

TABLE III
COMPARISON OF HYDROGEN AND GLASS ELECTRODE POTENTIALS USING THE GALVANOMETER WITH CONDENSER ATTACHMENT

Electrode (against calomel)	Potentials in volts Solution		ΔE
	0.1 N HCl	0.1 N Na ₂ HPO ₄	
Hydrogen (1)	0.3084	0.7780	0.4696
Hydrogen (2)	.3084	.7778	.4694
Corning glass	— .0433	.4261	.4694
MacI.-D. glass	— .0425	.4268	.4693

There is again no evidence of polarization and the results are as accurate as can be obtained with other methods of glass electrode measurements.

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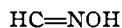
THE REACTIVE FORM OF GLUCOSE OXIME¹

BY M. L. WOLFROM AND ALVA THOMPSON

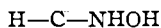
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The fact that glucose reacts with hydroxylamine to form an oxime has been used as proof for the presence of an aldehyde group in the molecule of this sugar. Glucose oxime was first prepared in crystalline form by Jacobi² and its structure was tacitly assumed to be of the true oxime type, R—CH=NOH. The establishment of the ring or cyclic hemiacetal structure for the crystalline forms of glucose raised the question whether this ring opened or remained intact in the nitrogen condensation products of the sugar. In the case of the oxime, these two possibilities are illustrated as follows



True oxime



Ring type oxime

Each of the above types could exist in two isomeric forms, *syn* and *anti* in the true oxime structure and alpha and beta in the other. In the latter case, the formation of more than one ring would indeed give rise to further isomers, but this possibility is not pertinent to the following discussion

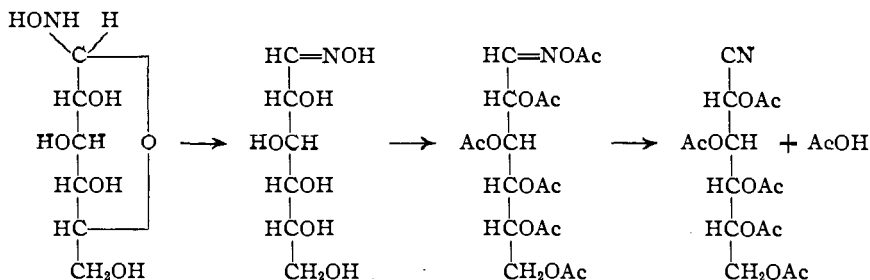
¹ (a) Constructed from a thesis presented by Alva Thompson in candidacy for the degree of Doctor of Philosophy in Chemistry at The Ohio State University. (b) Presented before the Division of Organic Chemistry at the 79th Meeting of the American Chemical Society, Atlanta, Georgia, April 7 to 11, 1930.

² H. Jacobi, *Ber.*, 24, 697 (1891).

and may be disregarded. Furthermore, the exact location of the lower end of any oxygen bridge present need not concern us.

Evidence as to the type of structure of the one known crystalline form of glucose oxime was produced by Miller and Plöchl,³ who found that glucose oxime did not add hydrogen cyanide, as is characteristic of a true oxime. The objection may be raised that this result is based upon negative experimental data, but positive evidence pointing to the same conclusion was obtained by Irvine and Gilmour.⁴ These workers found that on methylation and hydrolysis of this compound, crystalline tetramethylglucose of the lactal or ring structure was obtained instead of the open-chain pentamethylglucose,⁵ as would be the case if the substance were a true oxime.

Although the lactal structure for glucose and its derivatives explains many of the reactions of this important compound, it has been postulated by one of us⁶ and by others that in many reactions, and especially in those of a deep-seated nature, the glucose molecule reacts in the open-chain form. The lactal structure may then be considered as a resting or passive phase in these reactions, the ring opening to produce the highly reactive open-chain form as needed. The importance of this molecular behavior in biological reactivity is obvious. In the work herein reported we have verified this assumption by working with a nitrogen derivative, the oxime. The deep-seated reaction we have chosen to study is the formation of the acetylated nitrile from the oxime. This is the first step in the well-known Wohl sugar degradation. The agents used for this change are sodium acetate and acetic anhydride at relatively high temperatures. The end result of this reaction is, of course, an open-chain compound, but the point in question is whether the lactal structure opens to form the straight chain or true oxime as an intermediate, as follows



It is to be noted that the first reaction above is in the nature of a hydrogen shift and would probably be a prototropic change. Whether this is

³ W. v. Miller and J. Plöchl, *Ber.*, **27**, 1281 (1894).

⁴ J. C. Irvine and R. Gilmour, *J. Chem. Soc.*, **93**, 1429 (1908); *cf.* J. C. Irvine and Agnes Moodie, *ibid.*, **93**, 95 (1908).

