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

Estimation of O-Acetyl and N-Acetyl and the Structure of Osazone Acetates

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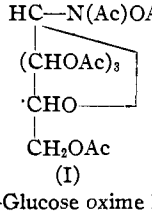
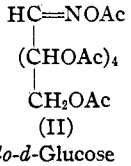
In previous work reported from this Laboratory[1] we have indicated the existence of both cyclic and acyclic structures in the acetates of the amino condensation products of the sugars. For example, with glucose oxime, two isomeric hexaacetates are known and the following structures have been assigned to them.



(I)

β-d-Glucose oxime hexaacetate



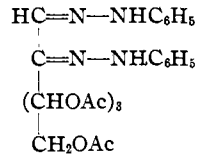
(II)

Aldehydo-d-Glucose oxime hexaacetate

The structures of type (II) have been well established through their synthesis from the *aldehydo*-forms of the sugar acetates. The cyclic structures of type I have been established through non-identity with the *aldehydo*-structures and through the use of certain results obtained from methylation and acetylation studies, in which it is necessary to assume that no structural shift has occurred during the substitution procedure. A more direct type of proof for these cyclic acetate structures is then desirable. It is to be noted that the two structural types contain the same number of acetyl groups but that in the cyclic structure (I) one acetyl group is directly attached to nitrogen. An analytical procedure which would distinguish between O-acetyl and N-acetyl would easily differentiate between the two structures. Such a procedure has been established.

The excellent method of Freudenberg and Harder[2] gives the combined O-acetyl and N-acetyl. We have found that the Kunz[3] procedure for the estimation of acetyl groups gives only the O-acetyl. Any difference is then due to the N-acetyl. The Kunz procedure is essentially an alkaline saponification carried out below 0° and has been used for the estimation of acetyl content on alkali-sensitive sugar structures. The saponified mixture obtained from these acetylated nitrogen structures by the application of the Kunz procedure, was titrated by the potentiometric method and it was found that phenolphthalein was not a satisfactory titration indicator but that phenolsulfonphthalein would give satisfactory results. The analytical procedures were established with acetanilide and with a mixture of acetanilide and *d*-mannitol hexaacetate of known composition. They were then applied to the acetylated structures previously reported from this Laboratory and the assigned cyclic structures confirmed in every case.

We had previously obtained in this Laboratory a crystalline acetate of *d*-glucose phenylosazone and also one of *d*-galactose phenylosazone.[4] The former was recently reported by Engel[5] and by Maurer and Schiedt.[6] These authors recorded elementary analyses for the substance. The Freudenberg analysis indicated a tetraacetate structure for both compounds. As both acetates were highly colored, the Kunz procedures were carried out by the potentiometric method and yielded the same acetyl values as were obtained by the Freudenberg method. This is proof that the acetates were acyclic compounds of the following structure



It is of interest to note that these acetylated osazones are open-chain compounds. We do not believe that the extension of this structure to the unacetylated osazones is at present justified. Percival and Percival[7] have concluded that a trimethyl phenylglucosazone obtained by them is a derivative of fructopyranose and is thus acyclic on the second carbon. This need not be considered as contradictory to our results, as the osazone may well be acyclic in its acetate and cyclic

(1) M. L. Wolfrom and A. Thompson, THIS JOURNAL, **53**, 622 (1931); M. L. Wolfrom and C. C. Christman, *ibid.*, **53**, 3413 (1931); V. Deulofeu, M. L. Wolfrom, P. Cattaneo, C. C. Christman and L. W. Georges, *ibid.*, **55**, 3488 (1933); M. L. Wolfrom, L. W. Georges and S. Soltzberg, *ibid.*, **56**, 1794 (1934).

(2) K. Freudenberg and M. Harder, *Ann.*, **433**, 230 (1923).

(3) A. Kunz and C. S. Hudson, THIS JOURNAL, **48**, 1982 (1926).

(4) L. W. Georges, Ph.D. Dissertation, The Ohio State University, 1934.

(5) L. L. Engel, THIS JOURNAL, **57**, 2419 (1935).

(6) K. Maurer and B. Schiedt, *Ber.*, **68**, 2187 (1935).

