acid added. The yellow crystals obtained had a m. p. of 194–195°; yield 50 mg.; mixed m. p. with diacetyl Rhein methyl ester prepared from authentic Rhein, 194–195°.

Acknowledgments.—Prof. Dr. R. Wasicky directed our attention to this problem. Prof. Dr. A. Stoll (Sandoz A. G., Basel, Switzerland) kindly supplied authentic Rhein. The Rockefeller Foundation supported this work by means of a grant. We wish to express our thanks to all.

Summary

Hydrolysis of extracts from *Cassia alata* leaves yields Rhein which occurs in the plant mainly in a reduced state as glycosides. Rhamnose and glucose were isolated as their phenylosazones.

SÃO PAULO, BRASIL RECEIVED AUGUST 4, 1949

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF MCGILL UNIVERSITY AND THE UNIVERSITY OF TORONTO]

The Extraction of Birch Lignins with Acetic Acid[1]

BY ALAN BELL AND GEORGE F WRIGHT

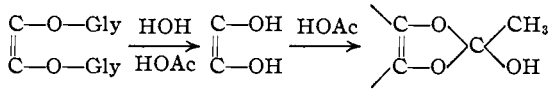
Comparison of the extracted lignins from various woods, and especially those from hard and soft woods, is of value in the elucidation of the structure of lignins. Since the extraction process always seems to alter the lignin from its original state in the wood, it is also useful to compare the lignins variously extracted from a certain wood. The extraction of birch wood with formic acid has already been reported.[2] Acetic acid has been used in the present investigation, and the extracted lignin separated into ether-soluble, benzene-soluble, methanol-soluble and chloroform-soluble fractions.

The expectation that lignins would be more easily extracted from a hard than from a soft wood, such as spruce, has been realized. The birch lignin was found to be extracted slowly in boiling glacial acetic acid over a four-day period. The products were notably more soluble in organic solvents than any heretofore obtained. This is owing, in part, to the presence of acetyl groups which apparently protect the lignins from undue decomposition during extraction. Moreover under completely anhydrous extraction conditions the least soluble part of the extract is found to contain 10–20% of carbohydrate chemically attached to the lignin. The isolation of this lignin-carbohydrate complex confirms previous conjectures concerning its existence.[3]

Most of the acetylation which occurs during extraction of birch lignin with acetic acid must take place on the attached carbohydrate. Such acetylation by boiling acetic acid (complete with monosaccharides and partial with polysaccharides) is not unknown.[4,5] The position of the acetyl groups is indicated by the elimination of about 7–10% of this group (compare Tables I and II) together with combined carbohydrate when the lignin fractions obtained by acetic acid extraction subsequently are digested with formic acid. This acid, stronger than acetic, would not be expected to cause de-acetylation (except perhaps by ester interchange) but it would tend toward glycoside hydrolysis. It would be especially effective in this action if the glycoside linkage were phenolic.

The acetic acid-extracted lignin fractions (hereafter called acetic lignins) have for convenience been designated as acetic-formic lignins after they have been digested with formic acid. The residual 7% of acetyl which remains after such digestion conforms with about one ester group per kilogram. This is approximately equivalent to the number of formyl groups which are found by analysis of birch lignins directly extracted by formic acid.[2]

These residual ester linkages seem unreactive toward Grignard reagent. Thus a lignin recovered from Grignard analysis of a sample containing 16% acetyl (1.2 RMgI per $COCH_3$ in dioxane) was found still to contain 9% of acetyl which would not react with the Grignard reagent. Since acetyl groups are not present in the original lignin as it exists in the wood, they evidently are introduced during the extraction process. Part of this acetylation must take place on the chemically bound carbohydrate, but the residual (and inert) acetyl seems to be present in the non-carbohydrate portion. A possible explanation would, in part, represent the lignin extraction process as



I II III

where Gly in I typifies any glycoside. The acetylation of an intermediate ene-diol, II, to form a 2-methyl-2-hydroxydioxole, III would explain the "hidden" hydroxyl groups which are suspected in fatty acid-extracted lignins.[2]

Not all of the glycosidically bound hydroxyl groups in lignin can, however, be explained in this way. Other acidic hydroxyl groups must be present. These have been demonstrated by "com-

(1) The authors are grateful for aid from the National Research Council of Canada and from the Canadian Pulp and Paper Association. They wish also to thank Dr. Harold Hibbert.