⁵ P. A. Levene and G. M. Meyer, *J. Biol. Chem.*, **69**, 175 (1926).

⁶ M. L. Wolfrom, *THIS JOURNAL*, **51**, 2188 (1929).

a true tautomeric phenomenon or whether it is a definite change is still open to question.

The isolation of the open-chain or aldehydo form of glucose penta-acetate⁶ has made possible the synthesis of the third compound represented above, as a reference substance for the study of this reaction.⁷ Aldehydo-glucose penta-acetate⁸ gave a crystalline oxime penta-acetate on reaction with hydroxylamine. Further acetylation yielded the crystalline hexa-acetate, which was isolated in two forms with melting points of 79 and 119.5°, respectively. The lower-melting compound was the form obtained first. This was very unstable and changed spontaneously into the other, after which all attempts to gain the 79° isomer failed. This latter substance showed a characteristic behavior on melting, first fusing at 79°, then, on continued heating, re-solidifying and again melting at about 119°. We believe that these isomers are probably *syn* and *anti* forms, although the possibility of an unstable pseudomorph has not been excluded, due to the fact that we failed to obtain the optical rotation of the 79° form.

In his classical work on the degradation of glucose, Wohl⁹ isolated an oxime hexa-acetate in the mother liquor from the crystallization of the acetylated gluconic acid nitrile. He gave the melting point of 109–110° for this compound and stated that it did not undergo nitrile formation. He considered this to be the *anti* form of the acetylated oxime and happily attributed the rather low yield of acetylated nitrile to the fact that half of the oxime was in this form which did not give the nitrile. Behrend¹⁰ obtained this same hexa-acetate by low temperature acetylation of glucose oxime with acetic anhydride and pyridine. He gave the melting point of 110–111° for this compound and showed that it possessed a normal molecular weight. We have repeated and confirmed the work of these two investigators. On rigorous purification of this compound we have obtained for this substance the melting point of 113–115° and the specific rotation of +7° in U. S. P. chloroform solution. The rotation of this substance has not been previously recorded. Behrend and Roth¹¹ had used this method of mild acetylation to correlate the two lactal penta-acetates of glucose with the two alpha and beta forms of the free sugar, which indicates that this treatment did not alter the lactal nature of the compound. Since Irvine and Gilmour⁴ have later shown that glucose oxime has a ring

⁷ Recently P. Brigl and H. Muehlschlegel, *Ber.*, **63**, 1551 (1930), have prepared aldehydo-*D*-glucose pentabenzoate mono-ethyl alcoholate from glucose mercaptal pentabenzoate by hydrolysis with formic acid. These authors use the prefix *al-* to denote the aldehydo form.

⁸ M. L. Wolfrom, *THIS JOURNAL*, **52**, 2464 (1930).

⁹ A. Wohl, *Ber.*, **26**, 730 (1893).

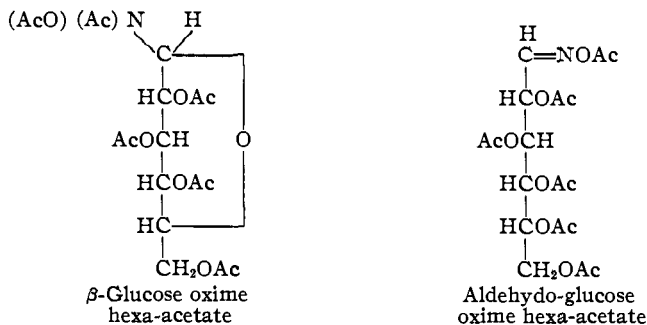
¹⁰ R. Behrend, *Ann.*, **353**, 106 (1907).

¹¹ R. Behrend and P. Roth, *ibid.*, **331**, 359 (1904).

structure, then it follows that this hexa-acetate also is the ring form. From their low rotations, these substances are very probably the beta isomers, according to the general classification of Hudson.¹² The constants of glucose oxime are given by Irvine and Gilmour⁴ as follows: m. p., 138°; specific rotation in aqueous solution, -7.8° , changing to the final value of -0.8° . By rigorous purification we have found the melting point of 142–143° and the specific rotation in 4% aqueous solution of -8.9° , changing to the final value of -2° .

