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by Magnus Erickson, Sam Larsson, and Gerhard E. Miksche

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Gaschromatographic analysis of products of lignin oxidation.
7. Improved procedure for characterization of lignins by methylation and oxidative degradation[1]

by

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Summary - An improved procedure is described for the structural characterization of lignins by means of methylation and oxidative degradation. Methylation of the phenolic hydroxyl groups is carried out with dimethyl sulfate-potassium hydroxide in dimethoxyethane-methanol-water (25:35:30) at pH 11. The methylated lignin is degraded first at 82° with potassium permanganate-sodium periodate in diluted sodium hydroxide solution, which contains 15% tert.-butanol, and then with hydrogen peroxide at pH 9 to 10 (50°). The aromatic carboxylic acids obtained in that way are methylated with diazomethane and the principal component of the ester mixture is determined quantitatively using gaschromatographic

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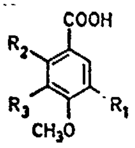
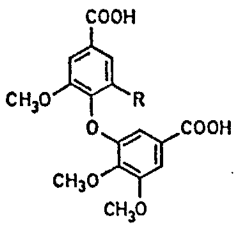
[1] Cf. Reference 1a for 6. Communication.

means. The yields of these esters are characteristic for the respective structures of lignin. Examples are presented for the oxidative degradation of lignins digested with sodium sulfide-cobtaining sodium hydroxide solution ("sulfate lignins"), and lignin models are discussed.

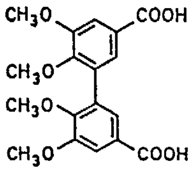
In a preceding investigation of this Series we have described a two-stage degradation of methylated lignin preparations (1b). First, the lignin was oxidized with potassium permanganate at pH 11 to 12; that step yielded a mixture of arylcarboxylic acids and artlglyoxylic acids. In a second oxidative step, the latter products were transformed into the corresponding arylcarboxylic acids. The methyl esters of these degraded acids obtained with diazomethane were separated by gaschromatographic means and identified by mass-spectrometric means (i.e. using a combined gaschromatograph—mass-spectrometer). The most important breakdown acids (1 - 9) could also be determined quantitatively (1c).



1 $R_1=R_2=R_3=H$
2 $R_1=OCH_3$, $R_2=R_3=H$
3 $R_1=R_3=OCH_3$, $R_2=H$
4 $R_1=OCH_3$, $R_2=H$, $R_3=COOH$
5 $R_1=OCH_3$, $R_2=COOH$, $R_3=H$
6 $R_1=R_3=OCH_3$, $R_2=COOH$



7 R = H
8 R = OCH_3



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The degradative procedure outlined in the present paper can be employed in a versatile manner in the chemistry of lignins and related natural substances. One of the possibilities of application is found in the chemotaxonomic comparison of lignins of different provenances. For that purpose, the lignin is first separated by heating the plant material with sodium sulfide-containing sodium hydroxide solution ("sulfate digestion"). Methylation and oxidative degradation of the "sulfate lignin" obtained in this manner, followed by quantitative determination of the methyl esters of the most important degradation acids, leads to a yield ratio of the methyl esters characteristic for the lignin in question (cf. Table 2). This yield ratio reflects the fractions of guaiacyl, syringyl and p-hydroxyphenyl propane structures in the construction of the lignin in question (1c). The procedure of nitrobenzene oxidation of lignins used in the past for determination of these particular aspects (cf. Ref. 2 for a critical review) is inferior to the two-stage oxidative degradation to the extent that it takes into consideration only the non-condensed aryl propane units.

In the comparison of the results obtained in lignins of different provenances, good reproducibility of the quantitative determinations of the breakdown acids is of significant importance. In order to ensure that reproducibility, we have worked out the experimental conditions for both the methylation and the oxidative degradation. The description of the improvements introduced at the present time as well as the application of this procedure in several sulfate lignins and a number of lignin models represent the subjects of the present communication.

Methylation

Hitherto, methylation of the phenolic hydroxyl groups of lignins has been carried out in the usual manner viz. by alternate addition of dimethyl sulfate and sodium hydroxide solution to a solution of the lignin preparation in aqueous dioxane (1b). The degree of effectiveness of this procedure—with regard to the considerable quantities of dimethyl sulfate and sodium hydroxide solution utilized in its course—is low. Due to the—at times—large excess of hydroxyl ions, we find that the undesired hydrolysis of dimethyl sulfate is promoted; the alternate addition of sodium hydroxide solution and dimethyl sulfate, furthermore, can be arranged in a reproducible manner only with a large expenditure of work. It therefore appeared desirable to carry out methylation under optimal conditions, i.e. at the most complete ionization of the phenolic hydroxyl groups possible and at the lowest concentration of hydroxyl ions possible. These conditions are given when the pH value during methylation lies within the order of magnitude of the <u>pK</u> value (about 10) of the phenolic hydroxyl groups of the lignin.

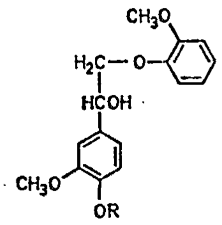
Stabilization of the pH values during methylation was achieved by addi- 129
tion of sodium hydroxide solution by means of a magnetic valve, which was controlled by a glass-calomel combination electrode coupled with a pH controlling device. The entire quantity of dimethyl sulfate is added at the start of methylation, and the pH value is kept at 11. We found a mixture of dimethoxyethane-methanol-water (35:35:30) to be suited as solvent system for methylation of lignin preparations and models. At 25°C methylation is completed after about 24 hours.

The advantage of this procedure compared to the one employed in the past is found in the very mild conditions for methylation of the phenolic hydroxyl groups. Completeness of methylation was checked by methylation of model compounds.