(2) M. Lieff, G. F. Wright and H. Hibbert, THIS JOURNAL, **61**, 1477 (1939).

(3) (a) E. E. Harris, E. C. Sherrard and R. L. Mitchell, *ibid.*, **56**, 889 (1934); (b) A. G. Norman and J. G. Shrikhande, *Biochem. J.*, **29**, 2259 (1935); (c) H. Hibbert and W. H. Steeves, THIS JOURNAL, **59**, 1768 (1937).

(4) C. J. Malm and H. T. Clarke, *ibid.*, **51**, 274 (1929).

(5) H. T. Clarke and H. B. Gillespie, *ibid.*, **54**, 2083 (1932).

pletely" acetylating the carbohydrate-rich methanol-soluble fraction of acetic birch lignin with pyridine and acetic anhydride. The acetylated product was alkali-insoluble. It was then boiled with formic acid, which might be expected to replace any ene-diolic diglycoside linkage I by the inert formyl linkage which has been typified as the hydroxydioxole group. Such a product should still be alkali-insoluble. On the other hand, if a monoglycoside linkage were present then hydrolysis by formic acid should yield a free acidic hydroxyl group. The lignin was readily alkali soluble after the formic acid treatment. When it then was treated with diazomethane, the methoxyl value was increased by 6–9%, in contrast to an increase of 4.5% before treatment with formic acid. It would thus appear that there were glycoside linkages present which cannot be classed as ene-diolic. They can be either monophenolic or carboxylic 1-glycosides. The latter is, of course, more probable since the monophenol might be expected to be blocked by formylation, but the carboxylic acid would not.

The nature of the carbohydrate which seems to be chemically bound to the acetic birch lignin has only partially been elucidated. The amount of carbon dioxide evolved during the treatment of acetic birch lignin with boiling formic acid is even greater than that evolved during the isolation of lignin from the wood with acetic acid. Furfural was produced, as might be expected if the carbon dioxide were formed by decomposition of uronic acids. However, if such acids are present, they are not completely decomposed either by the boiling acetic or formic acids, since some uronic acids are always found in the aqueous liquors, in which the lignins are precipitated together with a considerably larger amount of pentose (chiefly *d*-xylose).

When the carbohydrate was largely removed from acetic birch lignin by treatment with formic acid, the resulting sugar-free lignin was much less soluble than before. This is probably owing to removal of solubilizing acetyl groups together with the carbohydrate, since all acetic lignins, when hydrolyzed either with alkali or cold dilute mineral acid become correspondingly less soluble. The similarity in properties (methoxyl and Grignard machine analyses) between acetic and acetic-formic lignins indicates that no marked structural change has occurred during the formic acid treatment. However, the higher values for carbon found by elementary analysis do indicate that dehydration has taken place.

The removal of methoxyl-free carbohydrate from the methanol and chloroform-soluble acetic birch lignins ought to increase the methoxyl content. Comparison of Tables I and II shows that this expected increase is obtained. The first two fractions (ether and benzene soluble) each contains about 21% OCH_3 (23.4% on acetyl-free basis). Formic acid treatment ought not to affect these values appreciably since they are approximately the same when extraction of birch wood is carried out initially with formic acid.[1] The most insoluble (chloroform) acetic birch lignin, on the other hand, contains 16.0% methoxyl before treatment with formic acid and this is increased to 18.3% by such treatment (about 19% on acetyl and formyl-free basis).

