

TABLE IV (Concluded)

Sulfonate	Concn., moles/liter	Temp., °C.	% Splitting, sulfite basis	Av.
Sec.-butyl	0.9226	315	24.1	
		335	60.8	
		345	75.2	
		355	91.3, 91.8	91.5
Sec.-amyl	1.145	345	64.2, 64.2	64.2
		355	84.8	
Sec.-hexyl	1.015	335	35.0	
		345	54.2, 55.6	54.9
		355	75.0	
Benzene	0.9876	345	5.0	
		355	11.5	
		375	40.0, 40.2	40.1

Summary

1. A series of aliphatic sulfonic acids has been prepared.
2. The decomposition of the sodium salts of these acids in alkaline solution at high temperatures and pressures has been determined.
3. The order of the reaction has been determined and the course of the reaction indicated.
4. The velocity constants and heats of activation have been calculated.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

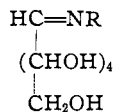
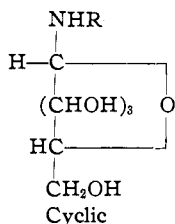
THE OCCURRENCE OF TRUE HYDRAZONE STRUCTURES IN THE SUGAR SERIES¹

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In the condensation reactions of sugars with amino compounds, it is of interest to determine whether the products possess a cyclic or an open-chain structure. These two types of structures are illustrated by the formulas



Open chain

¹ That part of this work concerned with the structure of *d*-galactose *p*-nitrophenylhydrazone was presented before the Division of Organic Chemistry at the 81st meeting of the American Chemical Society, Indianapolis, Indiana, March 31, 1931.

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Levene³ has shown that in the ammonia derivatives of the sugars the nitrogen is present as a primary amino group, since the substances react quantitatively with nitrous acid. These derivatives then have a cyclic structure, $\text{CH}_2\text{OH}-\text{CH}-\text{O}-\text{CH}(\text{CHOH})_3-\text{CHNH}_2$. Miller and Plöchl⁴ attempted to allocate open-chain structures to those sugar nitrogen condensation products which would add hydrogen cyanide. The fact that the ammonia condensation products possess the property of adding hydrogen cyanide, makes this method of doubtful value.

Amadori⁵ has obtained the condensation product of glucose and *p*-phenetidine (and also of several related aromatic amines) in two isomeric forms by varying certain experimental conditions. This worker has concluded that one of the isomers is a cyclic form and the other is an open-chain structure, basing this upon their differing stability toward acidity and alkalinity. Irvine and Gilmour⁶ have shown by methylation methods that glucose oxime has a ring structure. However, Wolfrom and Thompson⁷ have demonstrated by methods based upon the isolation of crystalline derivatives of known structure, that the cyclic structure of glucose oxime, $\text{CH}_2\text{OH}-\text{CH}-\text{O}-\text{CH}(\text{CHOH})_3-\text{CHNHOH}$, shifts to the open chain form, $\text{CH}_2\text{OH}-(\text{CHOH})_4-\text{CH}=\text{NOH}$, on undergoing nitrile formation.

Two isomeric forms of glucose phenylhydrazone are recorded in the literature. A cyclic structure for the so-called α -isomer⁸ has been definitely established by Behrend and Reinsberg.⁹ On mild acetylation of this very unstable α -hydrazone, Hofmann,¹⁰ working in Behrend's laboratory, had obtained a crystalline acetate. Behrend and Reinsberg determined that this acetate contained five acetyl groups and split it with benzaldehyde and also with dilute acid, obtaining acetylphenylhydrazine. This is definite evidence that the acetate and its mother substance are cyclic compounds, the acetate being formulated $\text{CH}_2\text{OAc}-\text{CH}-\text{O}-\text{CH}(\text{CHOAc})_3-\text{CH}-\text{NAc}-\text{NH}-\text{C}_6\text{H}_5$. Under the mild acetylating conditions used by these workers, the second nitrogen is not acetylated. α -Glucose phenylhydrazone is an elusive substance, tending to change to the stable β -form,¹¹ or to glucosazone.¹² Mild acetylation of the stable β -hydrazone by Hofmann produced only an

³ P. A. Levene, *J. Biol. Chem.*, **24**, 59 (1916).

⁴ W. v. Miller and J. Plöchl, *Ber.*, **27**, 1281 (1894).

⁵ M. Amadori, *Atti. accad. Lincei*, [6] **9**, 68, 226 (1929); **13**, 72, 195 (1931).