The second oxime hexa-acetate of glucose which we have now obtained has widely differing constants from the one previously known. The melting point is 119.5° and the specific rotation in U. S. P. chloroform solution is $+46^\circ$. From its method of synthesis it possesses the true oxime structure. On heating with acetic anhydride and sodium acetate it readily yielded the acetylated nitrile. On heating this hexa-acetate in the solid form to 135–140°, it began to decompose into acetic acid and acetylated nitrile. The sirup so obtained was colorless and gave a good yield of pure gluconic acid nitrile penta-acetate. These experiments show that this form of acetylated glucose oxime can lose acetic acid to form the acetylated nitrile, whereas the acetylated ring form of the oxime does not possess this property. It is of interest to note here that the penta-acetate of aldehyde-glucose oxime does not form the acetylated nitrile on heating. This indicates that acetic acid and not water is split out in the reaction.

To distinguish the ring form of the acetylated oxime from the open-chain form, the name β -glucose oxime hexa-acetate is suggested for the former, the prefix denoting the beta ring structure. In a parallel way, the prefix aldehyde is suggested for the other isomer, this prefix denoting the open-chain form. The differing structures for these two substances are illustrated by the following formulas



On repeating Wohl's⁹ procedure, we have confirmed his finding that at 135° or slightly above the reaction produces gluconic acid nitrile penta-acetate in about 40% yield, together with a small amount of the β -glucose

¹² C. S. Hudson, THIS JOURNAL, 31, 66 (1909).

oxime hexa-acetate. In this latter compound it is seen that the active hydrogen atom that is attached to nitrogen and is concerned in the shift to the aldehydo structure is here replaced by an acetyl group, which in this case blocks any change to the open-chain form. This compound is thus produced by direct acetylation of the ring form of glucose oxime before the ring has opened.

Gluconic acid nitrile penta-acetate has been rigorously purified and we have found the melting point of $83-84^{\circ}$ and the specific rotation in U. S. P. chloroform solution of $+48^{\circ}$. This optical rotation has been recorded for the first time.

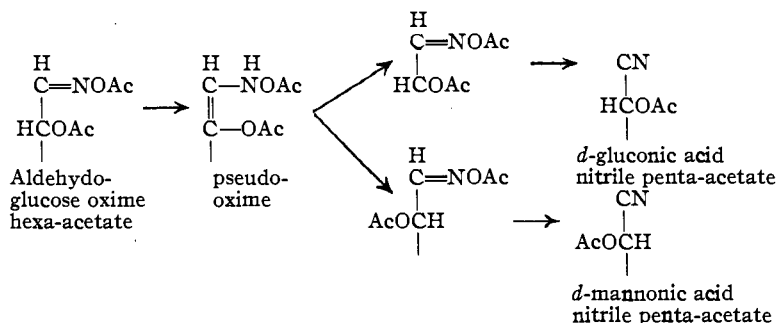
By using Wohl's procedure, but keeping the reaction temperature below 90° , aldehydo-glucose oxime hexa-acetate, melting point 119.5° , was formed from β -glucose oxime and was isolated along with a low yield of acetylated nitrile. This would seem to be definite proof that this open-chain acetate is an intermediate in the reaction. The ring form of glucose oxime then opens and undergoes acetylation, followed by the loss of acetic acid from the acetylated open-chain or true oxime form. We believe the experimental data reported confirm this statement. The reaction as a whole is a complicated one, as is indicated by the low yield, 40%, of acetylated nitrile formed under the best conditions known. The above mechanism may not account for the entire reaction, but it would certainly appear that it accounts for some of this yield of acetylated nitrile and perhaps for the major part of it. We are not interested in explaining the exact mechanism of this reaction, but only in proving that a shift from a lactal structure to the aldehydo or open-chain form does take place.

The question of the stability of the non-acetylated aldehydo-glucose oxime is of interest. Aldehydo-glucose oxime penta-acetate underwent hydrolysis on acid de-acetylation. A basic de-acetylation using methyl alcohol and ammonia produced only the previously known β -glucose oxime, thus showing that when the hydrogen atom concerned in the change between the two forms is not blocked, the solid phase separating from the solution is the ring form. In this connection it is to be noted that β -glucose oxime undergoes mutarotation, whereas none of the oxime acetates we have studied mutarotate, even in the presence of dry hydrogen chloride. In the case of these acetates the mobile hydrogen atoms are replaced by acetyl groups.

Recently, Busch and Kämmerer¹³ have isolated the crystalline pseudo oxime form of phenacyl-*p*-toluidine oxime, $C_6H_5C(NHOH)=CHNH-C_6H_4CH_3$. This is analogous to an enolic form. The possibility of such a pseudo oxime being a factor in the work herein reported is easily eliminated with sugar derivatives. Such a substance should produce both gluconic and mannonic acid nitrile penta-acetates, as it is known that the shift from

¹³ M. Busch and R. Kämmerer, *Ber.*, **63**, 649 (1930).

a double bond linkage to a new asymmetric carbon atom would produce both forms,¹⁴ as is illustrated below



The acetate of mannonic nitrile was not known. It was accordingly synthesized and found to be readily distinguishable from the acetate of gluconic nitrile. It was not found in the reactions studied and it then follows that no consideration of pseudo oxime forms need be made.

