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Summary

A procedure for isolating holocellulose from spruce and maple wood is given. The chemical characteristics of spruce holocellulose are compared with those of other wood fractions and with extractive-free spruce wood. Holocellulose, after hydrolysis with 1.0% sulfuric acid, leaves a carbohydrate residue comparable to Cross and Bevan cellulose.

By hydrolyzing the holocellulose with dilute acid there has been removed an easily hydrolyzable hemicellulosic fraction similar to a fraction that under the older methods of analysis has always been mixed with other wood constituents. It is composed of constituents that contain methoxyl, carboxyl, acetyl and formyl groups and that may be hydrolyzed to mannose, glucose, galactose, arabinose and xylose.

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Action of Aspergillus Niger on Normal 1,2-Diols

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The study of the action of *Aspergillus Niger* on sugars and other substrates has received a great impetus in recent years. This work is conveniently summarized by Bernhauer.[1] Since this fungus thrives particularly well on sugar solutions it was thought desirable to investigate its action on a series of homologous substances which resemble a hexose molecule in part of their molecules. We have in the molecule of glucose the grouping $CH_2OHCHOH$— in the open form as well as in the γ-form, at carbon atoms five and six. The simplest substances containing this grouping are the normal glycols of the type $CH_2OHCHOH$—R, *i. e.*, propane-, butane-, pentane- and hexanediol-1,2.

These glycols were prepared by hydrolyzing the corresponding 1,2-oxides. The oxides were obtained from the respective chlorohydrins,[2] which in turn were prepared by allowing chloroacetaldehyde to react with alkylmagnesium bromides. While butene-1,2-oxide and to some extent pentene-1,2-oxide was readily obtained from 1-chlorobutanol-2 and 1-chloropentanol-2, respectively, with the aid of hot aqueous alkali, such was not the case with the higher homolog hexene-1,2-oxide. To avoid this difficulty the chlorohexanol was refluxed in anhydrous ether solution with powdered potassium or sodium hydroxide with mechanical stirring. Under these conditions hexene oxide was obtained in a good yield. The racemic hexene oxide was transformed either into hexanediol-1,2 or into bromohexanol. Part of the pentene oxide was added to aqueous dimethylamine whereby 1-dimethylaminopentanol-2 was readily formed. With methyl iodide the latter compound yielded β-propylcholine iodide. This iodide was compared with a sample made in this Laboratory from 1-chloropentanol-2 directly.[3] Their melting points and mixed melting points agreed perfectly.

Propane-, butane-, pentane- and hexanediol-1,2 were subjected to the action of a known strain of *Aspergillus Niger*. The technique employed was the one recommended by several authors and consisted of subjecting the sterile substrates to well-developed mycelia of the fungus. With the first three glycols mentioned above in each case a substance was formed which reduced Fehling's solution very readily. These reducing substances were found to be the ketones formed by the oxidation of the secondary hydroxyl group. From propanediol-1,2, hydroxyacetone (acetol) was shown to be formed, from butanediol-1,2, 1-hydroxybutanone-2, and from pentanediol-1,2, 1-hydroxypentanone-2. These hydroxy ketones could be separated readily from the respective

(1) K. Bernhauer, "Die oxydativen Gärungen," Verlag Julius Springer, Berlin, 1932.

(2) (a) 1-Chlorobutanol-2, Helferich and Speidel, *Ber.*, **54**, 2634 (1921); (b) 1-chloropentanol-2, Levene and Haller, *J. Biol. Chem.*, **77**, 555 (1928); (c) 1-chlorohexanol-2, Levene and Haller, *ibid.*, **79**, 475 (1928); also Koelsch and McElvain, THIS JOURNAL, **52**, 1164 (1930).