⁶ J. C. Irvine and R. Gilmour, *J. Chem. Soc.*, **93**, 1429 (1908); cf. J. C. Irvine and Agnes Moodie, *ibid.*, **93**, 95 (1908).

⁷ M. L. Wolfrom and Alva Thompson, *THIS JOURNAL*, **53**, 622 (1931).

⁸ E. Fischer, *Ber.*, **20**, 821 (1887).

⁹ R. Behrend and W. Reinsberg, *Ann.*, **377**, 189 (1910).

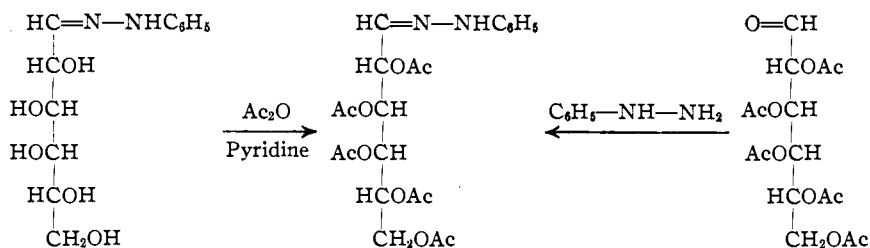
¹⁰ A. Hofmann, *ibid.*, **366**, 277 (1909).

¹¹ H. Skraup, *Monatsh.*, **10**, 401 (1889).

¹² C. L. Butler and L. H. Cretcher, *THIS JOURNAL*, **51**, 3161 (1929).

amorphous substance. Behrend and Reinsberg were unable to split this amorphous β -acetate without a short preliminary treatment with alkali. After such a treatment, they obtained un-acetylated phenylhydrazine as its benzal compound, and on this basis they concluded that the β -hydrazone had an open-chain or true hydrazone structure. We do not believe that this conclusion is valid, since an acetate group on the nitrogen atom could have been saponified by the treatment with alkali. Furthermore, conclusions based upon reactions of amorphous substances are always of doubtful value in carbohydrate work.

From the above literature review we may conclude that there is definite chemical evidence for the existence of cyclic structures for the amino condensation products of the unsubstituted sugars. There is only indirect evidence for the existence of open-chain structures. The isolation of crystalline open-chain or *aldehydo*-acetates of certain of the sugars¹³ has furnished a new method of approach to this problem. We wish to report now that we have obtained what we believe to be definite chemical evidence to the effect that several common hydrazones of galactose have an open-chain or true hydrazone structure. This conclusion is based upon the fact that the crystalline penta-acetate obtained by mild acetylation of the hydrazone is identical with that obtained by the interaction of aldehydo galactose penta-acetate^{13b} with the hydrazone. The reactions involved are illustrated.



The above set of reactions was applied to galactose phenylhydrazone and to three substituted hydrazones of galactose, the α -benzyl- α -phenyl, α -methyl- α -phenyl- and *p*-nitrophenylhydrazone. In each case the same crystalline penta-acetate was obtained by the two methods. Each of these crystalline hydrazones of galactose has been isolated in one form only. Hofmann¹⁰ made unsuccessful attempts to prepare isomers of the first two hydrazones named above. This same worker had prepared the crystalline acetate of the phenylhydrazone and α -benzyl- α -phenylhydrazone of galactose by mild acetylation. This method consists of adding the solid hydrazone to a mixture of acetic anhydride and pyridine, the latter being in excess. The mixture is previously cooled to 0° and maintained there

¹³ (a) M. L. Wolfrom, *THIS JOURNAL*, **51**, 2188 (1929); (b) **52**, 2464 (1930); (c) M. L. Wolfrom and Mildred R. Newlin, *ibid.*, **52**, 3619 (1930).

until the hydrazone has dissolved. The reaction mixture is then kept at room temperature to complete the reaction, which does not produce enough heat to raise the temperature of the solution above its surroundings. This is the method developed by Behrend and his students to acetylate a mutarotatory substance without changing its structure. Behrend and Roth¹⁴ correlated in this way the alpha and beta forms of glucose with their penta-acetates and showed that continued standing of the solution (fourteen days in one experiment) only increased the yield and did not affect the nature of the product obtained. This latter point was verified by us in the case of the hydrazones acetylated. That the method is applicable to the hydrazones without change of structure is further verified by the fact that Hofmann, and Behrend and Reinsberg obtained a characteristic crystalline acetate of cyclic structure from the very unstable α -phenylhydrazone of glucose. Certainly it is applicable in the case of the α -benzyl- α -phenylhydrazone of galactose, as neither this substance nor its acetate shows any mutarotation in pyridine solution.

