 and the solvent removed by film evaporation. The residue, dried at 20 mm Hg over KOH and P_2O_5 overnight, weighed 4.05 g.

Removal of the major part of the high molecular weight material. The acidolysis product was dissolved in 15 ml of dioxane and the solution was dropped into a stirred suspension of 15 g silica gel in 45 ml benzene. The mixture was added to a silica gel column (3.6 × 10 cm; 50 g SiO_2). For elution benzene-dioxane (3:1) was used. Elution was continued until no more ketol I could be detected in the eluate on examination by thin layer chromatography (total volume of eluate 830 ml). The solvent was removed by film evaporation and the residue dried at 20 mm Hg over KOH and P_2O_5 overnight to yield 1.02 g (26 % of the original lignin) of an oil.

Gel filtration. A column for gel filtration was prepared with 180 g of Sephadex G-25 (medium) using dioxane-water (1:1) as solvent. The final bed volume of the column (101 × 3.1 cm) was 760 ml. With this column a polymer, Björkman lignin from spruce (or Blue Dextran), was eluted at 330 ml, whereas a small molecule like acetone was eluted at 590 ml.

The product obtained from the silica gel column was dissolved in 5 ml dioxane and 5 ml water was added. The solution was added to the gel filtration column. Eluting solvent was applied to the column from a dropping funnel equipped as a Mariotte bottle and fitted to the column by a ground glass joint. The effluent was collected in 10 ml fractions; collection of fractions was started when the sample was added to the column. Flow rate of the column was 20 ml/h.

The fractions were examined by UV spectrophotometry. From each fraction 0.07 ml was taken by a microsyringe and diluted with 10 ml ethanol. The spectrum in the range 250–350 was recorded (Figs. 3 and 4). Investigation of the fractions by thin layer chromatography revealed the presence of coniferaldehyde (IX) in fractions 50–56. For the selective detection of IX the phloroglucinol-hydrochloric acid reagent described under *paper chromatography* was used as spraying agent. Similarly, fractions 45–50 were shown by thin layer chromatography to contain the stilbene XII by means of a selective spraying agent. In this case dilute aqueous hydrogen peroxide containing a

* The author expresses his thanks to Dr. R. Ryhage for taking the mass spectrum.
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small amount of copper sulphate served this purpose.[53] Spraying with this mixture made stilbene XII visible as a dark orange spot (see Ref. 53).

On the basis of the results of the examinations the effluent fractions containing UV absorbing material were divided into fraction groups A, B, and C (Fig. 3). Fraction group A (tubes 50—64, 150 ml) was extracted with one half of its volume of chloroform and then three times with one fourth the volume of chloroform. The UV absorption of the aqueous layer indicated that the extraction of lignin-related compounds was incomplete. Therefore further extractions alternately with dioxane-chloroform (1:1) and chloroform were made. This was continued until UV measurements of the aqueous layer showed that only negligible amounts of lignin-related materials remained. The combined extracts were dried over anhydrous Na_2SO_4 and the solvent removed by film evaporation. The residue, dried at 20 mm Hg over KOH and P_2O_5, weighed 0.42 g (11 % of the original lignin). This material was designated *fraction A*. Similar treatment of fraction group B (tubes 43—49, 70 ml) gave a product weighing 0.24 g (6 % of the original lignin). This material was designated *fraction B*. Fraction group C was not further investigated.

Examination of fraction A. Fraction A was subjected to silica gel chromatography according to the standard procedure.

Tubes 20—24 yielded 10 mg of an oil. Paper chromatography revealed the presence of *1-(4-hydroxy-3-methoxyphenyl)-1,2-propanedione* (V).

