α-D-Glucopyranose Pentaacetate and β-D-Glucopyranose Pentaacetate from Zone A.—A solution of 80 mg. of the material from zone A in 2 cc. of benzene was placed on a 170 × 35 mm. column[6] of 5/1 Magnesol/Celite and developed with 650 cc. of 250/1 benzene/ethanol. Two distinct zones were obtained at a distance of 116–130 mm. and 136–153 mm. from the top of the adsorbent column. The two zones were cut out and eluted with acetone; yields 27 mg. (upper zone) and 51 mg. (lower zone).

The above experiment was repeated several times in order to obtain further material. After several recrystallizations from ethanol (95%) the material from the lower zone was identified as β-D-glucopyranose pentaacetate; m. p. 132° (mixed m. p. unchanged), $[\alpha]^{24}\mathrm{D}$ +4° (c 3.9, chloroform); accepted values[11]: m. p. 132°, $[\alpha]^{20}\mathrm{D}$ +4°. The ethanol (95%) recrystallized material from the upper zone was identified as α-D-glucopyranose pentaacetate; m. p. 112–113° (mixed m. p. unchanged), $[\alpha]^{28}\mathrm{D}$ +101° (c 4.3, chloroform); accepted values[11]: m. p. 113°, $[\alpha]^{20}\mathrm{D}$ +101.6°.

Sucrose Octaacetate from Zone B.—The material (1.47 g.) from zone B was recrystallized from 10 cc. of ethanol (95%); yield 1.26 g., m. p. 85° unchanged on admixture with an authentic specimen of sucrose octaacetate of like m. p., $[\alpha]^{28}\mathrm{D}$ +60° (c 4.1, chloroform). These data identify the substance as the higher-melting dimorph of sucrose octaacetate for which the accepted constants are: m. p. 89°,[12] $[\alpha]^{20}\mathrm{D}$ +60° (chloroform).[13]

β-D-Fructopyranose Tetraacetate from Zone C.—The material (0.085 g.) from zone C was recrystallized from 3 cc. of ethanol (95%); yield 27 mg. of prismatic crystals, m. p. 130° (mixed m. p. unchanged), $[\alpha]^{25}\mathrm{D}$ −91.2° (c 3.9, chloroform). These data identify the substance as β-D-fructopyranose tetraacetate for which the accepted constants are: m. p. 131–132°, $[\alpha]^{20}\mathrm{D}$ −91.6° (chloroform).

Control Experiment with Pure Sucrose.—The acetylation and chromatographic procedure described above for the cane juice solids was applied to a sample of pure sucrose[14] (3 g.) There was obtained 4 mg. of crystalline material in zone C (β-D-fructopyranose tetraacetate zone) and 5 mg. in zone A (D-glucopyranose pentaacetate zone).

Acknowledgment.—Acknowledgment is made to the laboratory assistance of Mr. John M. Kolbas and Miss Eloise L. Carpenter. The cane juice was kindly supplied by Dr. A. G. Keller of Louisiana State University, Baton Rouge. We are indebted to the counsel of Dr. R. C. Hockett, Scientific Director of the Sugar Research Foundation.

Summary

D-Glucose (as α-D-glucopyranose pentaacetate and β-D-glucopyranose pentaacetate), D-fructose (as β-D-fructopyranose tetraacetate) and sucrose (as sucrose octaacetate) have been isolated from sugar cane juice by chromatographic methods.

COLUMBUS, OHIO RECEIVED MARCH 13, 1946

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[11] C. S. Hudson and J. K. Dale, THIS JOURNAL, **37**, 1264 (1915).

[12] R. P. Linstead, A. Rutenberg, W. G. Dauben and W. L. Evans, *ibid.*, **62**, 3260 (1940); *cf.* M. Frèrejacque, *Compt. rend.*, **203**, 731 (1936).

[13] C. S. Hudson and J. M. Johnson, THIS JOURNAL, **37**, 2748 (1915).

[14] The sucrose was obtained from the Coleman and Bell Co., Norwood, Ohio; its maximum invert sugar content was stated by the manufacturers to be 0.235%.

[CONTRIBUTION FROM THE INSTITUTE OF PAPER CHEMISTRY]

The Stability of the Methoxyl Groups in Methylated Hydrochloric Acid Spruce Lignin[1]

BY F. E. BRAUNS

In an earlier investigation on the decomposition of fully methylated spruce wood,[1a] it was found that lignin derivatives were obtained with a considerably lower methoxyl content than was expected. This might be caused by any one of the following possibilities: (1) the lignin was not completely methylated; (2) the lignin is chemically combined with another component of the wood through an oxygen linkage, thus covering the hydroxyl group and preventing it from being methylated; or (3) methoxyl groups are split off from the methylated lignin during its isolation. To study this question, the behavior of fully methylated hydrochloric acid lignin toward a number of reagents used for the isolation of lignin was investigated.

