ANHYDRO-OSAZONES: MASS SPECTRA, AND DIRECT FORMATION FROM GLYCOSULOSES AND HYDRAZINES

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(Received February 19th, 1973; accepted in revised form, April 28th, 1973)

ABSTRACT

The mass spectra of Diels 3,6-monoanhydro-osazones, Percival dianhydroosazones, and our own type of dianhydro-osazones show markedly different fragmentation patterns. This behavior was used to identify 3,6-anhydro-D-arabinohexosulose 1-(phenylhydrazone) 2-(methylhydrazone) (2) in the reaction products of D-arabino-hexosulose 1-(phenylhydrazone) with methylhydrazine, and a dianhydroosazone (4) of the Percival type among the products of reaction of D-arabinohexosulose with 2-hydrazinopyridine. The anhydro-osazones were separated from the nondehydrated osazones by chromatography. N.m.r.-spectral and c.d. studies revealed that, owing to the small size of the methyl group, compound 2 retained the D-arabino configuration during cyclization, and is thus the first anhydro-osazone of the Diels type that has the two bulky, hydrazone residues and the 4-hydroxyl group on the same side of the anhydro ring.

INTRODUCTION

Anhydro-osazones having 3,6-anhydro rings have recently attracted interest¹ as intermediates in the synthesis of C-nucleosides, which likewise possess fivemembered, anhydro rings. The anhydro-osazones can be cyclized to substituted triazoles and pyrazoles directly, or into a multitude of other heterocyclic rings² after conversion into the α -dioxo derivatives, the 3,6-anhydroglycos-2-uloses.

Three types of anhydro-osazone³ are known at present; these are (a) Diels 3,6-anhydro-osazones⁴⁻¹⁰ prepared by dehydrating osazones with methanolic sulfuric acid, (b) Percival dianhydro-osazones⁹⁻¹³ obtained by deacetylating osazone acetates, and (c) dianhydro-osazones of the pyrazole type¹⁴⁻¹⁶, first obtained by one of us by using boiling acetic anhydride as the dehydrating agent. It should be noted that elemental analysis does not necessarily distinguish between these compounds, because some anhydro-osazone crystallize with water of crystallization, and a hydrated monoanhydro-osazone, and a dianhydro-osazone might be mistaken for a hydrated monoanhydro-osazone.

In the present work, we have obtained mono- and di-anhydro-osazones in one *On leave from Faculty of Science, Alexandria University. step by treating glycos-2-uloses with alkyl or heterocyclic hydrazines. These anhydroosazones were, in some cases, the sole products obtained from the reaction and, in others, were accompanied by the nondehydrated bis-hydrazones. Their structures were established by comparing their mass spectra with those of authentic Diels anhydro-osazones, Percival dianhydro-osazones, and our dianhydro-osazones.

DISCUSSION

In order to use mass spectrometry to identify the anhydro-osazones obtained by treating glycos-2-uloses with alkylhydrazines and with heterocyclic hydrazines, it was necessary to study the mass spectra of representatives of each of the known types of anhydro-osazone. Mass spectrometry proved quite useful, not only in providing the molecular weight of these compounds (and thus distinguishing the mono- from the



Fig. 1. Mass spectra of Diels monoanhydro-osazones. Upper spectrum, 3,6-anhydro-*D-manno*-heptulose phenylosazone; middle, 3,6-anhydro-*D-ribo*-hexulose phenylosazone; lower, 3,6-anhydro-*D-arabino*-hexosulose 1-(phenylhydrazone) 2-(methylhydrazone) (2).

di-anhydro-osazones) but also in affording a reliable means of differentiating between the various types of anhydro-osazones, as they showed different fragmentation patterns.

The following is a discussion of the mass spectra of representatives of the different types of anhydro-osazone.