Tubes 25—30 yielded 34 mg of a partly crystalline product. Paper chromatography indicated that the major component was vanillin (VII). From a portion of the fraction the major component was separated by preparative thin layer chromatography and crystallized from benzene to give crystals of m.p. 75°, identical to *vanillin* (VII) (m.p. 81°[45]) by IR and mixed m.p. The fraction further contained *homovanillin* (VI) as shown by paper chromatography using the solvent system toluene-acetic acid-water (4:1:5).[11] (Further proof for the presence of homovanillin was achieved by its conversion to a derivative, the diacetate of 2-(4-hydroxy-3-methoxyphenyl)-ethanol, and identification of this compound by gas chromatography-mass spectrometry.[11]) Paper chromatography in this system further revealed the presence of a trace of p-*coumaraldehyde* (XI) (R_F 0.35). This compound was made visible, as a red-violet spot, by spraying with the phloroglucinol-hydrochloric acid reagent described under *paper chromatography*.

Tubes 31—48 yielded 32 mg of an oil. From a small amount of methylene chloride, a crystalline product of m.p. 200—202° was obtained. This was identified as *vanillic acid* (VIII) (m.p. 210°[45]) by IR and mixed m.p. By paper chromatography the mother liquor was shown to contain *coniferaldehyde* (IX) and a small amount of *1-(4-hydroxy-3-methoxyphenyl)-2-propanone* (IV). The amount of coniferaldehyde was determined by colorimetry using the reagent described by Adler and Marton.[54] Equal volumes of the reagent and an ethanol solution of the sample were mixed and the absorption spectrum recorded after 15 min. Under the conditions used coniferaldehyde gave an absorption maximum at 452 nm. The fraction was found to contain 14 mg coniferaldehyde.

Tubes 39—43 yielded 5 mg of an oil. The presence of a trace of coniferaldehyde was shown by paper chromatography.

Tubes 44—49 yielded 38 mg of a crystalline product, m.p. 102—105°. Recrystallisation from benzene raised the m.p. to 105°. By means of IR and mixed m.p. the product was identified as *2-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone* (III) (m.p. 109—110°[55]).

Tubes 50—58 yielded 28 mg of an oil. By paper chromatography the fraction was shown to contain ketol III and its isomer, *1-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-propanone* (II).

Tubes 59—60 yielded 4 mg of an oil. The fraction was dissolved in a small amount of benzene-methylene chloride (1:1). The solution was seeded with ketol X and placed in a refrigerator overnight. Crystals of m.p. 68° were obtained. In a second acidolysis experiment crystals of m.p. 72—73° were obtained from the corresponding fraction. The product was identified as *1-hydroxy-3-(4-hydroxyphenyl)-2-propanone* (X) (m.p. 74—75°, see description of synthesis above) by IR and mixed m.p.

Tubes 61—83 yielded 222 mg of a crystalline product, m.p. 78°. The product was identified as *1-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-2-propanone* (I) (m.p. 81—82°[20]) by IR and mixed m.p. Thin layer chromatography indicated that the product was contaminated with a minor amount of ketol X. In a second acidolysis experiment with

4.05 g lignin (methoxyl content 15.43 %) as starting material the corresponding fraction weighed 244 mg; recrystallisation from benzene gave 200 mg product of m.p. 81°.

Examination of fraction B. Fraction B was subjected to silica gel chromatography according to the standard procedure.

