

## Acid Degradation of Lignin

### IV.\* Analysis of Lignin Acidolysis Products by Gas Chromatography, Using Trimethylsilyl Derivatives

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A simplified procedure for the analysis of phenolic lignin acidolysis products of low molecular weight is described. Fractions of "monomeric" products were separated from lignin acidolysis mixtures by gel filtration, and examined by gas chromatography using trimethylsilyl derivatives.

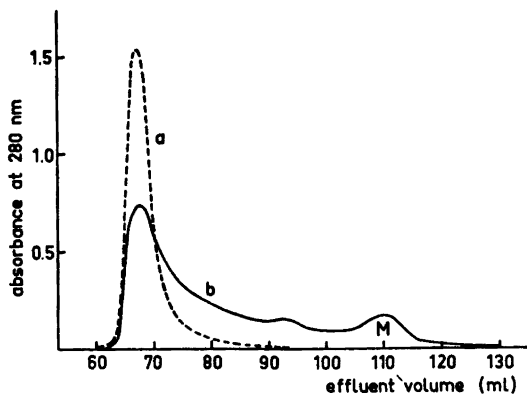
The identification of a number of low molecular weight phenols obtained from Björkman lignin of Norway spruce (*Picea abies* (L.) Karst.) on heating at reflux temperature with 0.2 M hydrogen chloride in dioxane-water (9 : 1)\*\*\* for 4 h has been described previously.<sup>1,2</sup> Fractionation of the acidolysis product mixture involved gel filtration on Sephadex and chromatography on silica gel columns. The fractions thus obtained were examined by various methods to identify individual components. Because the investigation of products formed on acidolytic degradation is of value for the structural characterization of lignin preparations, it is desirable to replace the tedious procedures for analysis used earlier with more convenient ones. In this paper a simplified method for the examination is described. The present investigation was limited to degradation products with one aromatic ring ("monomeric" products). A fraction consisting of these products was separated from the acidolysis mixture by gel filtration, and, after conversion of its constituents to their trimethylsilyl (TMS) derivatives, was examined by gas chromatography. Several of the compounds found to be components of the separated fraction have been analysed by gas chromatography as TMS derivatives in other connections.<sup>3</sup>

Gel filtration was accomplished using Sephadex G-25 with dioxane-water (1 : 1) as eluting solvent. A plot of the absorbance at 280 nm of the eluate

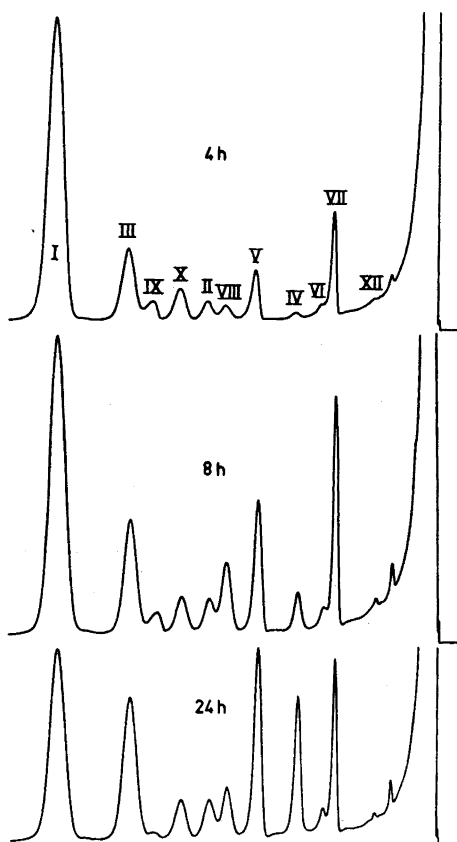
\* Part III, see Ref. 2.

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\*\*\* Throughout this paper the term "acidolysis" is used specifically for this treatment.



*Fig. 1.* Gel filtration of Björkman lignin from spruce (a) and its reaction product obtained on 4 h acidolysis (b) on Sephadex G-25 with dioxane-water (1 : 1) as eluting solvent.



*Fig. 2.* Gas chromatograms of TMS derivatives of "monomeric" products obtained on 4 h, 8 h, and 24 h acidolysis of spruce lignin. Silylation was accomplished by treatment with BSA in dioxane-pyridine (10 : 1) for 3 h.

versus elution volume for a 4 h acidolysis product mixture is shown in Fig. 1. Peak M contains the "monomeric" products, including compounds I–XI.<sup>1,2</sup> Nontreated lignin does not contain such low molecular weight components (see Fig. 1).

Gas chromatography of the TMS derivatives of the "monomeric" acidolysis products gave a chromatogram (Fig. 2) with peaks corresponding in retention times to all but one of the previously identified compounds. The compound not represented, *p*-coumaraldehyde (XI), presumably was present in too small an amount to give a distinct peak. The gas chromatogram showed two additional peaks which were relatively small; judging from its retention time, one of these could have been due to the TMS derivative of *p*-hydroxybenzaldehyde (XII) (see Fig. 2). The presence of minor amounts of XII is plausible, since XII is the *p*-hydroxyphenyl analogue of vanillin (VII), one of the earlier detected compounds of the guaiacyl series (see Ref. 1). Retention times of the TMS derivatives of compounds I–XII relative to that of the derivative of compound I (retention time 7 min) are given in Table 1 (for conditions used in the gas chromatographic analysis, see Experimental).