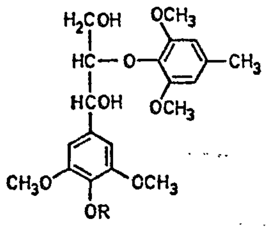
<u>Model experiments for methylation</u>

The phenolic Models <u>10</u>, <u>12</u>, and <u>14</u> were methylated at room temperature and pH 11 in the manner just outlined for lignin. The expected methyl ethers <u>11</u>, <u>13</u>, and <u>15</u> were isolated in high yields. The sole side reaction would have been the methylation of a small fraction of the alcoholic hydroxyl groups of

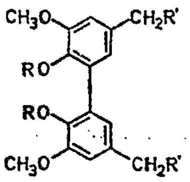
Models 10 and 12; among the products of the methylation reactions of Models 12 and 14, we detected only traces of the starting material (< 0.5%) (gaschromatography and thin-layer chromatography); in the case of the product obtained on methylation of Model 10, the quantity of the starting material present was below the limit of detection. Complete methylation of the two *o,o*-disubstituted Models 12 and 14 under these mild conditions is worthy of note. This procedure possesses also preparative value for the preparation of methyl ethers of sensitive phenols. Using a slightly modified procedure, it is possible to acylate phenolic hydroxyl groups also with carboxylic anhydrides, and to esterify carboxylic groups with dimethyl sulfate (3).



10 R = H
11 R = CH_3



12 R = H
13 R = CH_3



14 R = R' = H
15 R = CH_3, R' = H
16 R = H, R' = CH_2CH_3
17 R = CH_3, R' = CH_2CH_3

Oxidative degradation

In the past, the first step of oxidative degradation was carried out in the following manner: A diluted solution of permanganate was added in a dropwise manner to a suspension of the methylated lignin in a one per cent sodium 130 carbonate solution [reaction temperature for gymnospermian lignins, 100°C (1b), and for angiospermian lignins, 80°C (1c)] (1b). Appearance of violet coloration ten minutes after the concluding addition of potassium permanganate indicated the end of oxidation. In that procedure, both the drop rate and the duration of reaction (about six hours) had to be adjusted to the consumption of permanganate by the lignin preparation, requiring constant monitoring of the oxidative process. The reproducibility of that reaction step was impaired by the difficulty of assessing the excess of potassium permanganate present at a given moment in the solution and, thus, the optimal drop rate. It was also possible that the large quantities of manganese dioxide formed during the reaction would, under certain circumstances, either absorb or envelope undissolved lignin.

Standardization of the procedure of adding potassium permanganate as well as the prevention of formation of large quantities of manganese dioxide, thus, appeared to be desirable. Both these aspects have been settled on introduction of the modification of the degradation procedure described next. The second step of degradation with hydrogen peroxide in weakly alkaline solution was retained unchanged.

In alkaline solution IO_4^- oxidizes manganate(V) or manganate(VI) to MnO_4^-. The procedure described by Lemieux and Von Rudloff is based on that reaction, <u>viz.</u> cleavage of olefinic double bonds by oxidative means using a weakly alkaline solution of sodium periodate in the presence of catalytic quantities of potassium permanganate (4). The Lemieux-Von Rudloff procedure has since found use in the selective oxidation of double bonds.

We have now replaced the permanganate oxidation in the first step of the oxidative degradation by treatment of the methylated lignin with potassium permanganate-sodium periodate in diluted sodium hydroxide solution at 82°C. Using that approach we have been able to both simplify to a considerable degree the execution of this degradative step and prevent the formation of manganese dioxide during the course of oxidation; the permanganate concentration now is constant during the entire reaction period. A precondition for the success of oxidative degradation with permanganate-periodate is the employment of *tert.*-butanol (15%) as solvent additive. If that addition is omitted, we obtain greatly reduced yields (which, furthermore, are poorly reproducible) of all breakdown acids. Experiments on the action of potassium permanganate and/or sodium periodate on several of these breakdown acids revealed that these acids are degraded in the absence of *tert.*-butanol (Table 1). In the presence of *tert.*-butanol, on the other hand, these acids are largely stable; following a period of reaction of six hours, measurable attacks could be determined only in the cases of trimethylgallic acid (3) and meta-hemipinic acid (5).

The cause bringing about stabilization of the breakdown acids in the presence of *tert.*-butanol, perhaps, may be found in the fact that the alcohol acts as agent capturing hydroxyl radicals or other strongly oxodizing intermediate products. Hydroxyl radicals have been postulated as products of oxidation of hydroxyl ions by MnO_4^- (5). According to other authors, the reduction of MnO_4^- in sodium hydroxide solution requires the presence of transition metal ions (Co^{2+}, Ni^{2+}; in the present case, probably, present in traces) (6). In their absence, permanganate is stable also in strongly alkaline solutions (6; 7).

A further advantage of the employment of *tert.*-butanol as solvent additive (8) is found in the increased solubility of the lignin preparation to be 131
oxidized in the mixture obtained; in the majority of cases observed, the oxidative solution was homogenous either already prior to the onset of oxidation or after a short period of oxidation.

The overall yields of the most important aromatic breakdown acids obtained with the aid of this new procedure at a reation temperature of 82°C and a reaction time of 6 hours were slightly greater than those obtained using the

Table 1 - Stability of breakdown acids under the conditions of oxidative degradation (six hours at 82°C). Key: 1, Breakdown acids recovered (in per cent of the theoretical value); a, Determined gaschromatographically as methylester; b, Sodium carbonate; c, Sodium hydroxide solution; d, Precipitate of manganese dioxide during oxidation.