Hofmann made an elementary analysis of the two acetylated hydrazones of galactose prepared by him but was unable to distinguish between a penta-acetate and a hexa-acetate by this means. He was, of course, unable to draw any conclusions as to the structures of these acetylated hydrazones. We have found that the substances are penta-acetates by means of acetyl analyses. One of them, the acetate of galactose *p*-nitrophenylhydrazone, was suitable for a molecular weight determination by the camphor fusion method and was found to have a normal molecular weight.

Further work on the use of aldehydo sugar acetates in the determination of the structure of the nitrogen condensation products of the sugars is in progress.

Experimental

Preparation of the Penta-acetate of Galactose Phenylhydrazone from Aldehydo-galactose Penta-acetate.—In all condensations reported in this work the aldehydo-galactose penta-acetate was used in the form of its ethyl alcohol hemi-acetal, as it had been found previously^{13b} that the same semicarbazone penta-acetate was obtained from the alcoholate as from the free aldehyde form. Five grams (1 mole) of aldehydo-galactose penta-acetate ethyl alcoholate was dissolved in 50 cc. of warm water, cooled rapidly to room temperature and a solid mixture of 3 g. (1.8 moles) of phenylhydrazine hydrochloride and 2.5 g. (2.2 moles) of potassium acetate was added. The mixture was shaken until solution was effected. After a few minutes a light yellow amorphous solid was precipitated. This was filtered, washed with water and crystallized from hot absolute ethyl alcohol. The crystals were diamond-shaped and colorless. From four such runs a total of 9.4 g. of product melting at 130° was obtained. On further recrystallization the constant melting point of 135–136° was found. A mixed melting point of this material with that (m. p. 134–135°) obtained by acetylation of galactose phenylhydrazone according to the directions of Hofmann¹⁰ was 134–135°. The specific

¹⁴ R. Behrend and P. Roth, *Ann.*, **331**, 359 (1904); R. Behrend, *ibid.*, **353**, 109 (1907).

rotation of the product in pyridine solution was $[\alpha]_D^{27} +41.7^\circ$ (c , 4.029, α , $+3.36^\circ$, 2-dm. tube). No mutarotation was detected. The rotation of the acetylated galactose phenylhydrazone prepared according to the directions of Hofmann was $[\alpha]_D^{25} +42.0^\circ$ in pyridine solution (c , 3.983, α , $+3.35^\circ$, 2-dm. tube) without mutarotation. The two substances were therefore identical. The constants obtained are in good agreement with those given by Hofmann except that he records a melting point of $137-139^\circ$ with sintering at 135° and an initial specific rotation in pyridine solution of $+44^\circ$, changing in about two hours to the constant value of $+42^\circ$. We were unable to verify this higher melting point or the slight mutarotation. The substance is peculiar in that it undergoes a rapid decomposition in U. S. P. chloroform solution.

In the procedure used by Hofmann the mixture is allowed to stand for twenty-four hours after solution of the hydrazone at 0° in the acetylating mixture. In an experiment in which the hydrazone was held in the acetylating mixture at 0° for only two hours, the same crystalline product was obtained but in very low yield. The melting point was $134-135^\circ$, unchanged on admixture with an authentic specimen of the acetylated hydrazone.

As Hofmann was unable to determine whether this compound contained five or six acetyl groups an acetyl determination was made. All the acetyl determinations recorded in this work were made by distillation at constant volume with 15% phosphoric acid.

Anal. Subs., 0.4068: 42.38 cc. 0.1 *N* NaOH. Calcd. for $C_{12}H_{15}O_6N_2(COCH_3)_6$: 10.4 cc. of 0.1 *N* NaOH per 100 mg. Calcd. for $C_{12}H_{12}O_6N_2(COCH_3)_6$: 11.5 cc. Found: 10.4 cc.

Preparation of the Penta-acetate of Galactose α -Benzyl- α -phenylhydrazone from Aldehydo-galactose Penta-acetate.—Three grams (1 mole) of aldehydo-galactose penta-acetate ethyl alcoholate was dissolved in 50 cc. of 95% ethyl alcohol. To this was added a solution of 3 g. (2 moles) of α -benzyl- α -phenylhydrazine in 30 cc. of 95% ethyl alcohol. Water was added to incipient opalescence and the mixture allowed to stand overnight in the ice box. The crop of crystals so obtained was filtered and washed with dilute ethyl alcohol. On one recrystallization from ethyl alcohol 0.8 g. of colorless crystals melting at $128-129^\circ$ was obtained. On two further recrystallizations the melting point was $128-129^\circ$. A mixed melting point with the acetylated galactose α -benzyl- α -phenylhydrazone (m. p. $129-130^\circ$) prepared according to the directions of Hofmann¹⁰ was $128-129^\circ$. The rotation of the substance in pyridine solution was $[\alpha]_D^{25} +91^\circ$ (c , 1.983, α , $+1.80^\circ$, 1-dm. tube) without mutarotation. The rotation of the acetylated hydrazone prepared according to the directions of Hofmann was $[\alpha]_D^{25} +91^\circ$ (c , 1.902, α , $+1.72^\circ$, 1-dm. tube) without mutarotation. The two substances were therefore identical. Hofmann recorded for this compound a melting point of $128-130^\circ$ and $[\alpha]_D +93^\circ$ (c , 5.5) without mutarotation.

