The mixture of anomers was treated with acetic anhydride in pyridine in the usual manner. Two components were revealed by thin layer chromatography on silica gel G with ethyl acetate development. The slower moving component migrated at the same rate (R_f 0.47) as 2-*O*-(2-acetamidotri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-1,3-di-*O*-acetylglycerol (VIII), and the other component migrated at the same rate (R_f 0.53) as the naturally derived product VI. The latter component was isolated by preparative thin layer chromatography performed in the same manner and obtained in crystalline form from ethanol–petroleum ether (bp 30–60°); mp 123.5°, undepressed on admixture with crystalline, naturally derived VI; $[\alpha]^{20}\mathrm{D}$ +60° (*c* 1.99, chloroform); infrared and nmr spectra and X-ray powder diffraction pattern identical with those of crystalline, naturally derived VI.

Anal. Calcd for $C_{21}H_{31}NO_{13}$: C, 49.89; H, 6.17; N, 2.77. Found: C, 50.07; H, 5.93; N, 2.86.

Acknowledgment.— The authors thank the National Science Foundation for financial support of this work (Grants G13967 and GB1685). We also acknowledge the counsel of D. Horton and J. R. Vercelloti, and the assistance of the latter in the preparation of material.

The Configuration and Formation of Percival Dianhydroosazone[1a]

H. El Khadem and M. M. A. Abdel Rahman

Chemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt, United Arab Republic

Received May 18, 1965

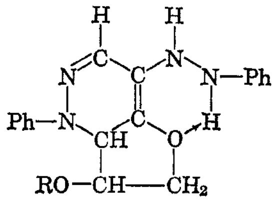
The nuclear magnetic resonance spectrum of Percival dianhydroosazone acetate agreed with structure III which possesses one imino proton and a *trans* arrangement between H_c and H_d and between H_d and H_e. Since the configuration of H_e is known, it was possible to determine the configuration of H_c and H_d and show that Percival osazone was a dianhydro-D-*lyxo* derivative. Dianhydro-D-*lyxo*-hexulose *o*-, *m*-, and *p*-tolylosazones and *p*-methoxyphenylosazone were also prepared from tetra-*O*-acetyl-D-*arabino*-hexulose arylosazones, but *N*-acetylated osazones and acetates of 3,6-anhydroosazones failed to give dianhydro derivatives.

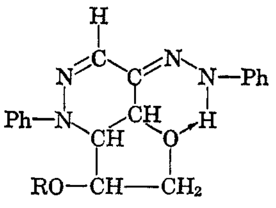
Nuclear magnetic resonance spectroscopy has recently[1-4] been used to establish the structure of osazones. We have now used this technique to prove the structure of Percival dianhydroosazone and to establish its configuration.

Percival dianhydroosazone[5] is formed by deacetylation of hexose phenylosazone tetraacetates with sodium hydroxide in aqueous acetone. It exists in two enantiomorphic forms depending upon the D and L configuration of the starting osazone. Thus, saccharides having different configuration on C-3 and C-4, such as D-glucose, D-galactose, and D-gulose, give the same dianhydro compound, and its enantiomer is obtained from the L sugars. It is therefore believed[5] that an inversion of configuration at C-3 and C-4 takes place in certain cases to assume a stable configuration and that only the asymmetric carbon atom at C-5 in the osazone retains its configuration during anhydride formation.

Percival dianhydroosazone gives a monoacetate and a monomethyl ether and upon treatment with nitrous acid loses only one hydrazone residue, suggesting that the other hydrazone residue is involved in anhydro-ring formation. This, and the fact that the anhydroosazone fails to give the formazan reaction, led Henseke[6] to suggest the tautomeric structures Ia and IIa for this anhydroosazone.