$c_{\mathrm{KMnO_4}}$ (mMol/l)	$c_{\mathrm{NaJO_4}}$ (mMol/l)	$c_{tert.\text{-}\mathrm{Butanol}}$ (Vol-%)	c_{Alkali} (Mol/l)	① wiedergewonnene Abbausäuren [a] (in % d. Th.)					
				2	*3*	*4*	*5*	*7*	*9*
3	30	0	0,1 [b]	0	0	30	–	–	–
0	30	0	0,1 [b]	31	30	–	–	–	–
0	30	0	0,14 [c]	30	28	59	–	–	–
3	30	0	0,1 [c]	38	31	70	–	–	–
3 [d]	0	0	0,1 [c]	83	61	97	–	–	–
3	30	15	0,1 [c]	96	86	103	–	95	95
3	30	15	0,1 [c]	98	86	99	83	93	95

Table 2 - Oxidative degradation of several sulfate lignins. Key: 1, Esters of the breakdown acids; 2, (mg ester per 100 mg Bjoerkman lignin or per 400 mg wood flour, respectively); 3, Wood flour. —— In instances where no yields are reported in this Table, the yields were below the limits of detection (0.1 mg per 100 mg Bjoerkman lignin or 400 mg wood flour). Yields of less than 2 mg per 100 mg Bjoerkman lignin are given with an accuracy of 0.05 mg; yields exceeding 2 mg per 100 mg Bjoerkman lignin are given with an accuracy of 0.1 mg; the corresponding is valid for the yields from wood flour (per 400 mg).

Lignin	① Ester der Abbausäuren ② (mg Ester per 100 mg Björkman-Lignin oder 400 mg Holzmehl)								
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Picea abies (Björkman-Lignin)	0,5	21,4	0,15	6,1	0,55	–	1,75	–	3,6
	0,5	20,6	0,15	6,0	0,55	–	1,75	–	3,9
	0,5	21,1	0,15	5,8	0,55	–	1,6	–	4,0
Picea abies (Holzmehl) –③	0,5	21,3	0,45	5,9	0,6	–	1,65	–	3,4
Torreya nucifera (Holzmehl) –③	0,7	23,6	–	6,8	0,7	–	2,9	–	5,9
	0,7	23,1	–	6,7	0,5	–	2,6	–	5,9
Taxus baccata (Holzmehl) –③	0,6	20,1	–	5,9	0,6	–	2,4	–	5,8
	0,5	21,0	–	6,2	0,5	–	2,5	–	5,9
Quercus robur (Holzmehl) –③	–	5,8	15,8	1,0	0,2	0,25	0,25	0,95	0,45
Syringa vulgaris (Holzmehl) –③	–	5,7	11,6	1,2	0,15	0,15	0,3	1,4	0,75

earlier procedure (1b). In breakdown experiments—not described further in the present paper—using methylated sulfate lignin of white birch (Betula verrucosa) obtained by sulfate cooking of Bjoerkman lignin, it was found that the oxidative breakdown of that lignin, in essence, was completed after a reaction time of four hours; prolonged reaction times—of five and six hours—led to only insignificant increases of breakdown acids.

Several results of oxidative degradation of sulfate lignins, obtained with the aid of our improved methylation and degradation procedures, are presented in Table 2. The sulfate lignins were obtained by means of sulfate cooking of Bjoerkman lignin (pine) and of the extracted wood flour (cf. in Experimental) of several gymonsperms and angiosperms. In several instances, we have carried out double and triple determinations; these experiments demonstrate the reproducibility of the results obtained with the aid of these breakdown procedures.

Model experiments on oxidative breakdown

In order to determine in some greater detail the behaviors of the most important structural types of lignin, we have subjected a series of model substances representative for these structures to oxidative degradation with alkaline potassium permanganate-sodium periodate, followed by treatment with hydrogen peroxide. Models with free phenolic hydroxyl groups—with the exception of Model 26—were methylated with dimethyl sulfate at pH 11 prior to degradation. The yields of methyl esters of breakdown acids 2, 3, 4, and 9, determined by gas-chromatographic means, are presented in Table 3.

Models of the guaiacyl propane type, on average, gave somewhat greater yields than those of the syringyl propane type. As already mentioned, that

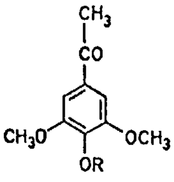
Table 3 - Oxidative degradation of lignin models. Yields (in per cent of the theoretical value) of the methylesters of the breakdown acids 2, 3, 4, and 9 (determined by gaschromatographic means). Key: 1, Model; 2, Breakdown acid; 3, Yield of methylester; a, Probably formed from a contaminant of 26 by 5,5'-dehydro-diveratum aldehyde (27).

① Modell	13	17		19	21	23	23	25
② Abbausäure	3	9	4	3	2	3	3	3
③ Ausbeute an Methylester	74	66	2	78	65	68	70	77

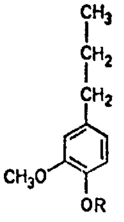
① Modell	26		28	31	33	35	37		39
② Abbausäure	4	9ⓐ	2	2	2	2	2	4	3
③ Ausbeute an Methylester	70	2	60	86	86	91	89	76	75

finding may be attributed to the slow degradation of trimethylgallic acid (3) 133

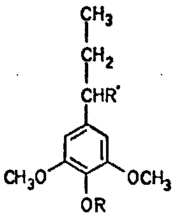
during oxidative breakdown. Models with benzylic oxygen function or ring-conjugated double bond (13, 19, 25, 26, 31, 33, 35, 37, and 39) were oxidized in very good yields to the corresponding carboxylic acids. Models without benzylic oxygen function (CH_2 group)—viz. Compounds 17, 21, 23, and 28—gave the expected breakdown acids in lesser yields, which, however, could still be described as good.



