

given as their sodium salts are presented in Table 1. The latter two acids have some activity, but it is very small in comparison with *o*-hydroxybenzoic (salicylic) acid. The antiulcer and antigastric secretory activity of various substances may not always go hand in hand; however, salicylic acid is not only a very potent antiulcer agent—it also reduces secretion of gastric juice. Long ago it was reported that sodium salicylate inhibits gastric acid secretion in man (1). It is interesting to note that of the three acids only the *o*-hydroxybenzoic acid (salicylic) gives relief in rheumatic fever. Stockman (3) showed that both the *m*-hydroxybenzoic acid and the *p*-hydroxybenzoic acid are practically inert as antiseptic and anti-rheumatic agents.

Acetyl salicylic acid (aspirin) is almost as active as salicylic acid in the prevention of gastric ulceration. The activity of other derivatives of salicylic acid, various dihydroxybenzoic acids, and related compounds is now under study.

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Constitution of Gymnosperm Lignin¹

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Freudenberg's deduction (5) of a benzopyrane ring constitution for gymnosperm lignin has recently been extended by Russell's (19) proposal of a polyflavanone structure as its specific form. However, the alleged synthesis offered as evidence is open to doubt (2). In fact, for substances such as lignin and its derivatives, which are unresponsive to most criteria for identity save ultimate and functional group analysis, even the best evidence of synthesis is contributory but scarcely conclusive. This is certainly the case when the reaction used is one so little suited to give predictable results as is the aluminum chloride-catalyzed Fries rearrangement, by means of which vanillin monoacetate is claimed to rearrange and condense to a polymer corresponding to gymnosperm lignin.

Evidence of a different and more reliable character has been accumulating in this laboratory, and we are prompted to report, perhaps somewhat prematurely, a summary of our investigations on the structure of lignin derivatives and the tentative conclusions drawn from our observations. The hypothesis that lignin from Western hemlock (*Tsuga heterophylla*) is a polyflavanone and that lignin sulfonic acid may be the polyflavanone 3-sul-

fonic acid is a concept upon which our experimental program was based as early as May 1947.

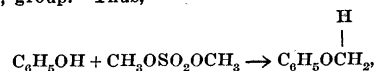
The evidence rests upon the use of periodic acid to determine the arrangement of oxygen substituted in the nonbenzenoid portion of lignin and its derivatives; upon the behavior of lignin sulfonic acid and other lignins in methylation and acetylation reactions, with particular attention to the influence of alkali; and upon the chemical and physical demonstration of the presence of carbonyl groups in the nonbenzenoid portion of the lignin molecule.

The use of periodic acid for structural determination of lignin rests upon the fact that lignin sulfonic acid is attacked by that reagent (14). It was soon understood that reaction with free phenols can scarcely be extensive, considering the small quantity present (15), but the large amount of demethylation with the formation of methanol does demonstrate that the reaction involves the aromatic ring (14, 15, 20). That phenols contribute to only a minor portion of the reactivity is shown also by the failure to block the oxidation by a single treatment with diazomethane, which should quantitatively methylate most phenolic substituents. Successive methylations with diazomethane fail to eliminate the oxidation until a composition corresponding to $C_9H_{7.75}O_{2.2}(SO_3NH_4)_{0.5}(OCH_3)_{1.52}$ (A) is reached. Reaction with periodate then ceases. Phenolic groups become available for methylation with diazomethane through an equilibrium promoted by alkali, in the presence of which successive methylations give the product (A), containing 19.6% methoxyl. The same number of methylations (five) carried out in neutral solution gives a product with only 14.5% methoxyl. More methoxyl can be introduced by dimethyl sulfate in cold aqueous alkaline solution, as found by Hibbert and his co-workers (11). This derivative, which has the composition $C_9H_{7.6}O_{2.2}(SO_3NH_4)_{0.42}(OCH_3)_{2.17}$, is not oxidized by periodic acid. A water-soluble acetyl derivative resistant to periodic acid oxidation has the composition $C_9H_6O_{2.6}(SO_3NH_4)_{0.5}(OCH_3)_{0.9}Ac_{1.2}$. During these reactions no alteration in molecular weight occurs.

Comparisons of the composition of ammonium lignin sulfonate, $C_9H_6O_{2.2}(SO_3NH_4)_{0.5}(OCH_3)$, and its methyl and acetyl derivatives show the introduction of a new oxygen atom for each methylene group.² Thus, during the methylation reactions there are added also the elements of water.

