

comparison with the theoretical. It can be concluded that D-lyxose and D-ribose will not introduce into total pentose determinations, much larger errors than those now encountered as a consequence of the difference between arabinose and xylose.

In 1932, Hughes and Acree showed that it is possible to obtain virtually quantitative yields of furfural from certain pentoses by a modified procedure. Salt was added to the reaction mixture to raise the temperature at which the reaction occurs, and the furfural was swept out with steam so that its time of exposure to the temperature of the reaction vessel was at a minimum. The furfural was determined by a volumetric method using standard "potassium bromide-potassium bromate solution."¹² In this way, it was possible to measure the rate of conversion of pentose to furfural as well as to obtain the aldehyde almost quantitatively.

Using essentially their procedure, we (A. G.), measured the rates of furfural formation from D-ribose and D-lyxose (Fig. 3). The curves are complex, but show a notable difference between these two pentoses.

(12) Hughes and Acree, *Ind. Eng. Chem., Anal. Ed.*, **9**, 318 (1937); *B. Standards J. Research*, **21**, 327 (1938); **23**, 293 (1939).

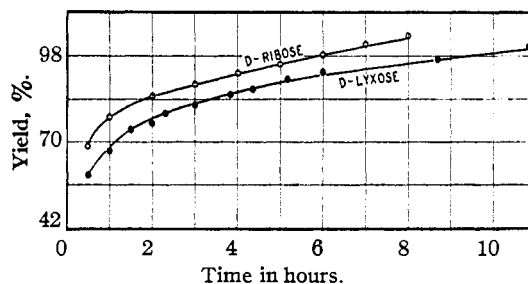


Fig. 3.—Yield of furfural vs. time of distillation.

We wish to express our indebtedness to Merck and Company of Rahway, New Jersey, for the gift of ribose sirups.

Summary

1. The standard procedure for determination of pentoses by conversion to furfural and weighing the latter as its phloroglucide has been applied to D-lyxose and D-ribose.
2. The ratio between weight of substance used and weight of phloroglucide obtained has been determined over a range from 0.0100 g. to 0.3000 g. of sugar.
3. The rates of furfural production from these sugars have been measured.

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[CONTRIBUTION FROM THE DIVISION OF CHEMISTRY, NATIONAL INSTITUTE OF HEALTH, U. S. PUBLIC HEALTH SERVICE]

The Alkaline Degradation of Phenylglycosides; a New Method for Determining the Configuration of Glycosides and Sugars¹

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The symbols α and β , originally assigned to anomeric pairs of sugars and their derivatives solely as distinguishing designations since the ring structures and the terminal configurations were not then known, are now generally in accord with the simple and logical system proposed in 1909 by one of us,² namely, that in the D-series of sugars the more dextrorotatory substance of an α,β -pair is designated the α -form, in the L-series the more levorotatory one is so named. This system is not wholly empirical as is sometimes stated, because it rests upon the idea that the additive rules of iso-

(1) A preliminary report on the phenylglycosides was presented in part at the meeting of the National Academy of Sciences, Washington, D. C., April 29, 1941; see *Science*, **93**, 438 (1941). The present work was presented at the Buffalo meeting of the American Chemical Society, Sept. 8, 1942.

(2) Hudson. *THIS JOURNAL*, **31**, 66 (1909); see also *ibid.*, **60**, 1537 (1938).

rotation hold with an approximation that is sufficiently close to correlate analogous configurations.

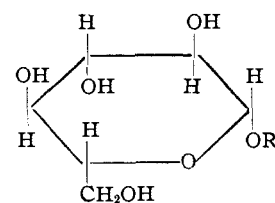
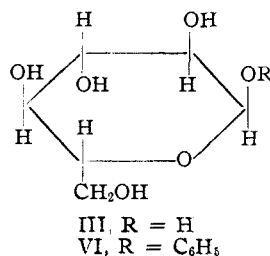
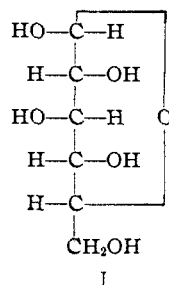
A number of methods have been proposed for determining experimentally the configuration of the glycosidic carbon atom in these α and β forms of the sugars and their glycosides.³ Most of these methods have been attempts to decide, either by physical measurements or by chemical reactions, whether the glycosidic group is on the same or on the opposite side of the pyranoid ring in respect to a hydroxyl or other group attached to one of the other carbon atoms in the ring. The configurations of these other carbon atoms were known previously from Emil Fischer's researches.