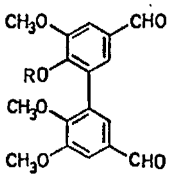
18 R = H
19 R = CH3



20 R = H
21 R = CH3

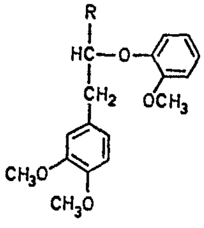


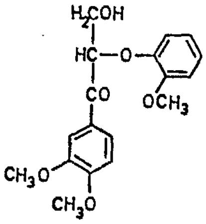
22 R = H, R' = H
23 R = CH3, R' = H
24 R = H, R' = OH
25 R = CH3, R' = OH



26 R = H
27 R = CH3

In the case of 5,5'-dehydro-divanilline monomethylether (26), the unetherified ring was completely opened on oxidation; the same should occur in the case of rings of lignin having a phenolic hydroxyl group as aroxyl residue in the etherified form in arylglycerol-β-aryl ether structures (the type of Model 12).


28 R = CH_2OH
29 R = $COOH$
30 R = $COOCH_3$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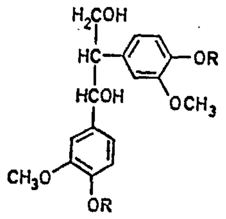
32 R = H
33 R = CH_3

While the C_2 bridge in the diphenylethane, 33, and in the stilbene, 37, is cleaved during oxidative degradation—with good yields of the corresponding breakdown acids—we find that the C_1 bridge in diphenyl methane structures is largely stable under the conditions of oxidative degradation (as long as the phenolic hydroxyl groups in the two rings are present as methyl ethers). The lignin models exhibiting diphenyl methane structures (40, 44, 48, and 54) yielded the breakdown acids 2 and 4 or 2 and 5, respectively, only in small quantities (Table 4). That finding must be attributed to the fact that these breakdown acids can be formed only on complete oxidative breakdown of one of the two rings.

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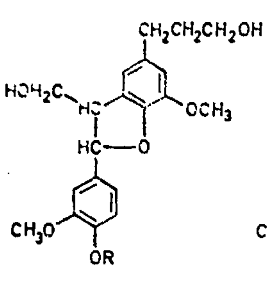
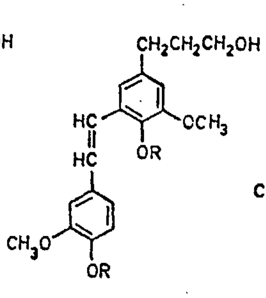

34 R = H
35 R = CH_3


36 R = H
37 R = CH_3

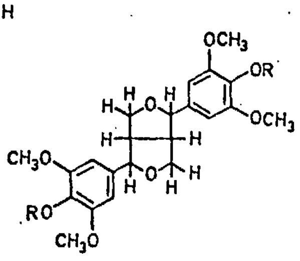
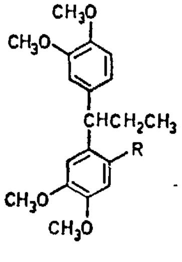

38 R = H
39 R = CH_3

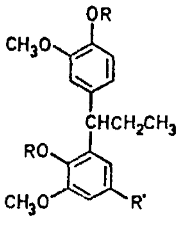
Table 4 - Oxidative degradation of lignin models with diphenylmethane structure. Yields (in per cent of the theoretical value) of methylesters of breakdown acids 2, 4, and 5 (determined by gaschromatographic means). Key: 1, Model; 2, Breakdown acid; 3, Yield of methylester.

① Modell	40		44		48		54
② Abbausäure	2	5	2	4	2	5	4
③ Ausbeute an Methylester	8	4,5	10	13	7	10	6,5

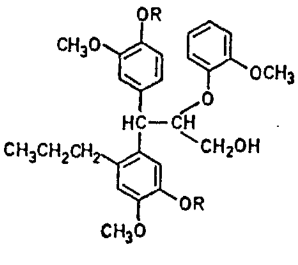
The mixture of breakdown products of Models 40 and 44, following methylation with diazomethane, exhibited in the gas-chromatogram several heavy volatile components in addition to small quantities of the methyl esters of breakdown acids 2, 4, and 5; one of these components always predominated (42, yield approximately 30%; 46, yield approximately 60%0. We are dealing here with the methyl esters of the monocarboxylic acids 41 and 45, which arose on degradation of the propyl side-chains of 40 and 44, respectively; in both cases, the ethyl residue on the bridge carbon remained intact. In addition, we have recovered 6-veratroyl veratrumic acid (49, from 40; yield approximately 5%), the lactone 59 (from 40; yield approximately 10%), and 5-veratroyl veratrumic acid (51, from 44; yield approximately 5%); in addition, we have in both cases obtained several unidentified breakdown acids in small quantities. The crude 135



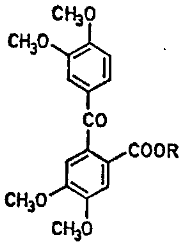
40 $R = CH_2CH_2CH_3$
41 $R = COOH$
42 $R = COOCH_3$