(7) E. E. Percival and E. G. V. Percival, *J. Chem. Soc.*, 1398 (1935).

in its methyl ether. Undoubtedly, both cyclic and acyclic structures are possible for sugar osazones and both are probably present in their mutarotated equilibrium mixture, although the mutarotation may be complicated by some hydrolysis.[5] Absorption spectra measurements recently made by Engel would support the presence of acyclic structures.

Experimental

d-**Galactose Phenylosazone Tetraacetate.**[4]—*d*-Galactose phenylosazone (1.5 g.) was dissolved in a mixture of 3 cc. of acetic anhydride and 8 cc. of pyridine by ten minutes of mechanical shaking at room temperature and allowed to stand overnight. The reddish-brown solution was then poured into 200 cc. of ice and water and the crude product separated by filtration; yield 2.3 g. Crystalline material was obtained from hot absolute ethanol; yield 1.3 g.; m. p. 178–179° (dec.); $[\alpha]^{24}D$ +87° (*c*, 4; U. S. P. $CHCl_3$; no mutarotation). Further recrystallization did not alter these constants. The substance was in the form of light yellow crystals and exhibited the usual sugar acetate solubilities.

Anal. Calcd. for $C_{26}H_{30}O_8N_4$: N, 10.64. Found: N, 10.66.

Preparation of *d*-**Glucose Phenylosazone Tetraacetate.**[4]—This substance was prepared in a manner similar to that described by Maurer and Schiedt.[6] We experienced the same difficulty in obtaining the substance in crystalline form; m. p. 115–117°; $[\alpha]^{25}D$ −55° ⟶ −45° (*c*, 3; U. S. P. $CHCl_3$); yellow needles.

Analytical Procedures.—The total acetyl value was determined exactly as described by Freudenberg and Harder[2] for N-acetyl. The analysis according to the Kunz procedure was performed by dissolving 100 mg. of the substance in 25 cc. (or more if required) of acetone, cooling in an ice–salt mixture and adding 25 cc. of 0.1 *N* sodium hydroxide dropwise. The solution was then kept below 0° for one hour and back-titrated with 0.1 *N* hydrochloric acid, using phenolsulfonphthalein indicator. A blank was run on the acetone used. The potentiometric titrations were performed on the Kunz saponification mixture by adding an excess of standard acid and back-titrating with standard alkali, using the quinhydrone electrode. The analytical results are expressed as cc. of 0.1 *N* sodium hydroxide per 100 mg. of substance.

TABLE I

ANALYSES OF ACETYLATED SUGAR AMINO CONDENSATION PRODUCTS FOR TOTAL ACETYL AND O-ACETYL

	Cc. of 0.1 *N* NaOH per 100 mg.			
	Total acetyl		O-Acetyl	
Substance	Calcd.	Found Freudenberg method	Calcd.	Found Kunz method
Acetanilide	7.40	7.2	0	−0.15
Acetanilide (44.4%) + *d*-mannitol hexaacetate (55.6%)	10.97	10.82	7.68	7.5
Aldehydo-d-glucose oxime hexaacetate	13.4	13.5	13.4	13.5
β-d-Glucose oxime hexaacetate	13.4	13.3	11.2	11.2
Aldehydo-d-galactose oxime hexaacetate	13.4	13.2	13.4	13.4
β-d-Galactose oxime hexaacetate	13.4	13.4	11.2	11.3
Aldehydo-d-glucose semicarbazone pentaacetate	11.2	11.3	11.2	11.4
d-Glucose semicarbazone pentaacetate (ring form)	11.2	11.3	8.95	8.9
Aldehydo - d - galactose methylphenyl - hydrazone pentaacetate	10.1	10.2	10.1	10.2
Aldehydo-d-glucose phenylosazone tetraacetate	7.6	7.6	7.6	7.6[a]
Aldehydo-d-galactose phenylosazone tetraacetate	7.6	7.5	7.6	7.1[a]

[a] Potentiometric titrations.

Summary

1. A procedure for the estimation of O-acetyl and N-acetyl is reported.

2. The ring structures previously assigned to various acetylated sugar amino condensation products have been verified.

3. A crystalline tetraacetate of *d*-galactose phenylosazone has been synthesized.

4. The phenylosazone tetraacetates of *d*-glucose and *d*-galactose have been shown to possess open chain structures.

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