The existence in birch lignin of two fractions with different methoxy contents designates the birch as different from the spruce, all lignin fractions from which contain approximately the same amount (20.6%) of OCH_3. A possible explanation may be found in the fact[6] that, whereas spruce ligninsulfonic acid yields only vanillin, yellow birch ligninsulfonic acid gives both vanillin and syringic aldehyde.

Except for this difference in methoxyl content, birch and spruce lignins are strikingly similar. Because of the high acetyl and carbohydrate content of isolated birch lignin the two are difficult of comparison, but both lignins, when hydrolyzed and methylated, will add (in dioxane) about 0.8 mole of methylmagnesium iodide per kg. This addition evidently involves a carbonyl group bound in lignin by a carbon–carbon rather than a carbon–oxygen linkage. This was shown by treatment of the benzene-soluble acetic birch lignin with phenylmagnesium bromide in ether–benzene. Subsequent oxidation of the "phenylated" lignin yielded 0.3 mole of benzoic acid per kg. Acetaldehyde was produced as well. The 0.3 mole of benzoic acid is less than would be expected if in the Grignard machine 0.8 mole of methylmagnesium iodide adds to the deacetylated lignin in dioxane. The choice of reaction medium is, however, critical; both active hydrogen and carbonyl addition values are much higher, for example, when pyridine rather than dioxane is used as the solvent in the Grignard analysis. Ether might be expected to limit the addition to less than that found in dioxane.

The authors wish to thank Mr. Cyril Marks for aid in the analytical work, and are grateful to Dr. Harold Hibbert, in whose laboratory major portion of the research was carried out.

Experimental[7]

Purification of the Woodmeal.—Yellow birch woodmeal (100–200 mesh), obtained from a tree about sixty years old and cut three feet above the ground, was continuously extracted with cold 1:1 benzene–ethanol mixture, followed by cold ethanol, and then by cold water, over a period of five days. The extracted woodmeal was air-dried and contained *ca.* 10% water.

Extraction of Yellow Birch Wood with Glacial Acetic Acid.—A suspension of 200 g. of the air-dried woodmeal in 1 liter of glacial acetic acid was stirred under reflux for one hundred hours. During this period 1.3 cc. (N.T.P.) of carbon dioxide was evolved per gram of woodmeal. After filtration by suction to remove 130 g. residual wood-

(6) (a) Bell, W. L. Hawkins, G. F. Wright and H. Hibbert, THIS JOURNAL, **59**, 598 (1937); (b) W. L. Hawkins, G. F. Wright and H. Hibbert, *ibid.*, **59**, 2447 (1937).

(7) All melting points have been corrected against known standards.

meal, the filtrate and acetic acid washing liquors were concentrated to a volume of about 100 cc. (15–20 mm.) and poured into 2 liters of distilled water. The precipitated lignin was filtered by suction, washed with water until free from soluble carbohydrate (Molisch's test), dried first by suction under a rubber dam[8] and then in the vacuum oven at 50–60°. The yield varied from 35–50 g. (25–29% of the weight of woodmeal on an oven dry basis), depending on the amount of combined carbohydrate. The higher yield (29%) was reflected in a lower methoxyl value (14.3%) and was obtained when glacial acetic acid and moisture-free woodmeal were used. The acetic acid lignin was soluble in acetic and formic acids, ethyl acetate, acetone, dioxane, chloroform, pyridine, dilute sodium hydroxide and partly soluble in ether, benzene, ethanol and methanol. In a typical run, the methoxyl value was 17.4%. Three additional one hundred-hour extractions of the residual woodmeal yielded 6, 2.5, and 1 g. of lignin containing 16.3, 16.5, and 9.8% OCH_3, respectively. When the extraction outlined above was carried out at 60° instead of 118° over a two-week period, only 2 g. of lignin was extracted.