43 $R = H, R' = CH_2CH_2CH_3$
44 $R = CH_3, R' = CH_2CH_2CH_3$
45 $R = CH_3, R' = COOH$
46 $R = CH_3, R = COOCH_3$

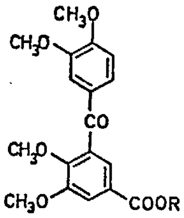


47 $R = H$
48 $R = CH_3$



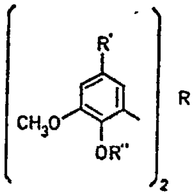
49 R = H

50 R = CH_3



51 R = H

52 R = CH_3



53 R = CH_2, R' = $CH_2CH_2CH_3$, R'' = H

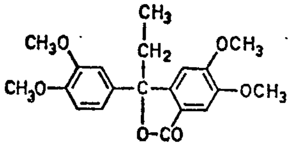
54 R = CH_2, R' = $CH_2CH_2CH_3$, R'' = CH_3

55 R = CH_2, R' = COOH, R'' = CH_3

56 R = CH_2, R' = $COOCH_3$, R'' = CH_3

57 R = CO, R' = COOH, R'' = CH_3

58 R = CO, R' = $COOCH_3$, R'' = CH_3



59

dimethyl ether 48 obtained on methylation of Model 47 also yielded several heavy volatile breakdown products in addition to the methyl esters of 2, 5, and 49; these products, however, were not investigated further.

Compound 53 is a model for the diphenyl methane structures formed during alkaline degradation of lignin on condensation of guaiacyl propane structures with formaldehyde. Its methyl ether, 54, yielded the expected iso-hemipinic acid (4) in small yield; the incompletely degraded acids 55 and 57 (principal product) predominated among the products of degradation.

It must be assumed that, at least, a part of the units of lignin yielding the *iso*- (4) and, in particular, the meta-hemipinic acid (5) as well as the 3,4,5-trimethoxyphthalic acid (6) (not treated further in the present paper) is present as structures of the type of Compounds 40 and 44. For that reason, we must regard the values for the corresponding condensed structures of lignin calculated on the basis of these breakdown acids as lower limiting values (cf. Ref. 1c).

The oxidative degradation of Model 28 led to formation of carboxylic acid 29 (at a yield of about 25%) in addition to veratrumic acid (2; cf. Table 3). In conjunction with the afore-outlined demonstration of the formation of Acids 41, 45, and 55 from Models 40, 44, and 54, that finding indicates that the oxidative degradation of the aliphatic side-chains of models and also of lignin, may under certain circumstances be subjected to steric hindrance. The propyl side-chains of Models 21 and 23, which are freely accessible to the attack of the oxidative agent, were completely degraded. Degradation of saturated alkyl residues probably is initiated by hydroxylation of the benzylic C 136
atom by MnO_4^- (cf. Ref. 7); this pathway of degradation is supported by the mass-spectrometric demonstration of the lactone 59 as product of degradation of 40.

The structures of breakdown acids 29, 41, 45, 49, 51, 55, and 57 have been derived from the mass-spectra of the corresponding methyl esters (30, 42, 46, 50, 56, and 58).

Experimental

Translator's note - Since the authors have made use of numerous abbreviations, I have employed the corresponding standard abbreviations used in *Chemical Abstracts* (1975). Non-standard abbreviations, like rel. int. (relative intensity) will be defined below on first use. V.N.N.

Methylation

Preparation of plant material. Sulfate cooking - The dried plant material roughly reduced to small pieces was—suspended in toluene—finely ground in a small vibration (swing) mill (manufactured by McCrone Research Associates, London). Grinding time: 30 minutes at room temperature; grinding body made of sintered corundum; grinding vessel made of polypropylene. The material to be ground was extracted in a Soxhlet apparatus for 24 hours with benzene-ethanol (2:1) and for 24 hours with 95% ethanol. The material was then passed through a sieve; only a small portion was retained on a 45-mesh screen. The finely ground main fraction was used for sulfate digestion. For the procedures used in sulfate digestion, cf. Ref. 1c.

Methylation - Methylation was carried out in a vessel made of Pyrex® glass; the lower part of this vessel was formed in a manner ensuring that—at a liquid quantity of 10 ml.—the electrode head is dipped, by means of a magnet rod (Teflon®), to an adequate extent into the mixed fluid. The combination electrode (glass calomel electrode No. GK 2302, manufactured by Radiometer, Copenhagen) was introduced by way of a tube attached at an angle on the side; a flange lid with four passage openings permitted introduction of the inert gas (N_2), of the liquor, and of dimethyl sulfate, and pressure equalization, respectively. The liquor was added by means of a magnetic valve (MNV 1, Radiometer), which was controlled by a pH controlling device (TTT1 or TTT2 'titrator,' Radiometer). The tubing system for the liquor, which was kept in a 50-ml. measuring cell, was made of Teflon (internal diameter: 1 mm), with the exception of a short piece of natural rubber for the magnetic valve. The outlet openings for the mother liquor must be located below the surface of the liquid close to the electrode head, in order to ensure a low pH band width at a lower pH limiting value of 11.

The solution of 80 mg of Bjoerkman lignin (or sulfate lignin from 50 mg of Bjoerkman lignin or 200 mg of wood flour, 25 mg of the model substances, and 10 mg of the case of Model 47, resp.; all test samples, with the exception of wood flour, were weighed with an accuracy of 0.01 mg) is introduced into the nitrogen-filled apparatus in 8 ml. of a mixture of 1,2-dimethoxyethane-methanol-water (35:35:30; the "methylation mixture"); the vessel was rinsed three times with 1 ml. of that same mixture. Once the magnetic stirrer is switched on, the addition of the liquir is strated, and 1 ml. of dimethyl sulfate is added by means of a pipet. As liquor we used an approx. 15% solution of KOH in the methylation mixture. Utilization of liquir was arrested after 18 to 24 hours. Addition of liquir was then interrupted, and the solution was acidified to pH 4 with the aid of 2 M H_3PO_4. The content of the vessel was then transferred into a separating funnel; the vessel was rinsed out with a total of 35 ml. of acetone-water (6:1). Following addition of 30 ml. of $CHCl_3$, the material was shaken twice more with 60 ml. of acetone-$CHCl_3$ (1:1) in each instance. The pooled extracts were washed with 5 ml. water and brought to dryness at room temperature in three-necked 250-ml. round-bottomed flasks subsequently used for oxidative degradation. We then added 10 ml. of *tert.*-butanol, and the solvent was once more removed by evaporation. The residue was subjected to oxidative degradation as described further below. Success of oxidation depends on the complete removal of acetone residues.