It is obvious that the nmr spectrum of structure I would reveal two imino protons, whereas that of structure II would reveal only one imino proton, thus affording a simple means of distinguishing between the two tautomeric forms. Our nmr study of Percival dianhydroosazone acetate (Figure 1) showed only one imino proton at δ 9.53 ppm, thus favoring tautomeric form II. The rest of the nmr spectrum was also in agreement with the carbon–hydrogen skeleton of IIb. The doublet at δ 5.08 ppm was assigned to the methine proton of C-3 of the osazone precursor, which is linked to the etheric oxygen; the coupling constant of this proton to the proton whose resonance appeared at δ 4.65 ppm and which was assigned to the C-4 proton of the osazone precursor was relatively large (J = 8 cps). This large coupling was explained by an axial–axial *trans* relationship between the C-3 and C-4 protons. The latter proton, appearing at δ 4.65 ppm, was split by a large coupling of J = 8 cps by the *trans* proton of C-3, and with a moderately large coupling of J = 6 cps by the proton at about δ 5.65 ppm, assigned to the methine proton of C-5 bearing the *O*-acetyl group. The moderate size coupling of J = 6 cps was also ascribed to a *trans* arrangement of the protons of C-4 and C-5, but probably quasi-axial–axial. The proton of C-5, appearing at δ 5.65 ppm, was split with a coupling constant of J = 6 cps by the *trans* proton of C-4 and the *trans* proton of the C-6 methene, and a small coupling of J = 3 cps by the *cis* proton at C-6. The geminal protons of the methene group at C-6 of the osazone precursor appeared at δ 4.3 and 3.7 ppm, both being split by a large geminal coupling of J = 11 cps. The *trans* proton at δ 4.3 ppm was split by the


I


II

a, R=H
b, R=CH_3CO

[1a] Note Added in Proof.—Preliminay communication: H. El Khadem and M. M. A. Abdel Rahman, *Tetrahedron Letters*, **No. 6**, 579 (1966).

(1) M. L. Wolfrom, G. Fraenkel, D. R. Lineback, and F. Komitsky, Jr., *J. Org. Chem.*, **29**, 457 (1964).

(2) O. L. Chapman, R. W. King, W. J. Welstead, and T. J. Murphy, *J. Am. Chem. Soc.*, **86**, 4968 (1964).

(3) L. Mester, E. Moczar, and J. Parello, *Tetrahedron Letters*, **44**, 3223 (1964); *J. Am. Chem. Soc.*, **87**, 596 (1965).

(4) H. El Khadem, M. L. Wolfrom, and D. Horton, *J. Org. Chem.*, **30**, 838 (1965).

(5) E. G. Percival, *J. Chem. Soc.*, 1770 (1936); 1384 (1938).

(6) G. Henseke, U. Müller, and G. Badicke, *Chem. Ber.*, **91**, 2270 (1958).

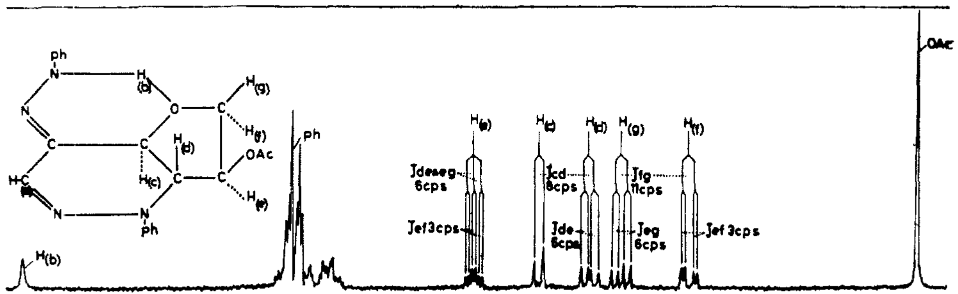
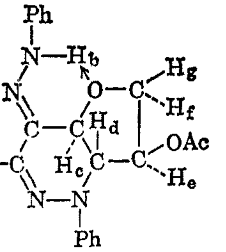


Figure 1.—Nuclear magnetic resonance spectrum of Percival dianhydroosazone acetate in deuteriochloroform.

C-5 proton with a spin-spin coupling constant of J = 6 cps and the *cis* methene proton at δ 3.7 ppm with a coupling constant of J = 3 cps.

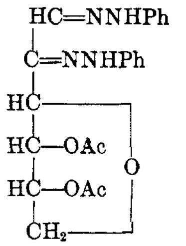
From the above, it is seen that we have in Percival dianhydroosazone a *trans* arrangement between the protons of C-3 and C-4 and a *trans* arrangement between those of C-4 and C-5. Since it is known that Percival dianhydroosazones possess the same configuration at C-5 as the starting osazone, we can now determine the configuration of C-3 and C-4. Thus, if we represent the dianhydroosazone sideways with the OAc group above the plane of the rings (D configuration) and the C-5 hydrogen below the plane of the rings, then the proton at C-4 will be above the rings and that at C-3 below the plane of the rings (III). This arrangement when represented in the Fischer projection formula with the C–C bonds below the plane and the hydrogen and oxygen (or nitrogen) above it would correspond to that of a dianhydro-D-*lyxo*-hexulose phenylosazone (galactose derivative). It seems that the osazones from D-glucose and D-gulose undergo inversion during anhydride formation to acquire the configuration of this galactose derivative.