Further work on the synthesis and reactivity of the open-chain forms of sugar acetates is in progress in this Laboratory.

Experimental

A.—Synthesis and Preparation of Reference Compounds

Aldehyde-glucose Oxime Penta-acetate.—Ten grams (1 mole) of aldehyde-glucose penta-acetate was added quickly to 100 cc. of boiling water, the solution filtered and cooled as rapidly as possible to room temperature. To this solution was added a mixture of 2.3 g. (1.3 moles) of hydroxylamine hydrochloride and 5 g. (2 moles) of potassium acetate in the dry form. In a few minutes the solution became milky and the oxime separated as an oil. The mixture was allowed to stand at room temperature and was then placed in the ice box to crystallize. The crystalline material was filtered and washed with water; yield, 7.3 g.; m. p. 95–97°. It was recrystallized by dissolving in the smallest possible quantity of methyl alcohol and adding water to opalescence. Crystals separated after standing overnight in the ice box. These were filtered and washed with cold water, m. p. 95–97°. After another recrystallization in the same manner, the substance melted at 97–98° and gave a polarization value of $[\alpha]_D^{28} +57.4^\circ$ (0.2104 g. of subs., 10.06 cc. of U. S. P. chloroform soln., $\alpha = +1.32^\circ$, 1.1-dm. tube). After two more recrystallizations the m. p. was 99–99.5° and $[\alpha]_D^{24} +57.2^\circ$ (0.2096 g. of subs., 10.06 cc. of U. S. P. chloroform soln., $\alpha = +1.31^\circ$, 1.1-dm. tube) was obtained. No rapid or appreciable mutarotation was observed in methyl alcohol solution containing 0.005% of dry hydrogen chloride. Under these conditions the anilides of tetramethylglucose and tetramethylmannose will mutarotate to a constant value.¹⁴ The polarization value of the substance in pure methyl alcohol was $[\alpha]_D^{24} +49.9^\circ$ (0.3487 g. of subs., 10.02 cc. of methyl alcohol soln., $\alpha = +1.91^\circ$, 1.1-dm. tube). A second value was $[\alpha]_D^{24} +49.7^\circ$ (0.3456 g. of subs., 10.06 cc. of methyl alcohol soln., $\alpha = +1.71^\circ$, 1.0-dm. tube).

The substance at room temperature is soluble in ethyl alcohol, very soluble in methyl alcohol, acetone and chloroform, but is practically insoluble in water and petro-

¹⁴ M. L. Wolfrom and W. Lee Lewis, *THIS JOURNAL*, **50**, 837 (1928).

leum ether. It is soluble in hot water but is practically insoluble in hot petroleum ether. It crystallizes in massive six-sided plates.

Anal. Subs., 0.2638: N₂, 9.03 cc. (749.5 mm., 23°). Calcd. for C₆H₈O₂N(CH₃CO)₆: N, 3.45. Found: N, 3.76. Calcd. for acetyl, 0.2000: 24.7 cc. 0.1 *N* NaOH. Found: 24.7 cc.

Aldehyde-glucose Oxime Hexa-acetate.—Thirty cubic centimeters of acetic anhydride was added to a solution of 10 g. of aldehyde-glucose oxime penta-acetate in 60 cc. of dry pyridine at 0°, and allowed to stand in an ice-bath for one hour. It was then poured into five times its mass of ice and water and stirred until all the acetic anhydride had been hydrolyzed. The mixture was then extracted with 50 cc. of chloroform. The chloroform solution was washed successively with a 3% sulfuric acid solution, saturated sodium bicarbonate solution and water, all solutions being kept near ice temperature. The chloroform solution when evaporated left a thick sirup which was crystallized by dissolving in the smallest possible volume of 95% ethyl alcohol and adding water to opalescence. The crude yield was 8.9 g. After one recrystallization the substance melted sharply at 79°; as the bath was heated, it solidified and melted again at 119°. After two recrystallizations, it melted at 119.5°. After four recrystallizations it melted at 119.5° and gave a polarization value of $[\alpha]_D^{25} +46.3^\circ$ (0.4043 g. of subs., 10.06 cc. of U. S. P. chloroform soln., $\alpha = +2.05^\circ$, 1.1-dm. tube). After five recrystallizations the m. p. was 119.5° and the polarization value $[\alpha]_D^{25} +45.9^\circ$ (0.4022 g. of subs., 10.06 cc. of U. S. P. chloroform soln., $\alpha = +2.02^\circ$, 1.1-dm. tube) was obtained. The polarization value of the pure substance in methyl alcohol was $[\alpha]_D^{25} +40.9^\circ$ (0.2010 g. of subs., 10.06 cc. of methyl alcohol soln., $\alpha = +0.90^\circ$, 1.1-dm. tube). A second value was $[\alpha]_D^{24} +41.0^\circ$ (0.2029 g. of subs., 10.06 cc. of methyl alcohol soln., $\alpha = +0.91^\circ$, 1.1-dm. tube). No rapid or appreciable mutarotation was observed in methyl alcohol solution containing 0.005% of dry hydrogen chloride.