1-(3,4-Dimethoxyphenyl)-2-(2-methoxyphenoxy)-ethanol (11) - A total of 173 mg of 11, m.p. 130 - 131° (Ref. 9 gives a m.p. of 133-134°), was obtained from 200 mg of 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-ethanol (10) (Ref. 9) following methylation at pH 11 (from ethyl acetate). The mother liquor contained chiefly Compound 11 [thin-layer on silica gel (Kieselgel HF_{254}, Merck; solvent system, acetone-hexane (1:3), developped twice].

2,2',3,3'-Tetramethoxy-5,5'-dimethylbiphenyl (15) - 15 was obtained in 137
the form of colorless crystals, m.p. 103 - 105°, on methylation of 200 mg of 2,2'-dihydroxy-3,3'-dimethoxy-5,5'-dimethylbiphenyl (14) (Ref. 10) at pH 11 and crystallization from ethyl acetate-hexane; yield: 183 mg. Compound 15 has been described as oil in literature (10).

Gas-chromatographic examination of the reaction product obtained in the preceding experiment. Condtions: Cf. quantitative determination of the methyl esters of the breakdown acids. Silylation of the reaction product: 5 µl. of an approx. 10% solution of the reaction product were heated for 5 minutes (min) to 120° in $CHCl_3$ with 5 µl. of bis-(trimethylsilyl-trifluoroacetamide (BSTFA, Pierce Chem. Co.) and 5 µl. of pyridine in a capillary; 1 µl. of that solution was used for gas-chromatographic examination. Retention times (in min): Bis-(trimethylsilyl)-ether of 14, 7.0; 15, 11.8. The product (15) was gas-chromatographically pure; starting material could not be detected with certainty.

Actually: N,O-bis-....

threo-3,4,5-Trimethoxy-phenylglycerol-β-(2,6-dimethoxy-4-methylphenyl)-ether (13) - Following recrsytallization, a total of 91 mg of Compound 13 was obtained in pure form from 102 mg of threo-3,5-dimethoxy-4-hydroxyphenylglycerol-β-(2,6-dimethoxy-4-methylphenyl)-ether (12) (Ref. 25) on methylation at pH 11; m.p. 79 - 81° (ethyl acetate-hexane). The thin-layer chromatographic examination of the reaction products (Kiselgel G, Merck; acetone-hexane, 1:1; spraying reagent, H_2SO_4-formalin, 9:1) revealed—in addition to 12—only traces of more rapidly migrating material, formed probably due to methylation of the alcoholic hydroxyl groups.

Gas-chromatographic examination of the reaction product obtained in the preceding experiment. Apparatus: Perkin-Elmer Model 900. Separating columns made of stainless steel, measuring 1 m in length with an external diameter of 0.3 cm. Carrier material: Chromosorb G, 80 - 100 mesh, washed with acid and treated with dimethyldichlorosilane. Stationary phase: Silicone elastomer SE-30 (5 per cent by weight of the carrier material). Working temperatures: Injector, 270°; separating column, 230°C. Carrier gas: N_2; flow rate, 30 ml./min. Retention times (in min): Bis-(trimethylsilyl)-ether of 13, 12.0; tris-

(trimethylsilyl)-ether of 12, 13.8. Only traces of the starting material could be detected by gas-chromatographic means following silylation of the mother liquor with BSTFA-pyridine (cf. further above).

Oxidative degradation

Performance: A total of 40 ml. of 0.5 M NaOH was added to the methylated sample dissolved in 40 ml. of tert.-butanol-water (3:1) (Merck, analytically pure); next, 100 ml. of 0.06 M $NaIO_4$ and 20 ml. of 0.03 M $KMnO_4$ were added (in that order). The flask was heated—with vigorous stirring of the content—in a thermostatted water-bath to 82° and kept at that temperature for six hours. Then we added 5 ml. of ethanol, and, after ten min, the MnO_2 formed was removed by suction through a layer of kieselguhr, and the residue was washed with a little 1% sodium carbonate solution. Following coolong, the material was shaken twice with 50 ml. of ether, and the pooled ether extracts were washed with 15 ml. of 1% sodium carbonate solution; the latter were pooled with the aqueous phase.

The aqueous solution was neutralized with 1 M H_2SO_4 and reduced to 30 ml. To this solution, we added 0.9 g of $NaCO_3$ (anhydrous) and 5 ml. of 30 to 35% H_2O_2. Next, 100 mg of activated MnO_2 were added, and the manganese dioxide was then removed by filtration following discontinuation of gas formation (checked by repeated addition of a little MnO_2). The container was rinsed with 10 ml. of water. The filtrate was acidified to pH 2 with concd. H_3PO_4 and shaken three times with the 1.5-fold volume of acetone-$CHCl_3$ (1:1). The pooled extracts were dried over Na_2SO_4, and the solvent was removed by evaporation. The residue was dissolved in 10 ml. of methanol, and an identical volume of an approx. 2% ethereal solution of diazomethane was added. After 30 min, the solvent was again expelled, and the residue was added to 1 ml. of a $CHCl_3$ solution containing

5 mg/ml. of pyromellitic acid tetramethyl ester (internal standard for gas chromatography). For gas-chromatographic determination of the methyl esters, 1 µl. of that solution was used.