(3) See especially Sørensen and Trumpp, *Kgl. Norske Videnskab. Selskabs, Skrifter*, **1934**, No. 6; Riiber and Sørensen, *ibid.*, **1938**, No. 1; also Micheel, "Chemie der Zucker und Polysaccharide," *Akademische Verlagsgesellschaft m. b. H.*, Leipzig, 1939, pp. 219-226.

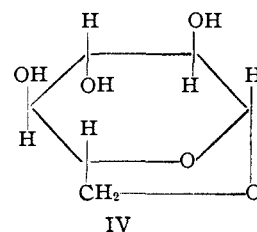
Armstrong, in 1903, was able to correlate the α - and β -methylglucosides with α - and β -glucose, respectively, through the use of enzymes.⁴ Recent oxidation studies with periodic acid have shown that α -methyl-D-glucoside, rannoside, galactoside, gulose, and altriose⁵ have identical configurations for their glycosidic carbon atoms. Hence, if we know the configuration of any one of these glycosidic carbon atoms we should be able to assume, with reasonable assurance, that we can determine the configuration of the glycosidic carbon atoms in each of the *alpha* and *beta* pairs of sugars and glycosides which can be correlated by enzymic, chemical or other means with the known reference compound.

One of the chemical methods which is deserving of attention is based upon the observation of Bergmann and Schotte⁷ that their presumed 1,2-anhydromannose sirup reacts with methyl alcohol at room temperature and in the absence of a catalyst, to form α -methylmannoside. Similarly, the crystalline 1,2-anhydro-3,4,6-triacetylglucose has been reported by Brigl,⁸ and later by Hickinbottom,⁹ to add methyl alcohol, thereby forming β -methylglucoside. In view of the universal tendency for ethylene oxide rings to add hydrogen compounds in such a way that the hydroxyl group and the other group are in a *trans* relationship, then in α -methylmannoside and in β -methylglucoside the methoxyl groups must be *trans* to the respective hydroxyl groups on the second carbon atom. These arguments, as well as others of a less conclusive nature,³ have led to the representation of β -D-glucose by the equivalent formulas I and II; by analogy, the " β -configuration" for the first carbon atom in other aldohexoses of the D-series, and their derivatives, is written similarly; in the " α -configuration" the H and OH groups have the opposite arrangement (III).

The fact that levoglucosan (IV) is formed readily by the destructive distillation of β -D-glucose has long been considered to be evidence of the β -configuration in β -D-glucose. Hann and Hudson¹⁰ have shown that levoglucosan is formed with equal ease from α -D-glucose, so this correlation is no longer valid. The present assignment



II, R = H
V, R = C₆H₅



of the β -configuration to levoglucosan (D-glucosan <1,5> β <1,6>) is based upon general stereochemical principles; the 1,6-anhydro ring seems readily possible if the glycosidic carbon atom has the same configuration as in β -D-glucose (II) because the reacting groups are on the same side of the pyranoid ring, but such a ring formation seems improbable when these groups are on opposite sides of the pyranoid ring, as in α -D-glucose (III).

The present research shows that it is possible to correlate levoglucosan and similar sugar anhydrides with the phenylglycosides, experimentally, and thus to determine the configuration of carbon atom 1 in those glycosides. C. Tanret,¹¹ in 1894, first observed that levoglucosan is formed by the action of aqueous barium hydroxide on picein, salicin and coniferin at 100° in a sealed tube. Vongerichten and Müller¹² were able to isolate levoglucosan from the action of boiling aqueous sodium hydroxide on glucoseapigenin, the probable intermediate being the β -glucoside of phloroglucinol. All of these substances are substituted β -phenylglucosides and they were hydrolyzed with the liberation of a phenol, the glucose moiety being transformed simultaneously into levoglucosan. The behavior of the alpha type of phenolic glycosides toward alkali seemed to us an interesting question but we could find no published data on the subject.