Fractionation of the Acetic Acid Birch Lignin.—Fractionation was effected in a Soxhlet extractor using the following solvents successively: (1) ether at 760 mm.; (2) benzene at 200 mm.; (3) methanol at 200 mm. and finally (4) cold chloroform in which the residue was almost entirely soluble. This procedure was applied to those preparations in which the yield indicated that only small amounts of carbohydrate were present. Alternatively, when a large yield of lignin showed considerable combined carbohydrate only the ether and benzene extractions were carried out, the residual lignin being used as an *ether and benzene-extracted-acetic birch lignin*. In each case the extraction was continued until the extract was colorless, each solvent being completely removed prior to further treatment. With each solvent the extract was allowed to cool to room temperature and decanted from the material, insoluble in a specific volume of solvent, thus insuring a fraction whose solubility was definitely known, the insoluble residue being transferred to the next extract.

Precipitation, following fractionation, of 34.5 g. of acetic acid lignin was carried out as follows: the second, or benzene fraction was evaporated (20 mm.) to remove the solvent; the residue then dissolved in 80 cc. of chloroform and precipitated into 800 cc. of light petroleum, b. p. 30–50°[9] (Skellysolve F) to yield 4.0 g. of *benzene soluble lignin*. The centrifuged liquors from that precipitation were evaporated to dryness and the residue, entirely ether-soluble, was added to the first or ether fraction. The 500 cc. of ether was then evaporated leaving a residue which was dissolved in 70 cc. of chloroform and when precipitated into 700 cc. of light petroleum gave 3.2 g. of *ether soluble lignin*. The centrifuged liquors from this precipitation upon evaporation yielded 6.8 g. of an oily residue from which 50 mg. of sulfur was removed by steam distillation. The non-volatile residue, upon oxidation with boiling 10% nitric acid, yielded 20 mg. of a substance m. p. 218°, not yet identified.

In like manner the filtered methanol and the chloroform extracts (500 cc.) were freed from solvent and the residual lignin after solution in chloroform, precipitated into a ten-fold volume of light petroleum. These precipitations were complete, no material remaining in the centrifuged liquors; yield, *methanol soluble* 15.5 g., *chloroform soluble* 9.6 g. For analyses, see Table I.

Removal of Carbohydrate with Formic Acid.—As has already been indicated a lignin-carbohydrate complex is isolated by extracting moisture-free woodmeal with glacial acetic acid. When this is fractionated under the conditions outlined above, the ether and benzene fractions have properties similar to those of the corresponding fractions obtained with glacial acetic acid and air-dried woodmeal containing 10–20% moisture. The methanol and chloroform fractions, on the other hand, contain only 15–16% methoxyl, and probably about 25–40% carbohydrate material. In order to remove the latter, 200 g. of ether and benzene extracted acetic acid lignin (12.8% OCH_3) was refluxed for eighteen hours with 1 liter of formic acid (95%). No methyl formate was evolved, but 4.2 cc. (N. T. P.) of carbon dioxide per gram of lignin was found in the effluent gases. The formic acid solution was evaporated to a volume of 250 cc. (20 mm.) and poured into 2 liters of water. After thorough maceration the lignin was filtered off, washed until free from soluble carbohydrates and furfural, and dried at 50–60° (15 mm.), wt. 135 g. (67%) OCH_3, 19.1%. The volatile acid obtained upon boiling this lignin with aqueous *p*-toluenesulfonic acid gave a good yield of *p*-nitrobenzyl acetate from *p*-nitrobenzyl bromide but no formic ester or acid could be detected.[10] The mother liquor was evaporated at 20° (15 mm.) to a thick sirup (wt. 98 g., trace of methoxyl only). The distillate from the evaporation contained 1 g. of furfural, weighed as the 2,4-dinitrophenylhydrazone. This proportion of 0.01 mole of furfural per 0.03 mole of carbon dioxide was obtained repeatedly.