Table 1. Retention times of TMS derivatives of compounds I–XII relative to the retention time of the TMS derivative of ketol I.

Compound <sup>a</sup>	Rel. ret. time of TMS derivative
R–CH <sub>2</sub> –CO–CH <sub>2</sub> OH (I)	1.00
R–CH(OH)–CO–CH <sub>3</sub> (II)	0.63
R–CO–CH(OH)–CH <sub>3</sub> (III)	0.82
R–CH <sub>2</sub> –CO–CH <sub>3</sub> (IV)	0.41
R–CO–CO–CH <sub>3</sub> (V)	0.51
R–CH <sub>2</sub> –CHO (VI)	0.34
R–CHO (VII)	0.32
R–COOH (VIII)	0.58
R–CH=CH–CHO (IX)	0.75
R'–CH <sub>2</sub> –CO–CH <sub>2</sub> OH (X)	0.69
R'–CH=CH–CHO (XI)	0.49
R'–CHO (XII)	0.22

<sup>a</sup> R = 4-hydroxy-3-methoxyphenyl; R' = 4-hydroxyphenyl.

Quantitative estimation of the main component, ketol I, showed that it amounted to 4.6 % of the original lignin. The amount of ketol III was 0.8 % of the original lignin. These yields are slightly lower than might be expected from earlier work,<sup>1</sup> but the use of a modified acidolysis procedure may explain the divergences.

As silylating reagent *N,O*-bis(trimethylsilyl)-acetamide<sup>4</sup> (BSA) was used. The gas chromatogram of the reaction mixture obtained with ketol I on prolonged treatment with BSA in dioxane (or chloroform) exhibited, in addition to the initial peak with rel. ret. time 1.00, a peak with rel. ret. time 1.60. By gas chromatography-mass spectrometry it was shown that the initially

formed product had a molecular ion at  $m/e = 340$ , corresponding to a derivative with two TMS groups. The product with rel. ret. time 1.60 had a molecular ion at  $m/e = 412$ , indicating that a third TMS group had been introduced. It is assumed that the two hydroxyl groups of compound I reacted first to give the product of rel. ret. time 1.00, and that this compound reacted further in enolized form to give a derivative with three TMS groups. Carbonyl compounds II, IV, V, VI, and X also gave additional products on prolonged reaction with BSA; this is similarly considered to be due to derivatization of enol forms. Formation of TMS derivatives of enol forms of carbonyl compounds on treatment with BSA has been reported previously.<sup>4</sup>

Examination of the reaction mixture obtained on brief treatment of ketol III with BSA in dioxane solution gave a chromatogram with three peaks (rel. ret. time 0.82, 0.63, and 0.52). Prolonged treatment resulted in an increase of the 0.82 peak, while the other two peaks decreased. When pyridine was used as solvent, only the 0.82 peak appeared, even on relatively brief treatment (15 min). The compound corresponding to this peak was found to have a molecular ion at  $m/e = 340$  by gas chromatography-mass spectrometry; this indicates that the derivative had two TMS groups. Therefore, pyridine is a more suitable solvent than dioxane for the analysis of ketol III. For the analysis of mixtures of compounds I–XII, however, pyridine has the disadvantage of being a catalyst for the above-mentioned formation of additional products from compounds I, II, IV, V, VI, and X. These difficulties were circumvented by the use of dioxane-pyridine (10:1) as solvent. Thus, in the reaction mixture obtained on treatment with BSA in this solvent mixture, ketol I existed quantitatively as the 1.00 derivative for several hours, and ketol III was, after 3 h, almost completely (in some experiments completely) converted to the derivative with rel. ret. time 0.82. For quantitative estimation of ketol III, however, separate experiments with pyridine as solvent were preferred.

The derivatization of the remaining compounds in Table 1 was not studied in detail. However, in the experiments with lignin acidolysis mixtures, comparison of the gas chromatograms obtained on 1, 2, and 3 h treatment with BSA (solvent: dioxane-pyridine (10:1)) revealed only slight differences.

The method described above was used to elucidate the variation with acidolysis time of the composition of that part of the acidolysis mixture which consisted of "monomeric" products. Fractions of "monomeric" products obtained from spruce lignin on 8 h and 24 h acidolysis were compared with that of the 4 h product mixture. As judged from the gas chromatograms (see Fig. 2), the variation with time of acidolysis can be described as quantitative rather than qualitative. Quantitative estimations of ketols I and III showed that the former decreased (4 h, 4.6 %; 8 h, 4.4 %; 24 h, 3.0 %), while the latter increased (4 h, 0.8 %; 8 h, 1.15 %; 24 h, 1.6 %) with increasing acidolysis time. From these determinations and the gas chromatograms (Fig. 2) it is also clear that the amounts of ketones IV and V increased with time of acidolysis. These observations are in accord with the previously described<sup>5</sup> interrelationships of compounds I–V on acidolysis:  $I \rightarrow II \rightleftharpoons III \rightarrow IV + V$ .