Gas-chromatographic determination of the methyl esters. Apparatus: Perkin-Elmer Model 900 with double flame ionization detector. Separating column made of stainless steel, measuring 1.75 m in length, with an external diameter of 0.3 cm. Carrier material: Chromosorb G, 80 - 100 mesh, washed with acid and treated with dimethyldichlorosilane. Stationary phase: Silicone elastomer 138
OV-17 (1.5 per cent by weight of the carrier material). Working temperatures: Injector, 300°; separating column, 160 to 255°, 5°/min, and then isothermal. Carrier gas: N_2; flow rate, 25 ml./min. The chromatograms were evaluated by graphic means (peak height x peak width at half-height). The yields reported represent mean values of—as a rule—three chromatographic determinations. The correction factors, determined on the basis of analyses of ester mixtures of known composition, must be checked at frequent intervals. It is furthermore necessary to reactivate the separating column from time to time by injecting 1 µl. of polyglycol (average molecular weight: 300) and passage under the aforementioned conditions.

Oxidative degradation of model compounds. References in literature for the preparation of the models: 2,2'-Dihydroxy-3,3'-dimethoxy-5,5'-dipropyl-biphenyl (16) (Ref.10); 3,5-dimethoxy-4-hydroxy-acetophenone (18) (Ref. 11); 2,6-dimethoxy-4-propyl-phenol (22) (Ref. 12); 1-(3,5-dimethoxy-4-hydroxyphenyl)-1-propanol (24) (Ref. 13); 6-hydroxy-5,5',6'-trimethoxy-biphenyl-3,3'-dicarbaldehyde (26) (Ref. 14); 1-(2-methoxy-phenoxy)-2-(3,4-dimethoxyphenyl)1-propanol (28) (Ref. 15); 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-3-hydroxy-1-propanon (31) (Ref. 16; erythro-1,2-bis-[4-hydroxy-3-methoxyphenyl]-1,3-propanediol (32) (Ref. 17); dihydro-dehydro-diconiferyl alcohol (34) (Ref. 18); *trans*-3,4'- dihydroxy-3,3'-dimethoxy-5-(3-hydroxypropyl)-stilbene (36) (Ref. 19); D,L-syringaresinol (38) (Ref. 20); 2-(2-methoxyphenoxy)-3-(5-hydroxy-4-methoxy-2-propylphenyl)-3-(4-hydroxy-3-methoxyphenyl)-1-propanol (47) (Ref. 15).

The model compounds with free phenolic hydroxyl groups—with the exception of 5,5'-dehydro-divanilline monomethyl ether (26)—were methylated prior to oxidative degradation. The methyl ethers obtained in this manner were degraded by oxidative means without further purification. In the degradation of methyl ethers 21 and 23, we examined the neutral fraction of the breakdown products by gas-chromatographic means; in both instances, that fraction weighed approx. 1 mg. Starting material (21 and 23, resp.) could be detected only in traces.

The methyl esters obtained on degradation of Models 28, 40, 44, and 54 were separated in micrograms by gas-chromatographic means, and collected in capillaries. For that purpose, we used a Perkin-Elmer Model 880 gas chromatograph.

Gas chromatography: For conditions, cf. examination on methylation of 12. Flow rate of carrier gas, 25 ml./min. *Retention times* (relative to 5,5'-dehydro-diveratrumic acid dimethyl ester, *t* = 19.8 min): 30, 0.41; 40, 0.43; 42, 0.65; 44, 0.40; 46, 0.67; 50, 1.00; 52, 1.01; 54, 0.46; 56, 1.32; 58, 1.86; 59, 1.14.

Mass spectra. Apparatus: AEI MS 902. Electron energy, 70 eV. *Ion source temperature*, 170°C. Partial mass spectra, only ions of mass numbers > 100 were taken into consideration.

2-(2-Methoxyphenoxy)-3-(3,4-dimethoxyphenyl)-propionic acid methyl ester (30). *m/e*, relative intensity (R.I.) (≥ 10%): 346, 59; 223, 98; 22, 12; 191, 16; 181, 26; 164, 19; 163, 40; 151, 100; 149, 14. The mass of the molecule ion was determined to be 346, 1416 (calcd. for $C_{19}H_{22}O_6$: 346, 1416).

1-(2-Carbomethoxy-4,5-dimethoxyphenyl)-1-(3,4-dimethoxyphenyl)-propane (42). m/e, R.I. (≥ 5%): 374, 42; 345, 100; 341, 19; 327, 11; 314, 8; 313, 16; 299, 7. (Mass of the molecule ion: Found, 374, 1741; calcd. for $C_{21}H_{26}O_6$, 374, 1729.)

1-(3-Carbomethoxy-5,6-dimethoxyphenyl)-1-(3,4-dimethoxyphenyl)-propane (46). m/e, R.I. (≥ 5%): 374, 50; 345, 83; 157, 8; 151, 100. (Mass of the molecule ion: Found, 374, 1712; calcd. for $C_{21}H_{26}O_6$, 374, 1729.)

3',4,4',5-Tetramethoxy-benzophenone-2-carboxylic acid methyl ester (50). Preparation obtained by synthetic means (Ref. 21). m/e, R.I. (≥ 10%): 360, 100; 329, 10; 223, 61; 165, 83.

3',4',5,6-Tetramethoxy-benzophenone-3-carboxylic acid methyl ester (52). m/e, R.I. (≥ 10%): 360, 100; 345, 14; 343, 21; 329, 24; 223, 31; 209, 93; 165, 74; 151, 46; 137, 14. (Mass of the molecule ion: Found, 360, 1215; calcd. for $C_{19}H_{20}O_7$, 360, 1209.)