TABLE I

FRACTIONATION OF ACETIC BIRCH LIGNIN

Fraction	Ether	Benzene	Methanol	Chloroform
Yield, %	10.6	12.6	45.0	27.4
Methoxyl, %	21.0	20.7	18.9	16.4
Acetyl, %	14.5	17.8	15.3	16.0
Acetyl groups/kg.	2.5	3.0	2.6	2.7
Act. H/kg. (dioxane)	4.1	4.2	3.7	3.0
RMgX added/kg. (dioxane)	1.1	2.2	1.8	1.9
Act. H/kg. (pyridine)	6.1	5.3		
RMgX added/kg. (pyridine)	5.2	6.1		
Carbon, %	62.4	61.9		
Hydrogen, %	5.95	5.80		

In a second preparation, 50 g. of *ether and benzene extracted* acetic birch lignin (OCH_3, 13.9%) was first acetylated with 500 cc. of acetic anhydride and 250 cc. of pyridine; yield 52 g. The methoxyl content (12.2%) indicated that about 2.5 acetyl groups have been introduced per kilogram. The product was insoluble in dilute sodium hydroxide.

Fifty grams of this product, dissolved in 600 cc. of formic acid (95%), was refluxed for twenty-four hours, yield of furfural 0.0035 mole; carbon dioxide 0.01; sirupy carbohydrate not weighed; acetic–formic lignin 25 g. (OCH_3 20.9%). The latter which was now readily soluble in dilute alkali was fractionated by the method outlined for spruce formic lignin,[11] except that the chloroform-soluble fraction was precipitated into petroleum ether (Table II)

TABLE II

Fraction	Chloroform	Acetone	Water-acetone 3:17
Yield, %	23.8	27.1	49.1
Methoxyl, %	22.1	19.0	17.8
Acetyl, %	7.1	7.0	6.7
Act. H/kg. (dioxane)	3.8	3.3	2.9
RMgX added/kg. (dioxane)	0.9	0.7	1.0
Act. H/kg. (pyridine)	6.1	7.0	7.7
RMgX added/kg. (pyridine)	3.2	3.1	2.6
Carbon, %	63.5	63.5	63.2
Hydrogen, %	5.5	5.8	5.6

Isolation and Identification of the Carbohydrate.—The above sirupy carbohydrate (freed from furfural by con-

(8) R. A. Gortner, THIS JOURNAL, **36**, 1967 (1914).

(9) This petroleum precipitating solvent was used throughout the investigation.

(10) Emich–Schneider, "Microchemical Laboratory Manual," John Wiley and Sons, New York, N. Y., 1932, p. 121.

(11) G. F. Wright and H. Hibbert, THIS JOURNAL, **59**, 125 (1937).

tinuous ether extraction), gave 13 cc. (N. T. P.) of carbon dioxide (0.0006 mole) and 1.16 g. of furfural 2,4-dinitrophenylhydrazone (0.004 mole) per g. of sirup, when boiled with 12% hydrochloric acid (Tollens method).

The carbohydrate (*ca.* 6 g.) obtained above from 50 g. of acetic lignin was dissolved in hot methanol (hot acetone in which it was less soluble is equally satisfactory). Upon cooling slowly, 0.6 g. of crystalline *d*-xylose, m. p. 147° (softening at 142°) separated $[\alpha]^{28}D$ +29.6°, at equilibrium in water. Evaporation of the filtrate yielded an additional, less pure product (0.38 g.) which was heated with an aqueous solution of 0.6 g. of phenylhydrazine hydrochloride and 0.5 g. of sodium acetate. After forty-five minutes *d*-xylosazone separated as yellow felted crystals, wt. 0.20 g.; m. p. 152–153°; re-crystallized from water ethanol, m. p. 159°, $[\alpha]^{28}_{5563}$ −25° in ethanol. From 2.62 g. of the original sirupy concentrate there was obtained, by this process, 1.51 g. of the osazone, m. p. 152°. Since a yield of 0.0030 g. of *d*-xylosazone (m. p. 153°) was obtained under similar conditions from 0.0050 g. of *d*-xylose the sirupy carbohydrate from 50 g. of lignin must contain about 3.5 g. of this pentose. The latter was also identified by mixed melting point and preparation of the tetraacetate, m. p. 126° (crystallized from ether).