A 4 h acidolysis product mixture of Björkman lignin of birch (*Betula verrucosa* Ehrh.) also was examined. The peaks corresponding to the TMS derivatives of ketol I and its syringyl analogue (1-hydroxy-3-(4-hydroxy-

3,5-dimethoxyphenyl)-2-propanone; rel. ret. time 1.48) dominated the gas chromatogram. Attempts to detect the syringyl compound in the acidolysis mixtures obtained from spruce lignin failed.

### EXPERIMENTAL

*Acidolysis.* A solution of 75–80 mg of Björkman lignin in 3 ml of 0.2 M HCl in dioxane-water (9 : 1) in a sealed glass ampoule was heated with refluxing acidolysis reagent as heating bath for the desired period of time. After being cooled, the ampoule was opened, and 2.4 ml of 0.225 M aqueous NaOH was added to reduce the acidity and to change the composition of the solvent mixture to dioxane-water (1 : 1). (On addition of the NaOH, the acidolysis mixtures became turbid, which probably was due to precipitation of high molecular weight material. This did not however, interfere with subsequent operations, and no attempt was made to eliminate it.)

*Gel filtration.* A column (2 × 50 cm) was prepared with 41 g of Sephadex G-25 (medium), using dioxane-water (1 : 1) as solvent. Acidolysis mixtures, prepared as described above, were applied to the column, and the elution was accomplished with dioxane-water (1 : 1) (flow rate 10 ml/h). The absorbance at 280 nm of the effluent, collected in 5 ml fractions, was continuously recorded with a Beckman DB spectrophotometer, equipped with a 1 mm flow cell and a recorder. The curve thus obtained with the 4 h acidolysis mixture from 5 mg spruce lignin is shown in Fig. 1. When 75–80 mg of lignin was used as starting material, the equipment used did not permit the complete curve to be recorded, but it was still possible to record the region containing the minimum prior to peak M (see Fig. 1). "Monomeric" products are eluted after this minimum,<sup>1</sup> so that those 5 ml fractions which included the "monomers" of the acidolysis mixture could be pooled on the basis of the UV measurements. Because the fractions were selected in such a way that the eluate, corresponding to the minimum, was included with certainty, small amounts of higher molecular weight material were included. Pooled fractions were extracted with chloroform (Na<sub>2</sub>SO<sub>4</sub> was added to facilitate extraction), until UV measurements showed that only minor amounts of lignin-related materials remained in the aqueous layer. The extract was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, the solvent removed by film evaporation, and the residue was dried over P<sub>2</sub>O<sub>5</sub> and KOH at 20 mmHg overnight, and weighed. In a series of experiments with spruce lignin (methoxyl content 15.2%), this fraction, consisting essentially of "monomeric" products, obtained on 4 h, 8 h, and 24 h acidolysis, weighed 14%, 17%, and 17% of the starting material, respectively.

*Preparation of trimethylsilyl (TMS) derivatives.* About 3 mg of the fraction of "monomeric" lignin acidolysis products (or a reference compound) were dissolved in 100  $\mu$ l dioxane and 10  $\mu$ l pyridine, and 50  $\mu$ l *N,O*-bis(trimethylsilyl)-acetamide (BSA) (Pierce Chem. Co., Rockford, Ill.) was added. Alternatively, the samples were treated with 60  $\mu$ l BSA in 100  $\mu$ l pyridine. Derivatives of reference compounds were also prepared with dioxane, chloroform, and dimethylformamide as solvent. Pyridine, and to a greater extent dimethylformamide, tended to accelerate the above discussed formation of additional products, presumed to be derivatives of enol forms, from the carbonyl compounds I, II, IV, V, VI, and X. Dimethylformamide therefore was used in certain experiments to study the formation of such products. All experiments were done at room temperature, and reaction mixtures were examined by gas chromatography after periods of time varying between 10 min and several hours.

*Gas chromatography* was accomplished on a Perkin-Elmer Model 880 Instrument. Column dimensions: 200 × 0.30 cm o.d. stainless steel tubing. Solid support: Chromosorb G, acid washed and treated with dimethyldichlorosilane, 80–100 mesh. Stationary phase: OV-1 (Applied Sciences Laboratories, State College, Pa.), 5% by weight of solid support. Temperatures: injection 285°, detector 230°, and column 220°. Carrier gas: N<sub>2</sub>, 25 ml/min. Detector: flame ionization. Quantitative determinations were made from standard curves, using docosane (ret. time 16.5 min) as internal standard. Mass spectra of some of the TMS derivatives were taken by the use of an LKB 9000 gas chromatograph-mass spectrometer unit.

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