The substance is soluble in hot ethyl alcohol and hot water. It is practically insoluble in cold alcohol, water, petroleum ether and ether but is soluble in cold acetone.

Anal. Subs., 0.3030: N₂, 9.4 cc. (760 mm., 26°). Calcd. for C₆H₈O₂N(CH₃CO)₆: N, 3.14. Found: 3.42. Calcd. for acetyl, 0.1000: 13.4 cc. 0.1 *N* NaOH. Found: 13.6 cc.

The acetyl value was determined by distilling 0.5 g. of the substance with 50 cc. of 20% sulfuric acid, water being added to replace that lost by distillation. The acetic acid was then determined in the distillate by titration.

For purposes of identification, a crystallographic description of this compound was made by Doctor V. H. Morris of the Ohio Agricultural Experiment Station.

Crystallographic Description of Aldehyde-*D*-glucose-oxime Hexa-acetate (119.5° Form).—The crystal is observed as colorless elongated prisms belonging to the orthorhombic system. The elongation is in the direction which is taken as crystallographic *c*. The crystal is characterized by the common occurrence of pyramid faces on one or both ends. The optical sign of the crystal is negative; the indices of refraction are $\alpha = 1.460$, $\beta = 1.497$ and $\gamma = 1.504$. Interference colors vary from first order gray when the acute bisectrix emerges, to third and fourth order colors when the optic normal emerges. The crystal shows parallel extinction in all positions. Since the vibration direction of (γ) is parallel to the direction of elongation, the sign of elongation is positive. The optic plane is parallel to the direction of crystallographic *c* and normal to one of the edges between two prism faces. When the crystal is observed lying on one of the edges between two of the prism faces, the acute bisectrix emerges; in this position beta (β) can be measured across the crystal and gamma (γ) along the elongation. When the crystal is observed lying on the edge between the other two prism faces, the optic normal emerges; in this position alpha (α) can be measured across the crystal and

gamma (γ) along the elongation. The optic angle is small. The vibration direction of the obtuse bisectrix is the direction of crystallographic c , the direction of elongation.

Properties of β -Glucose Oxime Hexa-acetate.— β -Glucose-oxime hexa-acetate was prepared by the method of Behrend.¹⁰ It was crystallized by dissolving the thick sirup obtained in ethyl alcohol and adding water to opalescence. After three crystallizations the m. p. was 113–115° and the polarization value was $[\alpha]_D^{25} +7.3^\circ$ (0.4014 g. of subs., 10.06 cc. of U. S. P. chloroform soln., $\alpha = +0.32^\circ$, 1.1-dm. tube). After four crystallizations the m. p. was 113–115°, and the polarization value was $[\alpha]_D^{25} +7.3^\circ$ (0.4018 g. of subs., 10.06 cc. of U. S. P. chloroform soln., $\alpha = +0.32$, after forty-eight hours, 1.1-dm. tube). In methyl alcohol the rotation of the pure substance was $[\alpha]_D^{24} -1.3^\circ$ (0.4026 g. of subs., 25.00 cc. of methyl alcohol soln., $\alpha = -0.04^\circ$, 2-dm. tube). Another determination gave the value $[\alpha]_D^{27} -1.3^\circ$ (0.4042 g. of subs., 25.00 cc. of methyl alcohol soln., $\alpha = -0.04^\circ$, 2-dm. tube). From its low rotation the substance is probably the beta form. No rapid or appreciable mutarotation was observed in a methyl alcohol solution containing 0.005% of dry hydrogen chloride.

The substance is soluble in methyl alcohol, ethyl alcohol, chloroform and acetone, but is practically insoluble in ether, petroleum ether and water at room temperature. It is slightly soluble in hot water, but insoluble in hot petroleum ether. It crystallizes in fine needles. The substance was analyzed, as no analysis was found recorded in the literature.