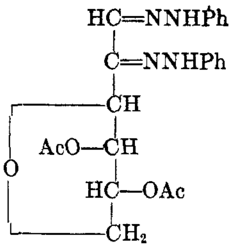
III

The mass spectrum of Percival dianhydroosazone acetate indicated a molecular weight of 364, in accordance with the molecular formula $C_{20}H_{20}N_4O_3$, and showed a strong M − 103 peak corresponding to the loss of PhN, also in agreement with structure III.

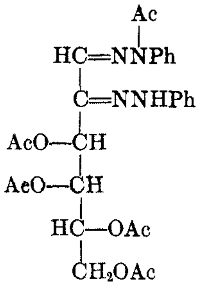
We have also found that osazone acetates, such as IV and V, having a 3,6-anhydro ring did not give Percival dianhydroosazone, irrespective of the configuration of their 3,6-anhydro ring; they were merely hydrolyzed to the starting 3,6-anhydroosazone. Similarly, N-acetylosazones, such as N-acetyltetra-O-acetyl-D-*lyxo*-hexulose phenylosazone (VI), failed to give a dianhydroosazone, probably because the difficultly hydrolyzable N-acetyl group delayed the anhydride formation between the hydrazone residue and the hydroxyl group of C-4, which must apparently precede the formation of 3,6-anhydro ring. This would explain the failure of 3,6-anhydroosazone acetates to give dianhydroosazones.



IV



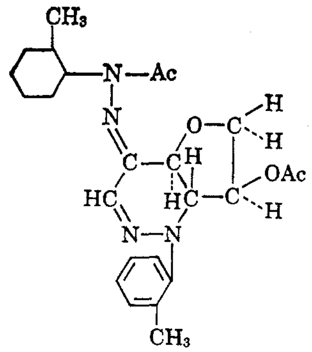
V



VI

We have also prepared a number of tetra-O-acetyl-D-*arabino*-hexulose arylosazones substituted in the phenyl ring with electron-releasing groups, such as *o*-, *m*-, and *p*-CH_3 and *p*-OCH_3, and electron-attracting groups, such as *p*-COOH and *p*-NO_2, and found that dianhydroosazones could readily be obtained from the former osazone acetates but not the latter. Upon acetylation of dianhydro-D-hexulose *o*-tolylosazone with pyridine and acetic anhydride we isolated a crystalline diacetate (VII) which showed two carbonyl bands, one ester band at 1730 cm^{-1} due to the O-acetate and an amide band at 1690 cm^{-1} due to the N-acetate. The isolation of this compound affords a further proof to Henseke's finding that one hydrazone residue is involved in anhydro-ring formation, while the other is only chelated.

We have ascribed to the substituted dianhydroosazones a *lyxo* configuration like that of Percival dianhydroosazone.



VII

Experimental Section

The nmr spectrum of Percival dianhydroosazone acetate was carried out on a Varian HA-100 spectrometer in deuteriochloroform, using tetramethylsilane as an internal standard. Infrared absorption spectra were measured on a Unicam SP-200 spectrophotometer.

Tetra-*O*-acetyl-D-*arabino*-hexulose *o*-Tolylosazone.—D-*arabino*-Hexulose *o*-tolylosazone (2 g) was treated with pyridine (15 ml) and acetic anhydride (10 ml), and the mixture was kept overnight at room temperature. It was then poured onto crushed ice (200 g) and the product was washed with water and crystallized from dilute ethanol as orange prismatic plates, mp 138–140°. These were soluble in acetone, ethanol, and methanol; ν_{max}^{KBr} 1740 (OAc) and 1595 (C=N) cm^{-1}. *Anal.* Calcd for $C_{28}H_{34}N_4O_8$: N, 10.5. Found: N, 10.4.

Dianhydro-D-*lyxo*-hexulose *o*-Tolylosazone.—Tetra-*O*-acetyl-D-*arabino*-hexulose *o*-tolylosazone (0.5 g) dissolved in acetone (30 ml) was mixed with 1.5% sodium hydroxide solution (35 ml) at room temperature. After 1 hr dianhydro-D-*lyxo*-hexulose *o*-tolylosazone separated. It was filtered and crystallized from ethanol as pale yellow needles, mp 208–210°, soluble in ethanol and methanol; ν_{max}^{KBr} 3400 (OH) and 1600 (C=N) cm^{-1}. *Anal.* Calcd for $C_{20}H_{22}N_4O_2$: C, 68.6; H, 6.3; N, 16.0. Found: C, 68.1; H, 6.7; N, 15.9.