Mass spectra of monoanhydro-osazones of the Diels type. — Two examples of Diels 3,6-anhydro-osazones, a hexose derivative⁴ and a heptose derivative⁹, were investigated by mass spectrometry (see Fig. 1). In both compounds, the molecular peak was the base peak, followed by a strong peak corresponding to $M^{\ddagger} - H_2O$, and a group of peaks, which included two strong ones, corresponding to the loss of Ph and Ph+2. Following this was a series of peaks at 249–250, corresponding to $M^{\ddagger} - PhNH_2$ and $M^{\ddagger} - PhNH$. In the heptose derivative, these peaks were followed by a group of peaks of similar intensity, centered at 249 and corresponding to the loss of the hydroxymethyl group. Farther down, we found peaks present in the spectra of the hexose and heptose 3,6-anhydro-osazones which included peaks at m/e 188, 174, 158, and 144. These were followed by peaks common to all osazones and corre-



Fig. 2. Mass spectra of Percival dianhydro-osazones. Upper spectrum, dianhydrohexulose phenylosazone; middle, dianhydroheptulose phenylosazone; lower, dianhydrohexulose 2-pyridylosazone.

sponding to HNCPh (104) and CNPh (103), and a series of peaks at m/e 93, 92, and 91, corresponding to PhNH₂, PhNH, and PhN, followed by the phenyl (77) and the cyclopentadienyl ions (65).

Dianhydro-osazones of the Percival type. — Two examples of this type of dianhydro-osazone have been examined by mass spectrometry, the first belonging to the hexose series¹¹, and the second to the heptose series⁹ (see Fig. 2). Both compounds were characterized by a strong molecular peak, which was the base peak for the hexose derivative and the second largest peak for the heptose derivative. The latter derivative also showed a peak, corresponding to the loss of CH_2O , located at exactly the same position as the molecular peak of the hexose derivative. From there on, the two mass spectra were practically identical. They showed a moderate peak at m/e 277, and a strong peak at m/e 262; this fragment is the second strongest peak for the hexose derivative and the base peak for the heptose derivative. This was followed by peaks at m/e 173 and 157, and at lower mass, we observed a rearrangement peak at m/e 103 and 104 corresponding to PhNC and PhNCH, and then a series of peaks at 92 and 91 corresponding to PhNH and PhN, followed by a phenyl peak (77) and the cyclopentadiene ion (65).

Dianhydro-osazones of the pyrazole type. — Two examples of these osazones have been studied (see Fig. 3), one belonging to the tetrose series (3-formyl-1-phenyl-



Fig. 3. Mass spectra of El Khadem anhydro-osazones. Upper spectrum, hexose derivative; lower spectrum, tetrose derivative.

pyrazole 2-acetyl-2-phenylhydrazone) and the other to the hexose series [the 5-(1,2dihydroxyethyl) derivativel^{14,15}. As these compounds are obtained by the partial deacetvlation of osazones dehvdrated with boiling acetic anhydride, they retain one N-acetyl group, and their molecular peaks are usually the second largest peaks after the M^{\ddagger} – CH₂CO peak, which is the base peak. It seems that the acetyl group in these compounds undergoes elimination by a McLafferty type of rearrangement, whereby the nitrogen atom adjacent to the formyl group is protonated, giving rise to an M^{+} -42 peak (instead of an M^{+} -43 peak). The hexose derivative showed a series of peaks, at 313, 303, 292, 262; the latter corresponds to the loss of the hydroxyalkyl group. From then on, the two spectra became identical showing peaks at 233, 208, 170, 157, and 143. At m/e 135, a peak corresponding to PhNHAc was observed. followed by one at m/e 130 corresponding to a methylenephenylazirine, and a 117 fragment assigned to the phenyldiazirine expected from five-membered. N-heterocycles. Like the other anhydro-osazones, these compounds showed peaks at 104 and 103, corresponding to PhNCH and PhNC, and a series of peaks at 91, 92, and 93, corresponding to PhN, PhNH, and PhNH₂, followed by the phenyl (77) and cyclopentadienvl (65) peaks.

Direct formation of anhydro-osazones from glycosuloses and hydrazines. — A. Monoanhydro-osazones. When D-arabino-hexosulose 1-(phenylhydrazone) (D glucosone 1-phenylhydrazone) was treated with methylhydrazine in the cold, the expected mixed osazone, namely, D-arabino-hexosulose 1-(phenylhydrazone) 2-(methylhydrazone) (1), was obtained. However, when the reaction mixture was heated we obtained a monoanhydro-osazone of the Diels type, which proved to be 3,6-anhydro-D-arabino-hexosulose 1-(phenylhydrazone) 2-(methylhydrazone); this compound (2) is of special interest, as it is the first example of a Diels 3,6-anhydro-osazone having the D-arabino configuration. For instance, it is known⁶ that, on dehydration, D-arabino-hexulose phenylosazone gives the 3,6-anhydro-D-ribo-hexulose derivative. The new 3,6-anhydro-D-arabino-hexulose osazone 2 may, therefore, be used to prepare C-nucleosides having the α -D configuration, as its two hydrazone residues are (and, accordingly, the base derived from them would be) below the plane of the furanoid ring, as depicted in formula 2.