An aqueous ethanol (3:7) solution of the sirupy carbohydrate mixture gave no precipitate with benzylphenylhydrazine. With *p*-bromophenylhydrazine, on the other hand, a compound m. p. 165° was obtained.

Saponification of Acetic Birch Lignin.—A solution of 120 g. of unfractionated acetic birch lignin (OCH_3, 16.9%) in 1 liter of aqueous sodium hydroxide (5%) was kept for thirty-six hours at 25°, then acidified with dilute hydrochloric acid and the precipitated lignin filtered, washed and dried: wt. 68 g.; OCH_3, 19.6%; $COCH_3$, 2.0%. After saponification, the lignin, formerly chloroform soluble, was now soluble only in water–dioxane. The final unfractionated product had an acetyl value of 2.0% showing that saponification was incomplete. Using aqueous alkali (0.5%) an acetyl-free product was obtained only with the ether soluble lignin (CH_3O, 18.8%, $COCH_3$, 10.6%). The product (OCH_3, 23.3%) was soluble in acetone, dioxane, and partially so in chloroform. A benzene soluble fraction, after saponification with 2.5% sodium hydroxide for forty-eight hours, gave an identical product; the methoxyl value upon treatment with diazomethane was increased to 33.4%.

Methylation.—The method was identical with that previously described.[9] The saponified, unfractionated lignin (13 g.; OCH_3, 19.6; act. H 7.9/kg.; RMgX added, 2.2 per kg.) after treatment with diazomethane in 4:1 dioxane-water solution, yielded a benzene-soluble fraction (wt. 3.8 g., OCH_3 35.0%; act. H, 4.8/kg.; RMgX added/kg. 1.8 in pyridine) and a dioxane soluble fraction (wt. 5.7 g., OCH_3, 23.1%; act. H, 7.3/kg.; RMgX added/kg., 1.3 in pyridine) each isolated by precipitation into petroleum ether. The latter fraction was found to contain carbohydrate. Both fractions were subsequently methylated with dimethyl sulfate and alkali (OCH_3 34.4, 36.0, respectively, with Grignard machine values identical; act. H, 3.5/kg.; RMgX added/kg., 3.3).

The benzene-soluble product of this dimethyl sulfate methylation (0.4 g.; OCH_3, 34.4%) was acetylated with 5 cc. of pyridine and 5 cc. of acetic anhydride, following the procedure outlined below. The product was reprecipitated from chloroform into petroleum ether; yield 0.3 g. (Fraction A). The mother liquor was evaporated to give 0.06 g. (Fraction B).

Anal. Fraction A: OCH_3, 34.6%; $COCH_3$, 3.4%; Grignard analysis in dioxane per kg.; act. H, 1.5; RMgX added, 2.5. Fraction B: OCH_3, 33.4%; $COCH_3$, 3.4%.

To show that these glycosidically substituted hydroxyls are phenol-acidic in type, 0.2 g. of the isolated benzene-soluble lignin (OCH_3, 20.7, Table I) was methylated with diazomethane. This alkali-insoluble product (OCH_3, 21.95%) dissolved in dioxane was treated overnight (at 25°) with 5% aqueous alkali, then acidified, the excess solvent removed (20 mm.) and the residue precipitated from dioxane into petroleum ether. The product (0.17 g.) was completely alkali soluble; OCH_3, 22.8%. The acetone-soluble acetic–formic lignin treated similarly gave a product (OCH_3, 27.65) which on saponification was likewise alkali soluble and contained 26.1%, OCH_3.

Acid Hydrolysis of Acetic Lignin.—A solution of 1 g. of ether-soluble lignin ($COCH_3$, 11.3%; OCH_3, 18.7%) in 16 cc. of dioxane and 20 cc. of 4% hydrochloric acid was kept at 25° for forty-eight hours. After evaporation with chloroform at 20 mm. to remove water and acid, the residue was partially dissolved in chloroform and precipitated into 10 volumes of petroleum ether; yield, 0.85 g.; $COCH_3$, 1.24%; OCH_3, 24.1%. The material insoluble in chloroform dissolved readily in water and evidently was carbohydrate.