Tubes 21—26 yielded 27 mg of an oil. This was dissolved in a small amount of ethanol and allowed to stand overnight in a refrigerator. Crystals of m.p. 195° were obtained, which were identified by IR and mixed m.p. as *4,4'-dihydroxy-3,3'-dimethoxystilbene* (XII) (m.p. 212—213° [21]). The residue obtained after removal of solvent from the mother liquor was dissolved in a mixture of 2 ml dioxane and 2 ml of a sodium borohydride solution (4 g $NaBH_4$ in 200 ml 0.25 M NaOH). After one day the reaction mixture was acidified with dilute hydrochloric acid and then extracted with chloroform. The chloroform extract was dried over anhydrous Na_2SO_4 and the solvent removed by film evaporation. The residue was acetylated by treatment with acetic anhydride-pyridine and then examined by gas chromatography. The gas chromatogram indicated that the product contained two main products, one of which (retention time 6 min) was identified by its mass spectrum as the diacetate of XII.[21] The second component (retention time 4.5 min) was found to be identical by its mass spectrum with a product which is formed on reduction and acetylation as described above of a compound, which has been obtained on acidolysis of 1,2-bis(4-hydroxy-3-methoxyphenyl)-1,3-propanediol (XXII) in addition to compounds XII, XIII, and XIV reported earlier.[3a,4] Based primarily on spectral and chromatographic data, the new acidolysis product from XXII has been assigned the structure XV. It would thus be expected that on reduction followed by acetylation the product XXVI would be formed (*via* XXV). In agreement with this assumption the peak in the mass spectrum with the highest *m/e* was 430, which corresponds to the molecular ion of XXVI. It could thus be assumed that *2,2-bis(4-hydroxy-3-methoxyphenyl)-propionaldehyde* (XV) is present in this fraction.

Tubes 27—35 yielded 30 mg of an oil. The presence of the ketone XIII was indicated by thin layer chromatography. Purification by preparative thin layer chromatography gave 24 mg of an oil. This was dissolved in benzene and the solution inoculated with XIII. On standing a minor amount of crystals of m.p. 111—114° was obtained. The product was identified by IR and mixed m.p. as *1,2-bis(4-hydroxy-3-methoxyphenyl)-1-propanone* (XIII) (m.p. 118—119° [3a]).

Tubes 36—42 yielded 14 mg of an oil. According to thin layer chromatography the oil contained the ketone XIV, which has been found in the reaction mixture obtained on acidolysis of XXII.[3,4] Reduction with sodium borohydride (by the method described above for the reduction of the material in tubes 21—26) gave a product which, as indicated by thin layer chromatography, contained 1,1-bis(4-hydroxy-3-methoxyphenyl)-2-propanol (XXIII), which has been synthesized.[3b] The reduction product was acetylated with acetic anhydride-pyridine and then examined by gas chromatography. The gas chromatogram indicated the presence of one major component (retention time 4 min), which by its mass spectrum was identical to the synthetic triacetate XXIV [3b]. Based on the data presented it is concluded that this fraction contained *1,1-bis(4-hydroxy-3-methoxyphenyl)-2-propanone* (XIV).

Tubes 43—48 yielded 16 mg of an oil. From methylene chloride/ether, crystals of m.p. 132—133° were obtained. The crystals were identified by IR and mixed m.p. as (±)-*3,4-divanillyltetrahydrofuran* (XVIII) (m.p. 132—133°, see description of synthesis above).

Tubes 49—54 yielded 11 mg of an oil. The presence of some 3,4-divanillyltetrahydrofuran was indicated by thin layer chromatography.

Tubes 55—68 yielded 32 mg of an oil. According to thin layer chromatography the major component was the phenylcoumarone XVI. Purification by preparative thin layer chromatography gave an oil weighing 23 mg. From methylene chloride/petroleum ether crystals of m.p. 110° were obtained. The product was identified by IR and mixed m.p. as the *phenylcoumarone XVI* (m.p. 110° [23]), which has been prepared from dehydrodiconiferyl alcohol [22] (XXVII) by oxidation with monoperphthalic acid followed by acidolysis (see Fig. 7).[23]

Tubes 69—79 yielded 14 mg of an oil. Thin layer chromatography indicated the presence of the stilbene XVII. Separation by preparative thin layer chromatography gave an oil weighing 7 mg. Crystallisation from a small amount of methylene chloride gave a product melting at about 155°. Recrystallisation raised the m.p. to 168—171°.

The crystals were identified by IR and mixed m.p. as the *stilbene XVII* (m.p. 169—172° [23]), which has been obtained as a byproduct in the synthesis of the phenylcoumarone XVI (see Fig. 7).[23]

Tubes 80—96 gave 18 mg of an oil. As yet no individual compound has been identified in this fraction.

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