Anal. Subs., 0.2005: 16.74 cc. 0.1 *N* NaOH. Calcd. for $C_{19}H_{19}O_6N_2(COCH_3)_5$: 8.8 cc. of 0.1 *N* NaOH per 100 mg. Calcd. for $C_{19}H_{18}O_6N_2(COCH_3)_5$: 9.8 cc. Found: 8.4 cc.

Aldehydo-*d*-galactose α -Methyl- α -phenylhydrazone Penta-acetate.—Five grams (1 mole) of aldehydo-galactose penta-acetate ethyl alcoholate was dissolved in 180 cc. of 95% ethanol and cooled to room temperature. Four cc. (3 moles) of α -methyl- α -phenylhydrazine was added to the solution. Water was then added to incipient opalescence and the solution kept overnight in the ice box. At the end of this period a crop of crystals had formed and a further quantity was obtained by adding more water and allowing the mixture to stand for a day longer in the ice box. The crystals were removed by filtration and washed with dilute alcohol; yield, 4.1 g.; m. p. $135-136^\circ$. On two recrystallizations from ethanol the melting point was $138-139^\circ$. After two

further recrystallizations the melting point remained at 138–139°. Material of this purity showed a rotation in pyridine solution of $[\alpha]_D^{25} +27.3^\circ$ (*c*, 4.014, α , +2.19°, 2-dm. tube) without mutarotation. The substance was colorless and was very soluble in chloroform, acetone, pyridine and benzene. It was soluble in ether, moderately so in ethanol and was practically insoluble in petroleum ether and water.

Anal. Subs., 0.2395: N₂, 13.55 cc. (739.4 mm., 25°). Subs., 0.2003: 20.48 cc. 0.1 *N* NaOH. Calcd. for C₁₃H₁₅O₅N₂(COCH₃)₅: N, 5.67; 10.1 cc. 0.1 *N* NaOH per 100 mg. Found: N, 6.11; 10.2 cc. 0.1 *N* NaOH.

Preparation of Aldehyde-galactose α -Methyl- α -phenylhydrazone Penta-acetate by Mild Acetylation of the Hydrazone.—Seven grams of *d*-galactose α -methyl- α -phenylhydrazone¹⁵ (m. p. 190–191°, corr.) was added to a solution of 36 cc. of pyridine and 18 cc. of acetic anhydride, previously cooled to 0°. The mixture was shaken mechanically at 0° for several hours and was then kept at ice box temperature with occasional shaking for two days. At the end of this period some undissolved hydrazone was removed by filtration and the filtrate poured into seven times its volume of water. The solid material precipitated was filtered and washed with water. It was then dissolved in hot ethanol and decolorized with norite. On cooling, a very pure, colorless and crystalline product was obtained; yield, 7.8 g.; m. p. 138–139°. On two further recrystallizations from hot ethanol, the melting point remained at 138–139°. A mixed melting point with the product (m. p. 138–139°) obtained from the aldehyde-galactose penta-acetate showed no depression. The rotation in pyridine solution of material of this purity was $[\alpha]_D^{25} +27.2^\circ$ (*c*, 4.022, α , +2.19°, 2-dm. tube) without mutarotation. The value found for the product obtained from the aldehyde-galactose penta-acetate was $[\alpha]_D^{25} +27.3^\circ$ in pyridine solution without mutarotation. The two substances were therefore identical.

In an experiment in which the hydrazone was held in the acetylating mixture at 0° for only two and one-half hours, the same crystalline product was obtained but in very low yield. The melting point was 138–139°, unchanged on admixture with an authentic specimen of the hydrazone acetate.