The methylated spruce lignin used was obtained by methylation of hydrochloric acid spruce lignin which was prepared according to Kalb and Lieser[2] and was kept in a moist condition. After five methylations, a methoxyl content of 32.26% was obtained, corresponding to 9.8 methoxyl groups per lignin building unit (Table I).

To obtain lignin derivatives soluble in organic solvents for further purification, the methylated hydrochloric acid lignin was treated with reagents under conditions previously used with unmethylated hydrochloric acid lignin and also in the isolation of lignin from wood.

Extracted spruce wood and hydrochloric acid spruce lignin, when treated with acetic acid in the presence of a small amount of magnesium chloride, give acetic acid spruce lignin.[3] This lignin still contains all of its original methoxyl groups, but two of its hydroxyl groups are acetylated. Because the acetic acid hydrochloric acid lignin is soluble in dilute sodium hydroxide, the phenolic hydroxyl group must still be free. Upon methylation with diazomethane, the acetic acid hydrochloric acid lignin becomes insoluble in dilute

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[1] Presented before the Division of Cellulose Chemistry at the 109th meeting of the American Chemical Society, Atlantic City, New Jersey, April 8 to 12, 1946.

[1a] F. E. Brauns and J. J. Yirak, in press.

[2] L. Kalb and T. Lieser, *Ber.*, **61**, 1007 (1928).

[3] F. E. Brauns and M. A. Buchanan, THIS JOURNAL, **67**, 645 (1945).

TABLE 1

No.	Lignin derivative	Newly added groups	CH_3O groups	Mol. wt., calcd.	Calculated, %		Found, %			
					CH_3O	S	C	H	CH_3O	S
1	Methylated hydrochloric acid lignin[a]	....	10	942	32.9	..	65.5	6.45	32.26	(Ash 0.8)
2	Acetic acid deriv. of 1[b]	1 CH_3CO	8	957	25.9	(CH_3CO 4.5)	66.25	6.07	25.3	(CH_3CO 4.75)
3	2 Methylated with diazomethane[b]	....	8	957	25.9	..	...	..	26.1	..
4	2 Toluenesulfonated[c]	2 Tosyl	8	1267	19.6	5.0	63.65	5.9	19.7	4.45
5	Phenol deriv. of 1[d]	3 C_6H_5OH	8	1160	21.43	..	69.3	6.2	21.6	..
6	5 Methylated with diazomethane[d]	2 CH_2	10	1188	26.16	..	70.9	6.9	26.05	..
7	5 Toluenesulfonated[c]	4 Tosyl	8	1780	13.96	7.2	..	..	13.5	7.17
8	Thiophenol hydrochloric acid lignin[f]	3 C_6H_5SH	4	1134	10.9	8.48	66.6	5.8	11.2	8.45
9	8 Methylated with diazomethane	2 CH_2	6	1162	16.0	8.27	66.8	5.8	16.7	8.2
10	8 Benzoylated	5 C_6H_5CO	4	1654	7.5	5.8	71.2	5.0	7.4	5.9
11	Thiophenol deriv. of 1[e]	3 C_6H_5SH	8	1226	20.2	7.8	67.15	5.85	21.0	7.6
12	11 Methylated with diazomethane	1 CH_2	9	1240	22.5	7.6	67.1	6.1	22.3	7.1
13	11 Nitrobenzoylated	1 $O_2NC_6H_4CO$	9	1376	18.04	(N 1.02)	...	..	18.2	(N 1.23)
14	Thioglycolic acid deriv. of 1[g]	2 H_2SCH_2CO	9	1112	25.15	5.7	60.6	6.1	25.2	5.2
15	13 Methylated with diazomethane	2 CH_2	11	1140	29.9	5.6	...	..	29.0	4.9

[a] Brauns and Hibbert, *Can. J. Research*, **13B**, 78 (1935). [b] Prepared in a way similar to acetic Willstätter spruce lignin; Brauns and Buchanan, THIS JOURNAL, **67**, 645 (1945). [c] Gray, King, Brauns and Hibbert, *Can. J. Research*, **13B**, 35 (1935). [d] Prepared according to a modified procedure of Buckland, Brauns and Hibbert, *ibid.*, 61 (1935). [e] Prepared in a similar way to 8. [f] Preparation given in experimental part. [g] Holmberg, *Ing. Vetenskaps Akad.*, *Handlinger*, No. 131, 85 pp. (1934).