Anal. Subs., 0.3045: N₂, 9.8 cc. (731.3 mm., 32°). Calcd. for C₆H₇O₅N(CH₃CO)₆: N, 3.14. Found: N, 3.28. Calcd. for acetyl, 0.5004: 67.0 cc. 0.1 N NaOH. Found: 67.3 cc. The acetyl value was determined as described for aldehydo-glucose oxime hexa-acetate.

Preparation of Mannose Oxime.—The directions of Fischer and Hirschberger¹⁵ were slightly modified to give a more convenient method of preparation. Ten grams of mannose was dissolved in 15 cc. of water. To this solution was added a mixture of 15 g. of potassium acetate and 7 g. of hydroxylamine hydrochloride. The solution was warmed very gently for a few minutes and then placed in an ice-salt bath for one-half hour. The mannosine oxime crystallized immediately upon scratching the sides of the flask with a glass rod. The crystals were filtered and washed with a little ice water; yield, 7.8 g.; m. p. 175–177°.

Mannonic Acid Nitrile Penta-acetate.—Six grams of mannose oxime was treated with 40 cc. of acetic anhydride and 6 g. of fused sodium acetate, by heating the reaction mixture to 85–90°. At this point the flask was removed from the source of heat and the reaction started with a rapid increase in temperature to the boiling point of the mixture. After the reaction was apparently over, the mixture was heated at its boiling point for one minute. It was then cooled and poured upon four times its volume of cracked ice with stirring. The sirup which separated was washed repeatedly with ice water and placed in the ice box. After four or five hours the sirup crystallized. This material had a very dark color which was removed by dissolving in boiling water and treating with norite. The water solution upon cooling gave a crop of almost white product. This material was recrystallized four times from a few cubic centimeters of alcohol; yield, 2.5 g.; m. p. 92–93°. This material gave a polarization value of $[\alpha]_D^{29} -1.8^\circ$

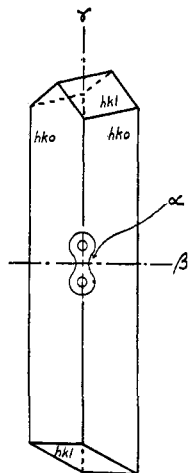


Fig. 1.—Optical orientation of aldehydo-*d*-glucose oxime hexa-acetate, 119.5° form.

¹⁵ E. Fischer and J. Hirschberger, *Ber.*, **22**, 1155 (1889).

(0.3878 g. of subs., 10.06 cc. of U. S. P. chloroform soln., $\alpha = -0.07^\circ$, 1.0-dm. tube). After one further recrystallization the m. p. $92-93^\circ$, and the polarization value $[\alpha]_D^{30} -1.1^\circ$ (0.3525 g. of subs., 10.06 cc. of U. S. P. chloroform soln., $\alpha = -0.04^\circ$, 1.0-dm. tube), were obtained.

The substance is soluble in cold chloroform, slightly soluble in cold alcohol, insoluble in cold water, slightly soluble in hot water, very soluble in hot alcohol and insoluble in petroleum ether. It crystallizes in plates.

Anal. Subs., 0.3030: N₂, 10.3 cc. (748.2 mm., 27°). Subs., 0.1993: CO₂, 0.3617; H₂O, 0.0993. Calcd. for C₆H₆O₆N(CH₃CO)₅: C, 49.56; H, 5.46; N, 3.61. Found: C, 49.49; H, 5.58; N, 3.68.

Measurement of Constants of Gluconic Acid Nitrile Penta-acetate.—Ten grams of gluconic acid nitrile penta-acetate which had been recrystallized once, after three further recrystallizations from the smallest possible volume of hot alcohol, had a melting point of $82.5-84^\circ$. After three more recrystallizations its melting point was $83-84^\circ$. The polarization value of the pure substance was $[\alpha]_D^{29} +47.8^\circ$ (0.9354 g. of subs., 24.88 cc. of U. S. P. chloroform soln., $\alpha = +1.80^\circ$, 1.0-dm. tube). A second determination gave the value $[\alpha]_D^{31} +48.3^\circ$ (0.3505 g. of subs., 10.02 cc. of U. S. P. chloroform soln., $\alpha = +1.69^\circ$, 1.0-dm. tube). The substance crystallizes in plates.