Acetylation of Acetic Birch Lignins.—A 1-g. sample of each of the lignin fractions was dissolved in a mixture of 10 cc. of pyridine and 10 cc. of acetic anhydride. After standing for one day at 20° the reaction mixture was poured into ice-water. The resulting precipitate was filtered, washed, dissolved in chloroform and re-precipitated as usual. Data are summarized in Table III.

TABLE III

ACETYLATION OF ACETIC BIRCH LIGNINS

Fraction	Yield, wt. %	Final OCH_3, %	Final $COCH_3$, %	Acetyl groups per kg.	Final Grignard analysis in dioxane per kg. Act. H	RMgX added
Ether	110	16.9	21.4	5.0		
Benzene[a]	102	17.5	20.2	4.7	2.3	6.8
Methanol	100	15.1	20.0	4.6	2.5	4.7
Chloroform	95	13.8	21.9	5.1	2.8	4.7

[a] A subsequent re-acetylation increased the acetyl value for the benzene-soluble fraction from 27.7–29.3% (5.0 acetyl groups per kg.).

Reaction with Phenylmagnesium Bromide.—A solution of 9.4 g. of benzene-soluble acetic birch lignin (OCH_3, 20.1%; $COCH_3$, 13.0%) in 400 cc. of dry benzene was treated with 200 cc. of 1 *N* phenylmagnesium bromide in ether. After reflux for four hours the reaction mixture was allowed to stand overnight. It still gave a positive Gilman test for RMgX. After precipitation and filtration, the product was washed with 200 cc. of benzene, then stirred with an acidified mixture of water and chloroform until solution was complete. The chloroform layer, washed with water and dried by evaporation at 15 mm., was precipitated into 1500 cc. of petroleum ether: wt. 7.6 g.; OCH_3, 21.0%; $COCH_3$, 4.8%; Grignard analysis in dioxane per kg.; active H, 2.0; RMgX added, 0.0.

A solution of 7 g. of this phenylated lignin in a liter of 2% sodium hydroxide was exhaustively extracted with ether, the latter removed and the alkaline solution oxidized at 20° over a three-day period with 65 g. of powdered potassium permanganate. The reaction mixture was boiled giving 300 cc. of distillate which yielded on treatment with 2,4-dinitrophenylhydrazine hydrochloride, a mixture of hydrazones (0.35 g.; m. p. 120–126°). Fractional crystallization separated this mixture into acetaldehyde 2,4-dinitrophenylhydrazone m. p. 163–165° (mixed melting point not lowered) and an unidentified fraction m. p. 105–107°.

The manganese dioxide in the reaction mixture was dissolved by addition of sulfur dioxide and the solution acidified and extracted four times with chloroform. Extraction of the evaporated chloroform solution (0.47 g.) with petroleum ether (60–70°) gave 0.39 g. benzoic acid, m. p. 122°, equivalent to 0.33 mole of benzoic acid per kg. of lignin.

Analytical Methods.—The method of Friedrich and Rappaport[12] was used for determination of acetyl or formyl content. The methoxyl content was determined as a

(12) A. Friedrich and S. Rappaport, *Biochem. Z.*, **251**, 432 (1932).

modified method of Viebock and Brecher.[13] The Grignard analyses were carried out in a modified Kohler machine.[14]

Summary

1. Glacial acetic acid has been shown to be a good extraction medium for removal of lignin from yellow birch.

2. A lignin-carbohydrate compound has been isolated from yellow birch. Most processes of lignin extraction involved a hydrolysis of this complex into isolated lignin and carbohydrate.

3. The carbohydrate material is shown to be partially *d*-xylose.

(13) M. Lieff, C. Marks and G. F. Wright, *Can. J. Res.*, **15B**, 529 (1937).