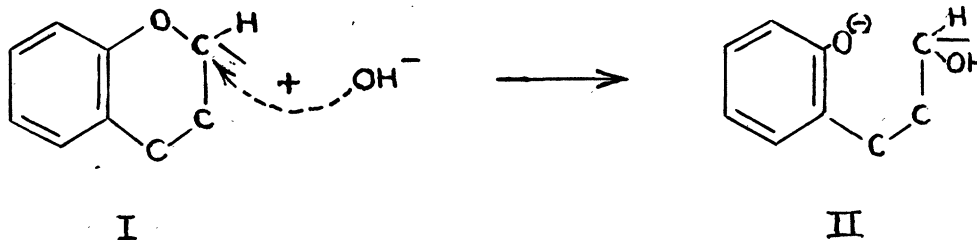
Since neither diazomethane nor cold dilute alkaline dimethyl sulfate will react with any but phenolic groups, phenols must be liberated during the methylations. For this to occur without alteration in molecular weight or change in the generic composition (*i.e.* no fragments lost) can mean only that the phenols are liberated from an intramolecular linkage. This means a ring opening. Assuming the benzopyrane structure, the phenolic libera-

² Methylation of a phenol adds not a methoxyl, but a methylene, group. Thus,



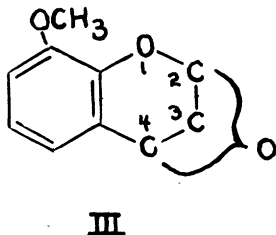
¹ Contribution from Pulp Mills Research Project, University of Washington, Seattle 5, Washington.

tion by alkali probably occurs through an S_N2 reaction thus:



The phenolate ion formed is ready for methylation. According to this mechanism, the new hydroxyl group is on the carbon atom originally bonded through the ether linkage.

The three oxygen atoms present in a C_9 unit of the lignin derivatives are obviously distributed with two attached to the aromatic ring and the third on one of the three nonbenzenoid carbon atoms, as is shown by isolation of such derivatives as the phenylpropanes (9), the propylcyclohexanes (7), vanillin (4), protocatechuic acid and isohemipinic acid (3) from lignin reactions; the arrangement can be generalized thus:



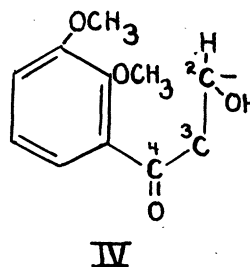
The third oxygen cannot be on carbon atom (2), because then the cleavage by an S_N2 reaction and methylation would give a ketone containing no more oxygen atoms than the original structure. The oxygen atom could then be on carbon atoms (3) or (4), and, as will be seen later, it must be on carbon atom (4).

The third carbon atom appears to be carbonyl. Experiments by Holmberg (10) have demonstrated the mercaptol character of a portion of the sulfur linkages in thioglycolic acid lignin by cleavage with acid mercuric chloride and by removal of sulfur on alkaline hydrolysis. We have confirmed this by hydrochloric acid catalyzed exchange between thioglycolic acid lignin and methylal in anhydrous methanol solution (12). Indication of carbonyl groups is also furnished by absorption spectra. The spectra of lignin derivatives are known to possess a prominent peak at 2,800 Å, a feature shared with several substances containing the guaiacyl acetophenone structure (13). By extending our observations to wave lengths below the 2,300-Å point, at which previous investigators have stopped, we have worked with a new feature in lignin spectrum comprising a band at 2,150–2,300 Å. The 2,800-Å peak, which previously has been assumed closely to obey Beer's Law, deviates slightly, and the newly studied absorption band increases in in-

tensity with dilution and with increase in pH; at the same time, the maximum of the band shifts. These fea-

tures are characteristic of spectra of lignin sulfonic acid, of thioglycolic acid lignin, and of their completely methylated and completely acetylated derivatives. Persistence of the effect is the absence of any other dissociable groups supports assignment of the phenomenon to functional groups involved in keto-enol tautomerism.