Measurement of Constants of β -Glucose Oxime.—We have been able to recrystallize glucose oxime in one-gram lots from 60% methyl alcohol by boiling with 15 cc. of the solvent for not more than one-half minute, decanting the supernatant liquor, and cooling it rapidly. If the solution is kept hot too long the glucose oxime will not crystallize. After two recrystallizations in this way the material gave a melting point of $139-140^\circ$, an initial polarization value of $[\alpha]_D^{25} -8.9^\circ$ and a final value of $[\alpha]_D^{25} -1.9^\circ$ (1.0260 g. of subs., 25 cc. of water soln., initial $\alpha = -0.73^\circ$, final $\alpha = -0.07^\circ$, 2.0-dm. tube). A melting point of $142-143^\circ$ was obtained for this substance when prepared by de-acetylation of aldehydo-glucose oxime penta-acetate. In this case an initial specific rotation of -8.3° and a final value of -3.0° were obtained.

B.—Reactivity of Glucose Oxime and its Acetates

De-Acetylation of Aldehydo-glucose Oxime Penta-acetate.—An attempt to de-acetylate aldehydo-glucose oxime penta-acetate in acid solution resulted in splitting the oxime linkage. The compound was successfully de-acetylated in alcoholic ammonia. Five grams of aldehydo-glucose oxime penta-acetate was dissolved in 25 cc. of methyl alcohol and dry ammonia bubbled through the solution for fifteen minutes at 0° . The solution was placed in the ice box for twenty-four hours. It was then evaporated under reduced pressure at 35° to a thick sirup, which was dissolved in 10 cc. of ethyl alcohol, nucleated with known glucose oxime and placed in an oven at 37° . Glucose oxime slowly separated out, taking several days to crystallize completely; yield, 0.85 g., m. p. $135-138^\circ$. A mixed melting point with known glucose oxime (m. p. 139°) was $135-138^\circ$. After one crystallization from 80% ethyl alcohol the melting point was $142-143^\circ$ and a mixed melting point with known material was $140-141^\circ$. It gave an initial polarization value of $[\alpha]_D^{29} -8.3^\circ$ and a final value of $[\alpha]_D^{29} -3.0^\circ$ (0.3380 g. of subs., 10.06 cc. of water soln., initial $\alpha = -0.28^\circ$, final $\alpha = -0.10^\circ$, 1.0-dm. tube).

Aldehydo-glucose Oxime Penta-acetate.—On heating aldehydo-glucose oxime penta-acetate slowly to 200° , it began to decompose. The heating was stopped at this point and the product dissolved in hot water. Only unchanged material was isolated.

Aldehydo-glucose Oxime Hexa-acetate, Nitrile Formation.—Two grams of aldehydo-glucose oxime hexa-acetate (m. p. 119.5°) was carefully melted and then heated to $135-140^\circ$. At this point the substance began to decompose and on removal from the source of heat the reaction continued spontaneously with a rapid rise in temperature.

After the reaction was completed, the resulting sirup was poured into cold water. It crystallized almost immediately. On one recrystallization from dilute alcohol, 1.4 g. of material melting at 82–83° was obtained. One further crystallization gave material melting at 83–84° and showing no depression when mixed with an authentic specimen of gluconic acid nitrile penta-acetate (m. p. 83–84°). It gave a polarization in chloroform of $[\alpha]_D^{27} + 47.8^\circ$ (0.4040 g. of subs., 10.06 cc. of U. S. P. chloroform soln., $\alpha = +1.92^\circ$, 1-dm. tube), in exact agreement with the value determined for the nitrile acetate.

Two grams of aldehydo-glucose oxime hexa-acetate (m. p. 119.5°), 2 g. of fused sodium acetate and 8 cc. of acetic anhydride were heated at the boiling point of the mixture for ten minutes and then poured on cracked ice. The resulting sirup was washed several times with cold water, recrystallized from water and then twice from dilute alcohol. The melting point was 83–84° and showed no depression on admixture with an authentic specimen of gluconic acid nitrile penta-acetate (m. p. 83–84°). The polarization value in chloroform was $[\alpha]_D^{27} + 46.8^\circ$ (0.2269 g. of subs., 10.06 cc. of U. S. P. chloroform soln., $\alpha = +1.06^\circ$ 1-dm. tube). The rotatory value previously determined for the acetylated nitrile was +47.8°.

β -Glucose Oxime Hexa-acetate.—This substance did not form gluconic acid nitrile penta-acetate when heated alone to high temperatures or with acetic anhydride and sodium acetate. The compound underwent profound decomposition when heated alone to high temperatures. If treated according to Wohl's procedure for the formation of the acetylated nitrile, the material was recovered unchanged in high yield.