The action of hot alkali on aromatic glucosides

(4) Armstrong, *J. Chem. Soc.*, **83**, 1305 (1903).
 (5) Jackson and Hudson, *THIS JOURNAL*, **59**, 994 (1937).
 (6) Richtmyer and Hudson, *ibid.*, **63**, 1727 (1941).
 (7) Bergmann and Schotte, *Ber.*, **54**, 1564 (1921).
 (8) Brigl, *Z. physiol. Chem.*, **122**, 245 (1922).
 (9) Hickinbottom, *J. Chem. Soc.*, 3140 (1928).
 (10) Hann and Hudson, *THIS JOURNAL*, **64**, 2435, note 2 (1942); cf. Karrer, *Helv. Chim. Acta*, **3**, 258 (1920); Pictet and Sarasin, *ibid.*, **1**, 87 (1918).

(11) C. Tanret, *Bull. soc. chim.*, [3], **11**, 949 (1894); *Compt. rend.*, **119**, 158 (1894).

(12) Vongerichten and Müller, *Ber.*, **39**, 241 (1906).

has long been known to cause hydrolysis,¹³ but most investigators, both before and after Tanret's production of levoglucosan from picein, seem to have assumed that the non-phenolic product was either glucose or its decomposition products. Even a very recent article¹⁴ gives no indication that levoglucosan is to be expected from the alkaline hydrolysis of the β -glycosides of phenol and acetovanillone.¹⁵

We find that β -phenyl-D-glucoside (V) is converted to levoglucosan (IV) by heating at 100° with 1.3 *N* aqueous barium hydroxide or potassium hydroxide. In the latter experiment, polarimetric measurements showed the reaction to be complete within nine hours, and the levoglucosan was isolated in nearly theoretical yield. On the other hand, a solution of α -phenyl-D-glucoside (VI) in 2.6 *N* aqueous potassium hydroxide showed no appreciable change in rotation when it was boiled for two weeks, and 85% of the original glucoside was recovered in crystalline form.

The case of the galactosides is equally significant. Under the same conditions as above, β -phenyl-D-galactoside was transformed quantitatively to D-galactosan <1,5> β <1,6> within nine hours. The α -phenyl-D-galactoside was boiled for sixteen weeks with 2.6 *N* aqueous potassium hydroxide; phenol was liberated slowly, the rotation changed gradually from a high positive to a low negative value, and the known crystalline D-galactosan <1,5> β <1,6> was isolated from the resulting solution in an 85% yield. The conversion of both β - and α -phenyl-D-galactosides, at so greatly different rates, to the same galactose anhydride is strikingly similar to the classical example of the conversion of maleic and fumaric acids to maleic anhydride. With these acids, and with other ethylenic and cyclic 1,2-dicarboxylic acids, it has been found that *cis* groups react more readily with each other than do *trans* groups; if the *trans* dibasic acid does form an anhydride, it does so with greater difficulty and the product usually rearranges to the *cis* form.¹⁶

(13) Among others, Bouchardat, *Compt. rend.*, **19**, 1174 (1844), on salicin; Piria, *Ann.*, **96**, 375 (1855), on populin and benzohelicin; Michael, *Am. Chem. J.*, **5**, 171 (1883), on β -phenylglucoside; Fischer and Strauss, *Ber.*, **45**, 2472 (1912), on β -2,4,6-tribromophenylglucoside.

(14) Fisher, Hawkins and Hibbert, *This Journal*, **63**, 3031 (1941).

(15) Other cases of glycosides unstable toward alkali have been reported, among others, by Goebel and Avery, *J. Exptl. Med.*, **50**, 520 (1929); Goebel, Babers and Avery, *ibid.*, **55**, 761 (1932); Robertson, *J. Chem. Soc.*, 1137 (1930); Helferich and Lutzmann, *Ann.*, **541**, 1 (1939).

(16) See, for example, Marvel, in "Organic Chemistry," edited by Gilman; John Wiley and Sons, Inc., New York, N. Y., 1938, Vol. I, p. 396.