(3) To be published shortly.

unchanged glycols by distillation at reduced pressure, the aqueous part as well as the hydroxy ketone distilling over, while the unattacked glycol remained in the distilling flask. In the case of propylene glycol the aqueous distillate was treated with semicarbazide hydrochloride and the resultant semicarbazone compared with the semicarbazone obtained from synthetic acetol. The two substances agreed in their melting points and the melting point of a mixture of the two acetol semicarbazones showed no depression.

Another sample of the aqueous distillate was subjected to phytochemical reduction with yeast. As expected, *l*-propanediol with a marked optical rotation was formed. In the case of the butane- and pentanediol-1,2 the aqueous distillate of each was treated with a solution of 2,4-dinitrophenylhydrazine. A crystalline precipitate was obtained readily. The analysis of the orange colored substances after two recrystallizations agreed well with that of a dinitrophenylhydrazone of the respective hydroxy ketone. The melting point of the dinitrophenylhydrazone of 1-hydroxybutanone-2 was 150°, that of 1-hydroxypentanone-2 was 165°.

The conduct of hexanediol-1,2 was surprising inasmuch as this higher homolog no longer was attacked by the mold under the conditions employed in the case of the lower members. It is possible that the higher glycol exerts a poisonous action on the mold.

Since it was reasonable to expect that the fungus would oxidize one form of the two enantiomorphous synthetic 1,2-glycols more rapidly than the other, the remaining glycols were recovered and examined for their optical activity. It was actually found that in all the solutions in which the hydroxy ketone was identified an optically active glycol could also be recovered. In the case of propylene glycol a larger absolute rotation was observed than in the case of the next two higher homologs. This is explained by the larger inherent rotation of propylene glycol compared with those of the butane- or pentanediol-1,2. On resubjecting a recovered propylene glycol of α_D^{27} $-1.2°$ to further fungus action a glycol was obtained of α_D^{27} $-4.6°$. Undoubtedly the procedure could be extended further and a more active glycol could be obtained in this manner. In the case of butane- and pentanediol-1,2 of which the rotations were small, the optical activity was determined also in the form of their crystalline diphenylurethan derivatives.

It is of particular interest to note that the active glycols thus obtained rotate in the same direction as the glycols obtained by the yeast reduction of the corresponding α-hydroxy ketones (or α-hydroxyaldehydes).[4] Levene and Haller[2c,4] have shown that the latter glycols are configurationally related. The active glycols produced by *Aspergillus Niger* are also of the same configuration.

In a case where a weak mycelium was allowed to become infected and non-sterile solutions of racemic butane- and pentanediol-1,2 were added it was observed that the mycelia decayed and the solutions became turbid. The same hydroxy ketones were formed as in the case of pure fungus cultures but the recovered glycols rotated in the direction opposite to that of those obtained with the pure *Aspergillus Niger* strain.

Experimental

Preparation of Normal 1,2-Oxides. Method 1.—This method consisted of adding powdered sodium or potassium hydroxide in small amounts to an anhydrous ether solution of the chlorohydrin in a three-necked flask while the mixture was mechanically stirred. The ether was allowed to reflux gently. Before each addition the flask was cooled. A total of about three moles of powdered hydroxide was introduced per mole of chlorohydrin. After the addition of the hydroxide the mixture was stirred for approximately another hour. The cooled mixture was filtered through a Buchner funnel, the solid being well washed with anhydrous ether. The solution was dried with anhydrous potassium carbonate or sodium sulfate. The ether then was distilled off with the aid of a Vigreux or preferably a Widmer column.

Method 2.—This procedure consisted of introducing the chlorohydrin under the surface of 1.1 moles of a concentrated solution of sodium hydroxide which was mechanically stirred and kept at such a temperature that the oxide vapors distilled rapidly into a well-cooled spiral condenser. The top layer in the receiving flask was separated from the aqueous bottom part and was dried over anhydrous sodium sulfate. Occasionally the top layer was hydrolyzed to the glycol without purification. This method for preparation of the homologous ethylene oxides performed well in the case of the lower oxides such as propene and butene oxide and to a lesser degree in the case of pentene oxide, but no hexene oxide was obtained in this manner from chlorohexanol.