caustic soda, and two new methoxyl groups are introduced, which indicates that two slightly acidic hydroxyl groups are methylated. When methylated hydrochloric acid lignin is refluxed with acetic acid and magnesium chloride, it readily dissolves and from the dark reddish-brown solution, a light orange acetic acid methylated hydrochloric acid lignin is isolated having a methoxyl content of 25.3% and an acetyl content of 4.8%. The relatively low methoxyl content is caused not only by the entrance of one acetyl group into the lignin building unit, but also by the loss of two methoxyl groups. A calculation of the molecular weight of the acetic acid methylated hydrochloric acid lignin on the basis of a molecular weight of 942 for the fully methylated lignin building unit[1a] gives a value of 957 for the lignin building unit of this acetic acid lignin derivative. On this basis and the presence of eight methoxyl groups, the calculated methoxyl and acetyl values are 25.9 and 4.5%, respectively (Table I). The difference of 0.6% in the methoxyl value may result from the fact that the methoxyl content of methylated hydrochloric acid lignin used was also 0.7% lower than the theoretical value. Upon methylation of the acetic acid methylated hydrochloric acid lignin with diazomethane, the methoxyl content increases to 26.0%, or almost to the theoretical value, indicating that no appreciable additional methylation takes place. The fact that acetic acid methylated hydrochloric acid lignin is insoluble in dilute sodium hydroxide proves that no acidic hydroxyl groups are present. This insolubility in caustic soda has caused difficulties in the saponification of the acetyl group. If acetic acid methylated hydrochloric acid lignin is treated with toluenesulfonyl chloride in pyridine, the solution, which at first is fairly light colored, soon becomes quite dark and a toluenesulfonyl derivative is obtained with a methoxyl content of 19.7%, and a sulfur content of 4.45%, indicating the entrance of two tosyl groups.

Phenol and a trace of hydrochloric acid dissolve lignin from spruce wood with the formation of a mixture of phenol lignins A and B, whereas hydrochloric acid spruce lignin is dissolved by these reagents with the formation of phenol lignin A only.[4] This reaction takes place under relatively mild conditions. It was hoped, therefore, that methylated hydrochloric acid lignin would dissolve readily in phenol without any deep-seated change. When methylated hydrochloric acid lignin is heated with phenol in the presence of a small amount of hydrochloric acid at 95°, it rapidly goes into solution. From this, a phenol methylated hydrochloric acid lignin is isolated in almost quantitative yield as a very light colored powder, having a methoxyl content of 21.6%. The decrease in methoxyl content from 32.26 to 21.6% is not caused merely by the entrance of phenol groups into the lignin molecule. If that were the case, between five and six phenol groups would have entered the lignin, whereas only four phenol groups react with the unmethylated hydrochloric acid lignin. It is more likely that, with the methylated hydrochloric acid lignin, fewer, rather than more, phenol groups react. When the theoretical methoxyl content of the phenol methylated hydrochloric acid lignin is calculated for the entrance of three phenol groups (one less than in the case of phenol lignin A from unmethylated hydrochloric acid lignin) and for the loss of two methoxyl groups, the methoxyl value is found to be 21.43% as compared with 21.6% actually found. Of these three phenol groups, one has reacted with the formation of a phenol ether, as is the case with phenol lignin A, and the other two have reacted in such a way that their two phenolic hydroxyl groups are still free. This is shown by methylation with diazomethane, whereupon two new methoxyl groups are formed. For such a compound, a theoretical methoxyl content of

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(4) I. K. Buckland, F. E. Brauns and H. Hibbert, *Can. J. Research*, **13B**, 61 (1935).

26.16% is calculated, which agrees well with that found (26.05%). The phenol methylated hydrochloric acid lignin has, in addition to the two hydroxyl groups newly introduced by the entrance of the two phenol groups, two free hydroxyl groups in the lignin molecule, as is shown by the entrance of four tosyl groups on toluenesulfonation. The calculated sulfur and methoxyl contents of 7.2 and 13.9% agree fairly well with those found.