Thus, in these cyclic phenylglucosides and phenylgalactosides we have two excellent examples of an application of the principle of anhydride formation to the determination of configuration in the pairs of anomeric glycosides. When the glycosidic OC₆H₅ and the terminal CH₂OH groups are in *cis* positions, a 1,6-anhydride is formed readily by the elimination of a molecule of phenol through the agency of the hot alkali. When the two groups are in *trans* positions, no anhydride formation was observed with α -phenyl-D-glucoside within two weeks, while with α -phenyl-D-galactoside the phenol was liberated extremely slowly, and the anhydride which was isolated after sixteen weeks of boiling was identical with the one which had been formed with relative ease from the β -phenyl-D-galactoside. Hence, the β -phenyl-D-glucoside and β -phenyl-D-galactoside must have the same " β -configuration" which had been assigned to them previously on other grounds. Conversely, the anomeric α -phenyl-D-glucoside and α -phenyl-D-galactoside must have the " α -configuration." As mentioned before, a combination of enzymic hydrolysis and oxidation with periodic acid may then serve to correlate these glycosides with many other glycosides and their corresponding sugar modifications. The relationship between certain sugars and their glycosides which are not hydrolyzed by any of the known enzymes rests only on analogy. In no case, however, are there any inconsistencies between the configuration as determined by these methods and that which would be assigned according to the system² proposed in 1909.

The action of caustic potash on some other glycosides is summarized in Table I. All the substituted β -phenylglucosides were transformed to levoglucosan; the non-phenolic β -glucosides were unchanged. As may be expected from the records of Goebel, Babers and Avery,¹⁵ the α -nitrophenylglucosides were hydrolyzed apparently even at room temperature by alkali. Other α -glucosides were unchanged. The alkaline degradation of β -phenyl-D-mannoside to D-mannosan <1,5> β <1,6> has been described in an earlier communication from this Laboratory.¹⁷ α -Phenyl-D-mannoside was attacked readily by hot alkali; three-fourths of the material seems to have been destroyed, and the remainder was a mixture from which only a very small amount of D-manno-

(17) Montgomery, Richtmyer and Hudson, *This Journal*, **64**, 1483 (1942).

TABLE I
SUMMARY OF THE BEHAVIOR OF SOME GLYCOSIDES TOWARD HOT 1.3 N AQUEOUS POTASSIUM HYDROXIDE

D-Xylosides	Time, hr.	Rotation ^a		Product	Yield, %
		Initial	Final		
α -Phenyl ^b	48	+189	+189	Xyloside	80
β -Phenyl ^b	3	- 49	\pm 0	Tar	..
D-Glucosides					
α -Methyl	24	+159	+159	Glucoside	87
β -Methyl	24	- 32	- 32	Glucoside	88
β -Cyclohexyl	24	- 41	- 41	Glucoside	87
β - <i>n</i> -Decyl ^c	24	- 28	- 28	Glucoside	82
β -Allyl	24	- 42	- 42	Glucoside	82
α -Phenyl	336 ^d	+180	+181	Glucoside	85
β -Phenyl	9	- 71	- 64 ^e	Glucosan	88
β - <i>o</i> -Cresyl ^f	22	- 68	- 62 ^e	Glucosan	85
β - <i>o</i> -Hydroxymethylphenyl	11	- 63	- 62 ^e	Glucosan	90
β - <i>p</i> -Xenyl ^g	10	^h	- 64 ^e	Glucosan	90
β - <i>p</i> -Acetylphenyl ^o	3	- 89	- 63 ^e	Glucosan	85
α - <i>o</i> -Nitrophenyl ^o	3	+ 58 ⁱ	...	Tar	..
α - <i>p</i> -Nitrophenyl ^o	3	+ 53 ⁱ	...	Tar	..
β - <i>o</i> -Nitrophenyl ^{b,j,l}	3	- 88 ⁱ	- 47 ^e	Glucosan	60
β - <i>p</i> -Nitrophenyl ^b	3	- 84 ⁱ	- 48 ^e	Glucosan	60
D-Galactosides					
α -Phenyl	2688 ^d	+217	- 15 ^e	Galactosan	85
β -Phenyl	9	- 43	- 22 ^e	Galactosan	91
D-Mannosides					
α -Phenyl	336	+113	- 9 ^e	Mostly undetermined	..
β -Phenyl ^k	120 ^d	- 71	-114 ^e	Mannosan	57