Pentene oxide-1,2 had a boiling point of 91–92°, analysis C, 69.7; H, 12.0%; calculated for $C_5H_{11}O$: C, 69.7; H, 11.7%. Hexene oxide-1,2 had a boiling point of 118–120°; analysis: C, 72.5; H, 11.9%; calculated for $C_6H_{12}O$: C, 72.0; H, 12.0%.

Preparation of Glycols.—The preparation of the glycols from the oxides was carried out by heating a mixture of the oxide and distilled water in a sealed tube. One part of

(4) Levene and Walti, *J. Biol. Chem.*, **94**, 361 (1931).

pentene oxide-1,2 was heated with one part of water at 90° for thirty-six hours, and one part of the hexene oxide-1,2 was heated with four parts of water at 180° for three hours. After hydrolysis the solutions were concentrated under reduced pressure. Pentanediol-1,2 distilled at 96–99° (11 mm.) and hexanediol-1,2 at 100° (5 mm.). At 760 mm. the pentanediol-1,2 boiled at 209–210° and the hexanediol at 223–224°.

After the distillation of the pentanediol at reduced pressure there remained a small amount of residue which distilled mostly at 130 to 132° (7 mm.). The composition of the substance agreed with that of a dihydroxypentyl ether.

Anal. Calcd. for $C_{10}H_{22}O_3$: C, 63.1; H, 11.7. Found: C, 63.5; H, 11.4.

The above glycols were completely miscible with water. Butane-, pentane- and hexanediol-1,2 were also miscible with ether.

Derivatives of Pentene and Hexene Oxide-1,2.—Besides the conversion of these oxides to their glycols another derivative of each oxide was prepared. Pentene oxide was transformed into 1-dimethylaminopentanol-2, hexene oxide to 1-bromohexanol-2.

1-Dimethylaminopentanol-2.—Four grams of the above pentene oxide was added in small amounts to 25 cc. of a somewhat cooled 30% aqueous solution of dimethylamine. After each addition the turbid mixture became limpid on shaking. The solution was put into a refrigerator for four hours and then was allowed to stand at room temperature overnight. Ether and anhydrous potassium carbonate then were added to the cooled solution. The ethereal part was decanted several times while more and more potassium carbonate was added until all of the water had been absorbed. After drying the united ether fractions over potassium carbonate the solvent was distilled off with the aid of a Widmer column. The remaining part distilled at 69° (24 mm.). It weighed 5 g. At atmospheric pressure the substance distilled at 165–166° (762 mm.). The refraction index of both distillates was n_{25} 1.4252.

Anal. Calcd. for: $C_7H_{17}ON$: N, 10.68. Found: N, 10.96.

β-Propylcholine Iodide.—A solution of a small amount of methyl iodide in ether was added to a solution of a few drops of the above dimethylaminopentanol. The precipitate formed was sucked off and dried. It melted at 198°. The melting point of a mixture of this substance with β-propylcholine iodide prepared previously[5] in this Laboratory from 1-chloropentanol-2 also was at 198°.

Anal. Calcd. for $C_8H_{20}ONI$: I, 46.47. Found: I, 46.76.

1-Bromohexanol-2 from Hexene Oxide-1,2.—3.5 grams of the hexene oxide described above was cooled in an ice-salt mixture; 10 g. of cooled 48% hydrobromic acid was added in small portions and stirred in the cooling mixture. The material was allowed to stand at room temperature for thirty minutes and the bottom layer was then separated and dissolved in ether. This solution was washed and dried over sodium sulfate. After removal of the ether 4.5 g. of substance distilled at 89–90° at 13 mm. This corresponded with the boiling point of *d*-1-bromohexanol-2.[6]