It was found earlier[5] that thiophenol in the presence of a trace of hydrochloric acid readily reacts with lignin. With sprucewood, the lignin is dissolved with the formation of a number of thiophenol lignin condensation products of various compositions. The principal product, the dioxane–ether insoluble thiophenol lignin A, contains four thiophenol groups, the same number as there are phenol groups in phenol lignin A. One of these thiophenol groups, however, is quite loosely combined with the lignin molecule as it is split off even on methylation with diazomethane. Thiophenol lignin from isolated native spruce lignin contains only three thiophenol groups. Thiophenol lignin from hydrochloric acid spruce lignin, hitherto unknown, has been prepared by heating hydrochloric acid lignin with thiophenol and a small amount of hydrochloric acid as a slightly pink powder. It has a methoxyl content of 11.2% and a sulfur content of 8.45%. From these values, the entrance of three thiophenol groups is apparent, but the analytical data do not permit a definite decision as to whether one or two molecules of water are split off in the reaction. The calculated values on the basis of the entrance of three thiophenol groups are 10.9 and 8.5%, respectively. On methylation with diazomethane, two methoxyl groups are introduced, whereas on benzoylation, five benzoyl groups enter the lignin building unit.

When methylated hydrochloric acid lignin is heated with thiophenol in the presence of a very small amount of hydrochloric acid, the lignin begins to swell but does not seem to dissolve. After heating for two hours, only a trace has gone into solution, as is shown by the faint color of the solution. A sample which was isolated has a methoxyl content of 26.3%. For a thiophenol methylated hydrochloric acid lignin containing two thiophenol groups, a methoxyl content of 26.7% is calculated. Since unmethylated hydrochloric acid lignin dissolves in thiophenol, it was expected that methylated hydrochloric acid lignin would also give a soluble product. The undissolved thiophenol methylated hydrochloric acid lignin was again heated with thiophenol and a somewhat larger amount of hydrochloric acid, whereupon the lignin readily went into solution. The thiophenol methylated hydrochloric acid lignin thus formed is a very light cream powder and soluble in the common lignin solvents. From its methoxyl content of 21%, it is calculated that three thiophenol groups have entered the lignin building unit with simultaneous splitting off of two methoxyl groups. On this basis, a theoretical methoxyl content of 20.2% and a sulfur content of 7.8% are calculated. When the compound is methylated with diazomethane, the methoxyl content increases to 22.3%; for the entrance of one methoxyl group, a methoxyl content of 22.5% is calculated. The difference of 0.8% for the thiophenol methylated hydrochloric acid lignin is probably caused by the fact that the reaction is not quite complete. This is also indicated by the low sulfur content of the diazomethane-methylated product.

In spite of the presence of a hydroxyl group capable of being methylated with diazomethane, the original product is insoluble in dilute sodium hydroxide. This is another proof that thiophenol reacts with lignin through its mercapto group as has already been reported by Holmberg[6] for other thio compounds.

Finally, the reaction between methylated hydrochloric acid lignin and thioglycolic acid was studied. Thioglycolic acid reacts with lignin in wood with the formation of a tetrathioglycolic acid spruce lignin.[7,8] Unmethylated hydrochloric acid lignin reacts with thioglycolic acid under similar conditions with the formation of a trithioglycolic acid derivative, insoluble in dilute sodium hydroxide and organic solvents.[9] When methylated hydrochloric acid lignin is treated with thioglycolic acid in 2 *N* hydrochloric acid, a product is obtained which is also insoluble. From its methoxyl content of 25.2% and its sulfur content of 5.2%, a molecular weight of 1112 is calculated (by addition of two thioglycolic acid groups and splitting off of one methoxyl group) (Table I). On this basis, a theoretical methoxyl value of 25.15% and a sulfur content of 5.7% are calculated. Because the reaction takes place in a heterogeneous mixture, the reaction is probably not complete, as is indicated by the low sulfur content. This is more clearly indicated by the diazomethane-methylated product. On methylation with diazomethane, two new methoxyl groups are introduced by methylation of the free carboxyl groups but, because of the incomplete reaction, both the methoxyl and the sulfur are somewhat lower than the calculated values.

The analytical data (Table I) show that, in the reactions of methylated hydrochloric acid lignin with acetic acid, with phenol and with thiophenol in which soluble condensation products are formed, two methoxyl groups are split off. This fact makes their use unsuitable for the isolation of lignin from methylated wood, because the precise goal of experiments with methylated wood

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(5) F. E. Brauns and W. Lane, *Paper Trade J.*, **122**, No. 8, 38 (1946).

(6) B. Holmberg, *Ing. Vetenskaps Akad. Handl.*, No. 103 (1930).