^a The rotations are calculated as the glycoside unless noted otherwise. ^b Montgomery, Richtmyer and Hudson, THIS JOURNAL, 64, 690 (1942). ^c Pigman and Richtmyer, *ibid.*, 64, 372 (1942). ^d In boiling 2.6 N aqueous potassium hydroxide. ^e Calculated as a hexosan. ^f Richtmyer and Yeakel, THIS JOURNAL, 56, 2496 (1934). ^g Richtmyer, *ibid.*, 56, 1636 (1934). ^h An initial rotation could not be obtained because of the slight solubility of the xenylglucoside in the cold alkaline solution. ⁱ These rotations of the nitrophenylglucosides, observed after about fifteen minutes in the alkaline solution, are considerably lower than the rotations in water, and indicate the instability of the compounds even at room temperature. The final solutions from the α -derivatives were too dark to measure; those from the β -derivatives could be read only after clarification with activated charcoal. ^j The melting point of β -*o*-nitrophenyl-D-glucoside, reported in ref. *b* as 152°, now appears to be 168-169°; the change is due presumably to a rearrangement to a more stable crystalline form. ^k The experimental details are described by Montgomery, Richtmyer and Hudson in THIS JOURNAL, 64, 1483 (1942). ^l The melting point of our tetraacetyl- β -*o*-nitrophenyl-D-glucoside was reported erroneously in ref. *b*, p. 693, as 150-152°. The correct m. p. is 160-162°.

san<1,5> β <1,6> could be isolated. α -Phenyl-D-xyloside was unaffected; β -phenyl-D-xyloside was converted to a dark, apparently decomposed sirup.

The extension of this work, both for configurational and preparative studies is now in progress with other types of sugars and their derivatives. Improved methods for preparing the desired phenylglycosides have been described in an earlier publication.¹⁸

Experimental Part

The Alkaline Degradation of β -Phenyl-D-galactoside to D-Galactosan<1,5> β <1,6>.—A 2.000-g. sample of β -

(18) Montgomery, Richtmyer and Hudson, THIS JOURNAL, 64, 690 (1942).

phenyl-D-galactoside,¹⁹ rotating²⁰ -43° in water, was dissolved in sufficient 1.3 N aqueous potassium hydroxide to make 100 cc., and heated in a bath at 98° for a period of nine hours. The reaction was followed polarimetrically, the rotation changing from -42.9°, calculated as the galactoside, to -22.0° (constant), calculated as a galactosan. The clear, pale yellow solution was cooled, neutralized to methyl orange²¹ with 3 N sulfuric acid, and concentrated *in vacuo* to dryness. The product was extracted from the

(19) Furnished through the courtesy of Dr. William Ward Pigman of the National Bureau of Standards.

(20) Throughout the article the rotations are specific rotations at 20° for sodium light; *c* designates concentration in grams per 100 cc. of solution.

(21) In all the alkaline degradations some coloring of the solution was observed. Although in many cases this color was only slight, the change produced on neutralization could often be used as an indicator and in no case did it interfere with the use of methyl orange as an external indicator.

potassium sulfate with warm, absolute ethyl alcohol. The solvent was removed, and the product was crystallized from 10 cc. of 50% ethyl alcohol, in a yield of 91%. It was identified as β -D-galactosan<1,5> β <1,6> by its rotation of -22.0° in water (*c*, 2) and by its melting point of 221° , a value which was not changed by mixing it with an authentic sample of this galactosan.

The Alkaline Degradation of α -Phenyl-D-galactoside.—A solution of 4.000 g. of α -phenyl-D-galactoside²² in 200 cc. of 2.6 *N* potassium hydroxide was heated in a conical flask of pure silver to gentle boiling under a reflux condenser for a period of sixteen weeks. The rotation changed from $+217^\circ$, calculated as the galactoside, to -15° (constant), calculated as a galactosan. By the same procedure as described above, the product was isolated as a sirup which was purified by acetylation and deacetylation. Crystalline β -D-galactosan<1,5> β <1,6>, of melting point and mixed melting point 221° , and rotation -22.0° in water (*c*, 2) was isolated in 85% yield.