Action of Aspergillus Niger on the Glycols.—In order to grow the mycelia of *Aspergillus Niger* required for the experiments, we had to raise enough spores of the fungus to seed the various 300-cc. Erlenmeyer flasks containing the sterile salt sugar mixture. The spores were grown on sterile bread in Kolle flasks by seeding it from an *Aspergillus Niger* strain 3528.7, obtained from Drs. Thom and Raper of Washington, D. C., and for which our thanks are expressed. Enough spores were introduced into the nutrient salt sugar solution to ensure the formation of a coherent mycelium. The nutrient solution was made up from Frey's salt mixture[7] and a 10% sucrose solution to which a small amount of a sterile yeast extract had been added. The mixture was kept at 35°. The mycelia were generally formed after twenty-four to thirty hours. The nutrient liquid then was removed by decanting carefully. Sterile distilled water was then introduced in such a manner that the lower side of the mycelia was washed. This was repeated several times until the wash water practically no longer reduced hot Fehling's solution. The various racemic glycols were introduced into several flasks thus prepared, in the form of 2% solutions. This solution had been sterilized before the careful addition to the fungus. All sterilizations indicated above were carried out at fifteen lb. pressure for twenty minutes. The flasks were kept at room temperature for several days. The fermentative change of the solutions was readily observed with the aid of Fehling's solution or 2,4-dinitrophenylhydrazine in the cold.

TABLE I

No.	Hydroxy ketone	Derivative	M. p., °C.
1	Hydroxyacetone from propanediol-1,2[a]	Semicarbazone[b]	199
2	1-Hydroxybutanone-2 from butanediol-1,2	2,4-Dinitrophenylhydrazone[c]	150
3	1-Hydroxypentanone-2 from pentanediol-1,2	2,4-Dinitrophenylhydrazone[c]	165

		Analyses, %					
		Calculated			Found		
No.	Formula	C	H	N	C	H	N
1	$C_4H_9O_2N_3$			32.05			31.58
2	$C_{10}H_{12}O_5N_4$	44.76	4.51	20.90	44.80	4.65	20.78
3	$C_{11}H_{14}O_5N_4$	46.79	5.00	19.86	47.05	4.91	19.60

[a] The aqueous distillate containing the acetol was distilled at ordinary pressure with the aid of a column containing glass beads in order to concentrate the hydroxy ketone in the distilling vessel. [b] This semicarbazone was compared with the semicarbazone of synthetic acetol (m. p. 198°).[8] A mixed melting point determination of the above semicarbazone with that of synthetic origin gave 198°. [c] A filtered solution of 2 normal hydrochloric acid containing 2% 2,4-dinitrophenylhydrazine was added to the aqueous distillate which was obtained from the fungus treated 1,2-diol. The hydrazones were recrystallized twice from dilute methyl alcohol.

(5) To be published shortly.

(6) Levene and Haller, *J. Biol. Chem.*, **79**, 484 (1928).

(7) Frey, *Arch. Microbiologie*, **2**, 272 (1931).

(8) Nef, *Ann.*, **335**, 213 (1904).

TABLE II

No.	Optically active diol-1,2	B. p., °C.	Mm.	Rotation homogeneous	Rotation/ $[\alpha]_D^{25}$	Derivative: diphenylurethane,/	M. p., °C.	Formula	N analyses, % Calcd.	Found
1	*l*-Propane-	81	10	$[\alpha]_D^{27}$ −1.20°						
				$[\alpha]_D^{27}$ −4.35°[a]						
2	*d*-Butane-[b]	91–92	11	$[\alpha]_D^{32}$ +0.50°	+2.6°	$[\alpha]_D^{30}$ +3.7°	117–118	$C_{18}H_{20}O_4N_2$	8.54	8.59
3	*d*-Pentane-[c]	98–100	10	$[\alpha]_D^{32}$ +0.05°	+0.5°	$[\alpha]_D^{26}$ +1.2°	100–101	$C_{19}H_{22}O_4N_2$	8.19	8.33
4	*l*-Butane-[d]	100	20		−1.01°	$[\alpha]_D^{30}$ −0.47°	118	$C_{18}H_{20}O_4N_2$	8.54	8.60
5	*l*-Pentane-[d]	112–113	20		−2.25°	$[\alpha]_D^{32}$ −2.3°	102	$C_{19}H_{22}O_4N_2$	8.19	8.40