β -Glucose Oxime: Nitrile Formation and Isolation of Intermediate Form.—Ten grams of β -glucose oxime, 10 g. of fused sodium acetate and 40 cc. of acetic anhydride were heated to a temperature of 75–80° for one hour. These conditions were identical with those used by Wohl except that the reaction was run for a longer time at a lower temperature. The solution was then poured into 200 cc. of ice water and allowed to stand overnight. The solid material from four such runs was combined, dissolved in the smallest possible volume of hot ethyl alcohol and water added to opalescence at room temperature. The material which separated was recrystallized four times from hot ethyl alcohol. It yielded 16 g. of gluconic acid nitrile penta-acetate, m. p. 83–84° and giving a polarization value of $[\alpha]_D^{29} + 47.6^\circ$ (0.4227 g. of subs., 10.06 cc. of U. S. P. chloroform soln., $\alpha = +2.00^\circ$ 1-dm. tube). The constants determined for the pure substance are m. p. 83–84°, $[\alpha]_D + 47.8^\circ$. The ice water used to hydrolyze the acetic anhydride solution was extracted with chloroform, the extract evaporated and the residue crystallized by dissolving in hot ethyl alcohol and adding water to incipient opalescence. The filtered material was recrystallized from alcohol-free ether. The alcohol mother liquor from the first recrystallization of the nitrile was diluted to 400 cc. with water, extracted with chloroform and the extract treated as above. The filtered material from these two sources was combined and recrystallized four times from alcohol-free ether and finally from dilute ethyl alcohol. The yield was 0.2 g. of material, m. p. 119°. A mixed melting point with an authentic specimen of aldehydo-glucose oxime hexa-acetate (m. p. 119.5°) was 119.5°, while on admixture with β -glucose oxime hexa-acetate (m. p. 113–115°) the melting point was depressed to 103–106°. The isolated material gave a polarization value of $[\alpha]_D^{31} + 45.9^\circ$ (0.2150 g. of subs., 10.06 cc. of U. S. P. chloroform soln., $\alpha = +0.98^\circ$, 1-dm. tube). The polarization value determined for the pure substance in this solvent was $[\alpha]_D + 46^\circ$. Doctor V. H. Morris of the Ohio Agricultural Experiment Station has made a comparison of the optical properties of the above isolated material with those of aldehydo-glucose oxime hexa-acetate synthesized through aldehydo-glucose penta-acetate and states that "the optical constants of the two samples are identical."

The yield of 0.2 g. reported above is probably not representative of the total amount

of this substance in the acetylation mixture, since its separation is difficult and most of the material is lost in purification.

β -Glucose Oxime Hexa-acetate. Isolation from Wohl's Acetylation Mixture.— Since Wohl⁹ merely reported a melting point for the oxime acetate obtained by him and gave no directions for its isolation, it was thought advisable to repeat this work. It was also of interest in our work to determine that when the acetylation of the oxime had gone to completion, no trace of the aldehydo-oxime acetate could be found. Five grams of glucose oxime was acetylated according to Wohl's procedure and the reaction mixture poured into 300 cc. of cold water. The liquid decanted from the sirup formed was extracted with chloroform and the evaporated extract dissolved in a small quantity of ethyl alcohol. To this solution was rapidly added about four volumes of water and the solution decanted from the sirup precipitated. A crop of crystals appeared in the decanted solution. After one recrystallization from alcohol and water the melting point was 113–115° and showed no depression on admixture with an authentic specimen of β -glucose oxime hexa-acetate (m. p. 113–115°) and gave a melting point of 100–105° on mixing with aldehydo-glucose oxime hexa-acetate (m. p. 119.5°)

Summary

1. The penta-acetate and hexa-acetate of the open-chain form of glucose oxime have been synthesized in pure crystalline condition.
2. Evidence is given that the ring form of glucose oxime shifts to the open-chain form on undergoing nitrile formation. This evidence is based upon the direct isolation of the intermediate open-chain oxime acetate from the reaction mixture when the reaction is stopped before completion.
3. Acetic acid and not water is split out on nitrile formation from glucose oxime.
4. The acetylated oxime isolated by Wohl and considered to be the *anti* form of glucose oxime hexa-acetate is shown to be a ring form, corresponding in structure to glucose oxime. It is shown that these ring forms are probably the beta isomers.
5. The open-chain form of glucose oxime penta-acetate yields the ring form of glucose oxime on de-acetylation.
6. A nomenclature is suggested which distinguishes between the ring forms and the open-chain forms of sugar oximes.
7. Evidence is given that glucose oxime does not react in the pseudo oxime form on undergoing nitrile formation.
8. Mannonic acid nitrile has been synthesized in pure crystalline condition.
9. Constants are given for β -glucose oxime hexa-acetate, β -glucose oxime, and gluconic acid nitrile penta-acetate.

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