In a parallel experiment, weekly determinations were made of the free phenol by titration with iodine. At the end of seven weeks, 97% of the phenol had been liberated, and the solution had a low positive rotation; 4% of the α -phenylgalactoside was recovered. Several attempts were made to secure evidence of a possible intermediate product in this reaction, but the amount of phenol-free intermediate present at any time appeared so small that the results must be considered inconclusive.

The Alkaline Degradation of β -Phenyl-D-glucoside.—A 2.000-g. sample of β -phenylglucoside in 100 cc. of 1.3 *N* aqueous potassium hydroxide was heated in a boiling water bath for nine hours, the rotation becoming constant at -64.4° , calculated as a glucosan. The product, isolated as above, was crystallized from 10 cc. of ethyl alcohol, in 88% yield. A melting point and mixed melting point of $179-180^\circ$, and rotation -66.2° in water (*c*, 2) identified it as β -D-glucosan<1,5> β <1,6>.

The Alkaline Degradation of α -Phenyl-D-mannoside.—A solution of 40 g. of α -phenylmannoside²³ in 2000 cc. of

(22) Tetraacetyl- α -phenyl-D-galactoside, melting at 131° and rotating $+177^\circ$ in chloroform (*c*, 2), was obtained in 65% yield by a modification (ref. 18) of the Helferich and Schmitz-Hillebrecht synthesis [*Ber.*, 66, 378 (1933)]. The pure α -phenyl-D-galactoside melted at $140-142^\circ$ and rotated $+217^\circ$ in water (*c*, 2), in agreement with the values reported by Helferich and Appel [*Z. physiol. Chem.*, 205, 231 (1932)].

(23) Tetraacetyl- α -phenyl-D-mannoside was obtained by the modified zinc chloride procedure in a 68% yield; it melted at 80° , and

aqueous 1.3 *N* potassium hydroxide was boiled gently in a stainless steel beaker fitted with a reflux condenser. The rotation dropped to -9° , calculated as a mannosan, at the end of seventeen hours, and remained constant as the heating was continued for two weeks. The product was isolated, and acetylated with acetic anhydride and pyridine at 0° , yielding only 13 g. of a sirup with a low positive rotation. Deacetylation produced 7.5 g. of a sirup with low negative rotation. This sirup was shaken overnight with 100 cc. of acetone and 10 g. of anhydrous copper sulfate. Upon removal of the solid, and concentration of the acetone solution, there was deposited 1.5 g. of elongated prisms, melting at 161° and rotating -59° in water (*c*, 2). This product was identified as 2,3-isopropylidene-D-mannosan<1,5> β <1,6> by direct comparison with the substance described by Knauf, Hann and Hudson,²⁴ and by hydrolysis of it with 0.1 *N* sulfuric acid at 20° to D-mannosan<1,5> β <1,6> of melting point and mixed melting point 210° and rotation -127.5° in water (*c*, 2).

Summary

β -Phenyl-D-glucoside and β -phenyl-D-galactoside have been degraded readily by the action of hot, aqueous potassium hydroxide to D-glucosan<1,5> β <1,6> and D-galactosan<1,5> β <1,6>, respectively. α -Phenyl-D-glucoside was unaffected and α -phenyl-D-galactoside was attacked only very slowly under considerably more drastic conditions. These differences can be used as a basis for determining the configuration of the glycosidic carbon atom in these compounds, and in the other anomeric glycosides and sugars with which they may be correlated.

The alkaline degradation of β -phenylglycosides appears to be of general application in the preparation of glycosans<1,5> β <1,6>.

The behavior of a number of other glycosides toward alkali has been described.

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rotated $+74.0^\circ$ in chloroform (*c*, 2). The α -phenyl-D-mannoside melted at 133° and rotated $+114^\circ$ in water (*c*, 1) in agreement with the data of Helferich and Winkler [*Ber.*, 66, 1556 (1933)].

(24) Knauf, Hann and Hudson, *THIS JOURNAL*, 63, 1447 (1941).