5,5'6,6'-Tetramethoxy-diphenylmethane-3,3'dicarboxylic acid dimethyl ester (56). This compound crystallized on collection in the glass capillary; m.p. 102 - 103°. m/e, R.I. (≥ 10%): 404, 100; 373, 41; 372, 37; 344, 27; 341, 19; 313, 21; 312, 12; 209, 45; 195, 31; 179, 18; 171, 13; 163, 11; 151, 19; 149, 10. (Mass of the molecule ion: Found, 404, 1486; calcd. for $C_{21}H_{24}O_8$, 404, 1471.)

5,5',6,6'-Tetramethoxy-benzophenone-3,3'dicarboxylic acid dimethyl ester (58). m/e, R.I. (≥ 10%): 418, 100; 387, 44; 369, 13; 358, 15; 357, 10; 223, 88; 209, 90; 180, 14; 178, 17; 149, 15; 135, 10. (Mass of the molecule ion: Found, 418, 1256; calcd. for $C_{21}H_{22}O_9$, 418, 1264.)

3-Ethyl-3-(3,4-dimethoxyphenyl)-5,6-dimethoxy-phthalide (59). m/e, R.I. (≥ 5%): 358, 18; 329, 100; 165, 6; 164.5, 5. (Mass of the molecule ion: Found, 358, 1397; calcd. for $C_{20}H_{22}O_6$, 358, 1416.)

Syntheses

1-(2-Hydroxy-3-methoxy-5-propylphenyl)-1-(4-hydroxy-3-methoxyphenyl)-propane (43). A solution of 2.0 g of 1-(4-hydroxy-3-methoxyphenyl)-1-propanol (Ref. 22) and 8 g of 4-propylguaiacol (20) in 30 ml. of 10% NaOH was heated in a steel autoclave under nitrogen for two hours to 140°. Following acidification to pH 2, the aqueous solution was shaken with $CHCl_3$, and the residue obtained following evaporation of the solvent was distilled in a bulb tube. At 0.01 torr/140 - 150°, a total of 1.41 g of a colorless, viscous oil passed over; that quantity was taken up in 50 ml. of abs. ethanol and, following addition of 150 mg of 10% Pd/C, hydrated at normal pressure; it took up only a little hydrogen. Compound 43 could not be crystallized. (Found: C 72.37; H 8.02; calcd. for $C_{20}H_{26}O_4$ [330.43]: C 72.70; H 7.93.)

1-(2,3-Dimethoxy-5-propylphenyl)-1-(3,4-dimethoxyphenyl)-propane (44) was obtained by methylation of 43 with dimethyl sulfate-KOH. Colorless oil; b.p. 135 - 140°/0.01 torr (bulb tube). (Found: C 73.67; H 8.44; calcd. for $C_{22}H_{30}O_4$ [358.48]: C 73.71; H 8.44.) NMR (60 MHz, 10% in $CDCl_3$, tetramethylsilane as internal standard). δ-Values: 0.89 (3) t, $C-CH_3$: 0.92 (3) t, $C-CH_3$; 1.62 (2) m, $CH_2-\underline{CH_2}-CH_3$; 1.99 (2) m, $CH-\underline{CH_2}-CH_3$; 1.99 (2) m, $CH-\underline{CH_2}-CH_3$; 2.61 (2) t, $Ar-CH_2$; 3.61 (3)s, OCH_3 ; 3.78 (9) s, 3 OCH_3 ; 4.20 (1) t, Ar-CH; 6.54 (1) d, H_4 or H_6 ; 6.64 (1) d, H_4 or H_6 ; 7.20 (3) m, protons on the tri-substituted ring. $J_{4,6}$ = 2.0 Hz; J_{CHCH_2} = 7.8 Hz. In the case of the multiplets of the protons of the propyl side-chains, we have reported the mid points (and not the centers of mass).

2,2'-Dihydroxy-3,3'-dimethoxy-5,5'-dipropyl-diphenylmethane (53). A solution of 1.0 g of 2-methoxy-4-propylphenol (20), 980 mg of 2-hydroxy-3-methoxy-5-propylbenzyl alcohol (Ref. 23) and 0.5 g of NaOH in 14 ml. of ethyleneglycol monomethyl ether was heated in a steel autoclave under nitrogen for 3 hours to

170°. From ether-hexane, we obtained, on processing of the material in the usual manner, 0.8 g of 53 in the form of colorless, hexagonal leaflets, m.p. 100 - 101°. A reference (24) in literature gives a m.p. of 76- 77° for 53. (Found: C 73.11; H 8.05; caclcd. for $C_{21}H_{28}O_4$ [344.45]: C 73.23; H 8.19.)

2,2',3,3'-Tetramethoxy-5,5-dipropyl-diphenylmethane (54) was obtained from 53 with dimethyl sulfate-NaOH as a colorless oil, which was homogenous on gas-chromatography. NMR (60 MHz, 10% in $CDCl_3$, tetramethylsilane as internal standard). δ-Values: 0.88 (6) t, 2C-CH_3; 1.57 (4) m, 2 C-CH_2-C; 3.70 (6) s, 2 OCH_3; 3.80 (6) s, 2 OCH_3; 3.95 (2) s, Ar-CH_2-Ar; 6.48 (2) d, 2 H_4 or H_6; 6.55 (2) d, 2 H_4 or H_6. $J_{4,6}$ = 2.0 Hz. In the case of the multiplets of the protons of the propyl side chains, we have reported the mid points (and not the centers of mass).

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