[a] A glycol of this rotation was obtained when the above glycol of $[\alpha]_D^{27}$ −1.20° was resubjected to a new mycelia of the fungus. [b] Two-thirds of the glycol applied was recovered. [c] Three-fourths of the glycol applied was recovered. [d] Obtained from *infected* mycelia. [e] The diphenylurethans were prepared by treating one equivalent of the optically active glycol with two equivalents of phenyl isocyanate at 100°. The substances obtained were usually recrystallized twice from dilute alcohol. [f] Specific rotation in absolute alcohol.

Separation of Hydroxy Ketones.—The separation of the volatile hydroxy ketones from the unchanged glycols was effected by distillation at ordinary pressure or at reduced pressure (about 20 mm.) from a water-bath of about 50°. For this purpose the solutions were filtered. The water vapors carried along the respective hydroxy ketones during the distillation. The evaporated water in the distilling flask was renewed a few times.

Phytochemical Reduction of Acetol.—The aqueous distillate obtained from the fungus treated propanediol was subjected also to phytochemical reduction. To the distillate containing the highly reducing substance was added a concentrated sugar solution to bring the concentration of sugar to approximately 10% of the total solution. The same amount of bakers' yeast as there was sugar present was then added. The mixture was allowed to stand for three days after which time it was worked up in the usual manner.[9] The glycol formed distilled at 81° (9 mm.). The specific rotation of the substance in water was $[\alpha]_D^{29}$ −14.56°.

Diphenylurethan of *l*-Propylene Glycol.—This urethan was prepared from the above glycol as described in footnote *e* of Table II. It melted at 146°, $[\alpha]_D^{29}$ +9.3°.

Anal. Calcd. for $C_{17}H_{18}O_4N_2$: N, 8.91. Found: N, 8.89.

Isolation of Optically Active Glycols.—The residue obtained from the above distillation containing the glycol was taken up in absolute alcohol, filtered or centrifuged if necessary. After distilling off the solvent at reduced pressure the distillation residue again was taken up in absolute alcohol to which there was added approximately one-half of its volume of anhydrous ether. If a precipitate was formed it was filtered in a Buchner funnel or centrifuged off. The glycol then was freed from the solvents by distillation at reduced pressure. The glycol distilled at the temperature given in Table II, at reduced pressure.

(9) "Organic Syntheses," Vol. X, p. 84.

Appreciation is expressed to Dr. Randolph T. Major for suggesting the problem.

Summary

Normal racemic propane-, butane- and pentanediol-1,2 have been subjected to the action of a known strain of *Aspergillus Niger*. The fungus caused oxidation of the secondary hydroxyl group of the glycol, forming thus the corresponding α-hydroxy ketone. In the case of hydroxybutanone and the hydroxypentanone the 2,4-dinitrophenylhydrazone was prepared. The glycols which had been recovered were found to be optically active. They were configurationally related.

In a case where infected mycelia were allowed to act on butane- and pentanediol-1,2 solutions the same hydroxy ketones were formed but the recovered glycols rotated polarized light in a direction opposite to that found for the above described glycols.

Normal pentene oxide-1,2 and normal hexene oxide-1,2 have been prepared. Pentene oxide-1,2 was transformed into pentanediol-1,2 and into 1-dimethylaminopentanol-2. From the latter compound β-propylcholine iodide was prepared. Hexene oxide-1,2 was transformed into hexanediol-1,2 and into 1-bromohexanol-2.

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