(7) B. Holmberg, *ibid.*, No. 131 (1934).

(8) E. C. Ahlm and F. E. Brauns, THIS JOURNAL, **61**, 277 (1939).

(9) F. E. Brauns and M. A. Buchanan, *Paper Trade J.*, **122**, No. 12, 49 (1946).

was to isolate the lignin with all the methoxyl groups intact.

Since methylated spruce wood is not delignified by heating with 5% sodium hydroxide solution at 175–180°, it was of interest to study the behavior of methylated hydrochloric acid spruce lignin under these conditions. It was found that the unchanged lignin is recovered almost quantitatively.

In order to test the stability of some of the lignin derivatives toward 72% sulfuric acid, they were treated under conditions used in the lignin determination. The results are given in Table II. These lignin derivatives are completely insoluble in 72% sulfuric acid and do not even swell. For this reason, the conditions used may not be suitable for the completion of the reaction. Fully methylated hydrochloric acid spruce lignin loses about two of the methoxyl groups which are attached in an acetal-like linkage to the lignin. Acetic acid methylated hydrochloric acid lignin loses a part of its acetyl groups which causes an increase in the methoxyl content. Phenol and thiophenol methylated hydrochloric acid lignins are not changed in their methoxyl contents because they had already lost 2 methoxyl groups during their preparation. Thiophenol hydrochloric acid lignin is also unchanged. Thioglycolic acid methylated hydrochloric acid lignin loses one methoxyl group because, in its preparation, it had lost only one of the two acetal-like methoxyl groups and the second is split off on treatment with 72% sulfuric acid.

TABLE II

ACTION OF 72% SULFURIC ACID UPON VARIOUS LIGNIN PREPARATIONS

	Before treatment			After treatment	
Lignin	MeO, %	S, %	Yield, %	MeO, %	S, %
Fully methylated HCl	32.2	..	92.0	26.9	..
AcOH methylated HCl	25.3	..	91.5	26.2	..
Phenol methylated HCl	21.6	..	98.7	21.9	..
Thiophenol HCl	11.2	8.45	97.9	11.4	8.6
Thiophenol methylated HCl	21.0	7.58	97.5	20.8	7.34
Thioglycolic acid methylated HCl	25.2	5.0	93.4	23.98	4.84

Experimental[10]

Thiophenol Hydrochloric Acid Spruce Lignin.—Hydrochloric acid spruce lignin (CH_3O 15.5%), prepared according to Kalb and Lieser and kept in a moist state, was sucked as dry as possible and dehydrated by suspending the lignin (15 g., oven dry basis) in 150 cc. of thiophenol and distilling off the water with thiophenol as an azeotropic mixture under reduced pressure. The suspension was made up to 150 cc. by the addition of fresh thiophenol. A slow stream of dry hydrogen chloride was passed into the mixture through the capillary for three minutes, after which the mixture was heated on a boiling water-bath for one hour. The lignin swelled but did not seem to go into solution. Hydrochloric acid was therefore passed into the mixture for five minutes and heating was continued. The lignin then went rapidly into solution. After heating for another hour, all the lignin had dissolved, forming a reddish-brown solution. The excess of thiophenol was distilled off, leaving the lignin compound as a reddish-brown resin. This was dissolved in 120 cc. of dioxane, centrifuged, filtered, and the thiophenol hydrochloric acid lignin precipitated by dropping the solution into 2.5 liters of anhydrous ether. The product was washed twice with ether and with high- and low-boiling petroleum ether, and dried in a vacuum desiccator over sodium hydroxide and sulfuric acid. A second purification did not change the methoxyl content. The yield was 16.5 g. Thiophenol hydrochloric acid lignin is a tan-colored powder, soluble in dioxane, pyridine, acetone and dilute sodium hydroxide, and insoluble in ether and petroleum ether.

Acknowledgment.—The author is indebted to Miss Virginia West for carrying out the carbon and hydrogen determinations.

Summary

Methylated hydrochloric acid spruce lignin, when treated with acetic acid in the presence of magnesium chloride or with phenol or thiophenol in the presence of a small amount of hydrochloric acid, loses two methoxyl groups, with formation of the corresponding partially methylated lignin derivatives. With thioglycolic acid, only one methoxyl group is split off. When heated with 5% sodium hydroxide solution at 175–180°, the methylated hydrochloric acid lignin is recovered unchanged.

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[10] The experimental details as to the methods of preparation of the various lignin fractions are similar to those reported in the references given in Table I and are omitted at the request of the Editor.