Aldehyde-*d*-galactose *p*-Nitrophenylhydrazone Penta-acetate.—Five grams (1 mole) of aldehyde-galactose penta-acetate ethyl alcoholate was dissolved in 150 cc. of hot water and cooled to room temperature. Three grams (1.4 moles) of *p*-nitrophenylhydrazone hydrochloride and 2.5 g. (2.2 moles) of potassium acetate was dissolved in 300 cc. of hot water. This solution was cooled to just above room temperature and the aqueous solution of the penta-acetate added to it. An amorphous substance was precipitated. This was filtered, washed with water and was obtained crystalline by dissolving in hot ethanol and cooling. The substance crystallized in diamond-shaped, complicated, light yellow crystals; yield, 2.8 g. Further recrystallization was effected by dissolving in warm chloroform, cooling to room temperature and adding petroleum ether. It may also be recrystallized from hot ethanol. The melting point of the substance was 194–195° (corr.) with decomposition, unchanged by further recrystallization. The rotation of the purified material in U. S. P. chloroform solution was $[\alpha]_D^{25} +21.2^\circ$ (*c*, 3.974, α , +0.84°, 1-dm. tube). The substance is moderately soluble in chloroform, alcohol and benzene and is practically insoluble in ether, petroleum ether and water.

Anal. Subs., 0.4008: 37.39 cc. of 0.1 *N* NaOH. Subs., 0.2212: N₂, 16.03 cc. (739.6 mm., 23°). Calcd. for C₁₂H₁₂O₇N₃(CH₃CO)₅: N, 8.00; 9.5 cc. 0.1 *N* NaOH per 100 mg. Found: N, 7.91; 9.4 cc. 0.1 *N* NaOH. *Molecular weight* (Rast). 0.0102

¹⁵ W. Alberda van Ekenstein and C. A. Lobry de Bruyn, *Rec. trav. chim.*, **15**, 97, 225 (1896).

g. of subs. in 0.1335 g. of camphor depressed the m. p. 6° . Mol. wt. calcd. for $C_{27}H_{27}O_{12}N_3$: 525. Found: 510.

Preparation of Aldehydo-galactose *p*-Nitrophenylhydrazone Penta-acetate by Mild Acetylation of the Hydrazone.—A mixture of 5.5 cc. of acetic anhydride and 10.3 cc. of pyridine was cooled to 0° and 2 g. of *d*-galactose *p*-nitrophenylhydrazone¹⁶ (m. p. 196° , corr.) added. The mixture was kept at 0° with occasional shaking until the hydrazone had dissolved. Solution was effected in about two hours. The mixture was then allowed to stand at room temperature for two days. The acetylated product crystallized out of the reaction mixture in the form of large and complicated spear-shaped crystals of a light yellow color. These were removed by filtration and washed rapidly with petroleum ether; yield, 1.8 g. Recrystallization was effected by dissolving in warm chloroform, cooling to room temperature and adding petroleum ether. The melting point was 194 – 195° (corr.) and the rotation in U. S. P. chloroform solution was $[\alpha]_D^{26} +21.1^{\circ}$ (*c*, 3.981, α , $+0.84^{\circ}$, 1-dm. tube). This melting point and rotation were unchanged by further recrystallization. A mixed melting point with the product (m. p. 194 – 195° , corr.) obtained from the aldehydo-galactose penta-acetate showed no depression. The rotation for the product obtained from the aldehydo-galactose penta-acetate was $[\alpha]_D^{26} +21.2^{\circ}$ in U. S. P. chloroform solution. The two substances were therefore identical.

In one experiment the hydrazone was held in the acetylating mixture at 0° for only two and one-half hours, the solution poured into water, the precipitated amorphous substance crystallized from ethanol and recrystallized several times from the same solvent. There was so obtained a very low yield of product melting at 194 – 195° (corr.) and showing no depression on admixture with an authentic specimen of the acetate previously obtained.

Summary

1. The open chain or *aldehydo* forms of the penta-acetate of the α -methyl- α -phenylhydrazone and *p*-nitrophenylhydrazone of *d*-galactose have been synthesized.

2. The crystalline penta-acetates obtained by mild acetylation of the phenylhydrazone, α -benzyl- α -phenylhydrazone, α -methyl- α -phenylhydrazone and *p*-nitrophenylhydrazone of *d*-galactose have been found to be identical with those obtained by condensing the open-chain or *aldehydo* form of *d*-galactose penta-acetate with the hydrazine.

3. Evidence based upon the isolation of crystalline derivatives of known structure is thus obtained that these hydrazones of galactose have the open-chain or true hydrazone structure.

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¹⁶ W. Alberda van Ekenstein and J. J. Blanksma, *Rec. trav. chim.*, **22**, 434 (1903).