***N*-Acetylmono-*O*-acetyldianhydro-D-*lyxo*-hexulose *o*-Tolylosazone.**—A solution of dianhydro-D-*lyxo*-hexulose *o*-tolylosazone (0.2 g) in pyridine (5 ml) was treated with acetic anhydride (5 ml) and kept overnight at room temperature. It was then poured onto crushed ice (100 g), and the solidified product was washed repeatedly with water and crystallized from dilute ethanol as orange prismatic plates, mp 215–216°. These were soluble in acetone, ethanol, and methanol; ν_{max}^{KBr} 1730 (OAc), 1690 (NAc), and 1630 (C=N) cm^{-1}. *Anal.* Calcd for $C_{24}H_{26}N_4O_4$: C, 66.4; H, 6.0; N, 12.9. Found: C, 66.2; H, 6.0; N, 13.0.

Dianhydro-D-*lyxo*-hexulose *m*-Tolylosazone.—D-*arabino*-Hexulose *m*-tolylosazone (1 g) in pyridine (10 ml) was treated with acetic anhydride (10 ml) and kept overnight at room temperature. It was then poured onto crushed ice, and the solidified acetate was filtered and washed thoroughly with water (0.8 g). It was dissolved in acetone (35 ml) and deacetylated with 1.5% sodium hydroxide (30 ml). The dianhydro derivative which separated crystallized from ethanol as yellow prismatic plates, mp 204–208°. These were soluble in ethanol and methanol; ν_{max}^{KBr} 3400 (OH) and 1600 (C=N) cm^{-1}. *Anal.* Calcd for $C_{20}H_{22}N_4O_2$: C, 68.6; H, 6.3; N, 16.0. Found: C, 68.9; H, 6.4; N, 16.0.

Tetra-*O*-acetyl-D-*arabino*-hexulose *p*-Tolylosazone.—D-*arabino* Hexulose *p*-tolylosazone (2 g) in pyridine (15 ml) was treated with acetic anhydride (10 ml) as usual, and the product was crystallized from dilute ethanol as yellow prismatic needles, mp 153–154°, soluble in acetone, ethanol, and methanol; ν_{max}^{KBr} 1725 (OAc) and 1605 (C=N) cm^{-1}. *Anal.* Calcd for $C_{28}H_{34}N_4O_8$: N, 10.5. Found: N, 10.7.

Dianhydro-D-*lyxo*-hexulose *p*-Tolylosazone.—Tetra-*O*-acetyl-D-*arabino*-hexulose *p*-tolylosazone (0.4 g) was dissolved in acetone (30 ml) and treated with 1.5% sodium hydroxide (35 ml) at room temperature. After 2 hr dianhydro-D-*lyxo*-hexulose *p*-tolylosazone was separated, filtered, and crystallized from ethanol as yellow needles, mp 265–266°. It was soluble in ethanol and methanol; ν_{max}^{KBr} 3490 (OH) and 1605 (C=N) cm^{-1}. *Anal.* Calcd for $C_{20}H_{22}N_4O_2$: C, 68.6; H, 6.3; N 16.0. Found: C, 68.4; H, 6.4; N, 15.8.

Dianhydro-D-*lyxo*-hexulose *p*-Methoxyphenylosazone.—D-*arabino*-Hexulose *p*-methoxyphenylosazone (1 g) was acetylated with pyridine (10 ml) and acetic anhyride (10 ml) overnight and then poured onto crushed ice. The amorphous acetate was filtered, washed with water, and dried (0.8 g), then dissolved in acetone (30 ml) and deacetylated as before. Dianhydro-D-*lyxo*-hexulose *p*-methoxyphenylosazone which separated was crystallized from ethanol as yellow prismatic needles, mp 241–243° dec, soluble in ethanol and methanol; ν_{max}^{KBr} 3500 (OH) and 1595 (C=N) cm^{-1}. *Anal.* Calcd for $C_{20}H_{22}N_4O_4$: C, 62.8; H, 5.8; N, 14.7. Found: C, 63.0; H, 6.0; N, 14.6.