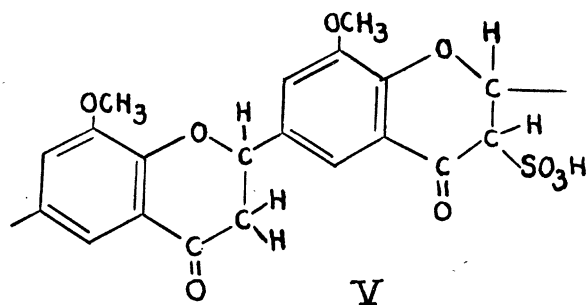
There is now to be considered the relation between the methylation reactions and the periodate oxidation. Diazomethane reacts under ordinary conditions to methylate only carboxylic acids, phenols or their precursors (8), and dimethyl sulfate in cold, dilute alkaline solution reacts only with phenols and flavanones. The elimination of periodate oxidation by reaction with those reagents and the demethoxylation, which accompanies the reaction when it does occur, can mean only that the potential phenolic group was involved in the oxidation. Accordingly, there are present in lignin sulfonic acid no other centers of periodate reactivity, *i.e.*, there are no vicinal aliphatic hydroxyl or carbonyl groups, either in lignin sulfonic acid or in its methyl derivatives. This has an important bearing on the arrangement of the oxygen atoms in these substances. In the methyl derivatives the original nonbenzenoid oxygen and the oxygen atom introduced during methylation cannot be on adjacent carbon atoms. They must then be arranged thus:⁸



The structure is narrowed down to that shown in IV, and a choice must be made between the unsaturated γ -benzopyrone or flavone structure and the γ -benzodihydropyrone or flavanone constitution. The absence of basic character in lignin derivatives tends to eliminate the flavone from consideration, but the methylation and acetylation reactions can be regarded as decisively indicative of the flavanone structure. Neither in the methylation

⁸ This arrangement also rules out all five-atomic heterocyclic rings, since the added oxygen in that case would be on carbon atom (3).

by alkaline dimethyl sulfate of such flavones as myricetin (16), quercetin (18), or apigenin (1, 17) nor in the

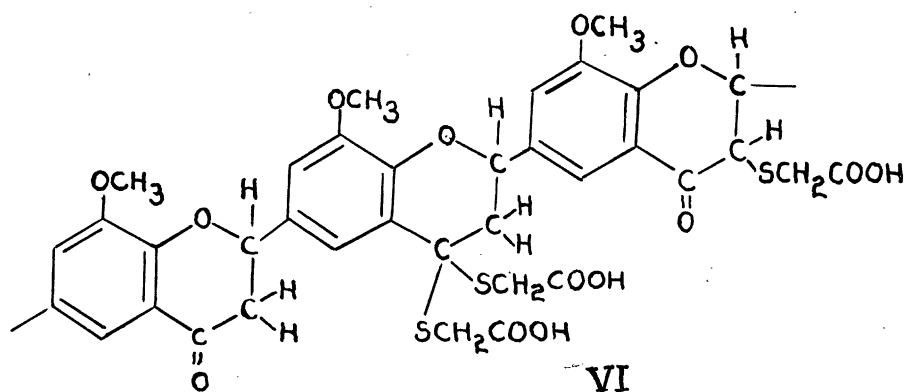


sodium acetate catalyzed acetylation of apigenin (1) is the ring opened. The products are the methoxy or acetoxy flavones.

genin (6). There appear to be in lignin sulfonic acid flavanone rings of two different degrees of lability: (a) those opened by very dilute alkali accompanied by diazomethane methylation and oxidized by periodic acid, and (b) those resistant to periodic acid and opened by alkaline dimethyl sulfate.

According to the evidence presented here, it appears that the constitution of lignin sulfonic acid is that of a polyflavanone as shown by V, and VI represents the analogous thioglycolic acid lignin. It is easily shown that the sulfonic acid group must be on carbon atom (3). If on (2), cleavage should give a ketone bisulfite, and it could be on (4) only as a ketone bisulfite. It has none of those properties.

One verification of the reliability of the polyflavanone structure proposed will be the degree to which the extensive literature on lignin derivatives will be found to



For ring-opening of flavones the more drastic conditions of elevated temperature and concentrated alkali are required. In contrast, ring-opening of the flavanones is easy; for many, only the presence of water or alcohol is required to establish ring-chain equilibrium. Reaction with dimethyl sulfate in cold alkali leads to ring-opening and methylation with such typical flavanones as narin-

conform to its requirements. The case must finally rest upon the results of quantitative degradation reactions carried to a point where, by means of tractable compounds, the criteria of identity can be met. It is work toward that objective which has been in progress in this laboratory for the past three years and which is proceeding to fruition.

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