(14) M. Lieff, G. F. Wright and H. Hibbert, THIS JOURNAL, **61**, 865 (1939).

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[CONTRIBUTION FROM THE SOUTHERN REGIONAL RESEARCH LABORATORY[1]]

The Shape of Pyranoside Rings

BY RICHARD E. REEVES

In addition to isomerizations between α- and β-pyranose, aldehydo-, and α- and β-furanose forms, many sugars containing pyranose rings are capable of another type of isomerization which is displayed not only by reducing sugars, but by their glycosides and substituted derivatives as well. Instead of ring shift or change of configuration, this other type of isomerization involves changes in ring shape (conformation) which vastly alter the relative position of various groups within the same molecule. This type of isomerization is of very great importance in determining the properties and reactions of sugars. Since the actual shapes of sugar molecules and the rules governing those shapes have been only poorly understood, it is the purpose of this manuscript to review existing information regarding pyranose ring conformations and to present speculations which may facilitate future work in this field.

In a series of communications dealing with cuprammonium–glycoside complexes[2] the writer has regarded the pyranose ring as a regular skew hexagon theoretically capable of being oriented in any one of the eight Sachse strainless ring conformations. It is apparent that small deviations from this regular structure could exist without obscuring the recognizable ring comformations. The most probable such deviations are the possibilities of the ring C–O bonds being slightly shorter than the C–C bonds and of the oxygen valence angle being slightly less than the tetrahedral angle. Either or both of these deviations would produce minor but important changes in the relative position of neighboring ring substituents. In a skew hexagon of this slight distortion, adjacent *cis* hydroxyl groups would always be a bit closer together than the closest approach for adjacent *trans* groups. This difference is in agreement with a large amount of information dealing with the chemical reactions of sugars. Acetonation, oxidative glycol cleavage, and complexing with cuprammonium require a close approach of two hydroxyls, and these reactions proceed more readily with groups that are *cis* in the Fischer projection formula. With this concept of the slightly distorted skew hexagon still capable of forming the recognizable strainless ring conformations (Fig. 1) the rules governing pyranose ring shapes will be considered.

The Case against Boat-Form Rings

It has been suggested by Scattergood and Pacsu,[3] Gorin, Kauzmann and Walter,[4] and recently by Hassel and Ottar[5] that boat-form pyranose rings are unlikely, or energetically unstable. Scattergood and Pacsu reject the unsymmetrical boat forms B1, 1B, B2 and 2B on the grounds that there would always be interference between adjacent groups. Hassel and Ottar state that "all experimental evidence indicates that the 6-membered pyranose ring found in many sugars will generally have the staggered form [the chair form]." Gorin, Kauzmann and Walter state that the "boat forms would seem to be unstable due to large repulsions both in the ring and among the subsidiary groups."

Yet there can be no doubt that boat conformations are structurally possible for the pyranose ring. The substance methyl 2,6-anhydro-α-D-altropyranoside[6] strikingly illustrates this fact. Molecular models show this substance to be capable of existence only in the boat form B2, and this shape has been confirmed by the chemical reactions of the substance.[2a] Furthermore, whenever an ethylene oxide type of anhydride occurs on a pyranose ring, and many such substances are known, a boat form must be ascribed to the ring. This assignment follows because it is necessary to regard the two C–O valence bonds

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) Reeves, (a) THIS JOURNAL, **71**, 212 (1949); (b) **71**, 215 (1949); (c) **71**, 1737 (1949); (d) **71**, 2116 (1949).

(3) Scattergood and Pacsu, *ibid.*, **62**, 903 (1940).

(4) Gorin, Kauzmann and Walter, *J. Chem. Phys.*, **7**, 327 (1939).

(5) Hassel and Ottar, *Acta Chem. Scand.*, **1**, 929 (1947).

(6) Rosenfeld, Richtmyer and Hudson, THIS JOURNAL, **70**, 2201 (1948).