Deacetylation of 3,6-Anhydro-D-*ribo*-hexulose Phenylosazone Acetates.—4,5-Di-*O*-acetyl-3,6-anhydro-D-*ribo*-hexulose phenylosazone[7] (0.3 g) or *N*-acetyl-4,5-di-*O*-acetyl-D-*ribo*-hexulose phenylosazone[8] (0.3 g) in acetone (20 ml) was deacetylated with 1.5% sodium hydroxide (40 ml). The solution upon standing overnight was neutralized with a stream of CO_2, whereby 3,6-anhydro-D-*ribo*-hexulose phenylosazone separated. It was identified after crystallization from dilute ethanol by comparison with an authentic specimen; both showed identical melting point and mixture melting point (156°), as well as identical infrared spectra.

***N*-Acetyl-4,5-di-*O*-acetyl-3,6-anhydro-D-*lyxo*-hexulose Phenylosazone. A. With Boiling Acetic Anhydride.**—3,6-Anhydro-D-*lyxo*-hexulose phenylosazone (2 g), prepared from D-*lyxo*-hexulose phenylosazone and methanolic sulfuric acid,[7] was refluxed with acetic anhydride (30 ml) for 1 hr and then poured onto crushed ice (500 g). The crystalline acetate was filtered, washed with water, and crystallized from ethanol as yellow plates, mp 173–175°, soluble in acetone, ethanol, and methanol; ν_{max}^{KBr} 1730 (OAc), 1690 (NAc), and 1600 (C=N) cm^{-1}. *Anal.* Calcd for $C_{24}H_{26}N_4O_6$: C, 61.80 H, 5.6; N, 12.0. Found: C, 62.0; H, 5.9, N, 12.5.

B. With Acetyl Chloride.—The same product was obtained by treating 2 g of 3,6-anhydro-D-*lyxo*-hexulose phenylosazone with dimethylaniline (20 ml), followed by acetyl chloride (10 ml) at room temperature. The mixture was kept overnight, then poured onto crushed ice, and the acetate was crystallized from ethanol; yield 1 g, mp 173–175°.

Deacetylation of 3,6-Anhydro-D-*lyxo*-hexulose Phenylosazone Acetates.—When either 4,5-di-*O*-acetyl-3,6-anhydro-D-*lyxo*-hexulose phenylosazone[7] (0.2 g) or *N*-acetyl-4,5-di-*O*-acetyl-3,6-anhydro-D-*lyxo*-hexulose phenylosazone (0.2 g) in acetone (20 ml) was deacetylated with sodium hydroxide and the solution kept overnight at room temperature, then neutralized with a stream of CO_2; 3,6-anhydro-D-*lyxo*-hexulose phenylosazone separated. This compound was identified after crystallization from dilute ethanol by comparison with an authentic specimen; both showed identical melting point and mixture melting point (155–158°), as well as identical infrared spectra.

Tetra-*O*-acetyl-D-*arabino*-hexulose *p*-Carboxyphenylosazone.—A solution of D-*arabino*-hexulose *p*-carboxyphenylosazone (2 g) in pyridine (15 ml) was treated with acetic anhydride (10 ml) and the mixture kept overnight at room temperature. It was then poured onto crushed ice (200 g) and the acetate was filtered, washed with water, and crystallized from dilute ethanol as yellow prismatic plates, mp 215–217° dec, soluble in acetone, ethanol, and methanol; ν_{max}^{KBr} 1740 (OAc) and 1605 (C=N) cm^{-1}. *Anal.* Calcd for $C_{28}H_{30}N_4O_{12}$: C, 54.7; H, 4.9; N, 9.1. Found: C, 54.8; H, 5.3; N, 9.4.

Deacetylation of tetra-*O*-acetyl-D-*arabino*-hexulose *p*-carboxyphenylosazone and *N*-acetyltetra-*O*-acetyl-D-*lyxo*-hexulose phenylosazone[9] as well as the amorphous tetra-*O*-acetyl-D-*arabino*-hexulose *p*-nitrophenylosazone in acetone using 1.5% sodium hydroxide in the usual manner resulted in amorphous products.

Acknowledgment.—The authors wish to express their thanks to Professor M. L. Wolfrom, The Ohio State University, Columbus, Ohio, for the nmr and mass spectra.

(7) O. Diels and R. Mayer, *Ann.*, **519**, 157 (1935).

(8) H. El Khadem, Z. M. El-Shafei, and M. M. Mohammed-Ali, *J. Org. Chem.*, **29**, 1565 (1964).

(9) H. El Khadem, Z. M. El-Shafei, and M. M. A. A. Rahman, *Carbohydrate Res.*, **1**, 31 (1965).