The structure of 3,6-anhydro-D-*arabino*-hexosulose 1-(phenylhydrazone) 2-(methylhydrazone) was determined in the following way. (a) The results of elemental analysis agreed with the values calculated for a monoanhydro-osazone ($C_{13}H_{18}N_4O_3$). (b) Its mass spectrum (see Fig. 1) showed a molecular peak at 278. This peak was followed by an $M^{\ddagger} - H_2O$ peak, similar to that of other Diels anhydro-osazones. At m/e 217, it showed a peak, corresponding to CH_3NN , which is not present in the spectra of the phenyl derivatives; but, at lower mass, the pattern of fragmentation was quite similar to that of the other Diels osazones studied. Thus, it showed $M^{\ddagger}-Ph+2$, $M^{\ddagger}-PhN$, and $M^{\ddagger}-Ph$ peaks followed by peaks at 174, 158, 144, and 119 which were identical with those of the other Diels osazone.

(c) It was now necessary to determine the configuration at C-3, as this center was, in certain cases, found to undergo inversion during the formation of the anhydro ring (as with the phenylosazones). The two, bulky, phenylhydrazone groups tend to assume a sterically favorable structure having the two hydrazone residues on the opposite side of the ring with respect to the adjacent, 4-hydroxyl group. We studied the 100-MHz, n.m.r. spectrum of the new anhydro-osazone. The spectrum (see Fig. 4)



Fig. 4. N.m.r. spectrum of 3,6-anhydro-D-*ribo*-hexosulose 1-(phenylhydrazone) 2-(methylhydrazone).

showed a methyl peak, at δ 3.18, which, at high resolution, could be resolved into two peaks of almost equal intensity, assigned to the *N*-methyl group split by the adjacent NH by a very small coupling-constant (0.03 Hz). This was followed at lower field by the ABX system of the methylene group at C-6, which appeared as two quadruplets having coupling constants of J4 and J 5.5, respectively, with a geminal coupling of 10 Hz. The protons at C-4 and C-5 appeared as a multiplet, of two-proton intensity, at $\delta \sim 4.5$ -4.7. At δ 5.1, the proton attached to C-3 appeared as a doublet having a coupling constant of J 6.7. This large coupling-constant for a five-membered ring is indicative of a *cis* arrangement between the protons on C-3 and C-4, and is in agreement with the D-*arabino* configuration. Thus, the 3,6-anhydro-osazone was, presumably, formed without occurrence of inversion at C-3; this is remarkable, because (as mentioned earlier), in order to assume a more favorable structure, D-*arabino*-hexulose phenylosazone undergoes inversion to give the D-*ribo* derivative. It would appear that the methyl group in the 2-(methylhydrazone) is not sufficiently bulky to cause the molecule to assume the *trans* configuration and that, therefore, the original configuration was retained. The rest of the n.m.r. spectrum showed the hydroxyl groups at δ 6.5–7.1, the phenyl group at δ 7.3–7.6, and two NH groups at δ 10.0 and 10.65 p.p.m.

(d) Further proof that the molecule had retained the D-arabino configuration was provided by its circular dichroism (c.d.) curve (see Fig. 5). The c.d. curves of anhydro-osazones having various configurations have been studied in detail¹⁷, and



Fig. 5. C.d. spectrum of 3,6-anhydro-D-ribo-hexosulose 1-(phenylhydrazone) 2-(methylhydrazone).

it was found that the sign of the Cotton effect depends upon the configuration of the asymmetric center adjacent to the chromophore (C-3); osazones having a hydroxyl group or the oxygen atom of the furan ring to the left in the Fischer projection formula show a negative Cotton effect, whereas those having the hydroxyl group or the oxygen atom of the ring to the right in the Fischer projection formula show a positive Cotton effect. The c.d. curve of our anhydro-osazone showed a negative Cotton effect similar to that of osazones having the hydroxyl group or the oxygen atom of the furan ring involving C-3 to the left in the Fischer projection formula, suggesting that the D-arabino configuration had been retained. The only difference between the c.d. curve of this compound and that of the phenyl compounds studied earlier is that the higher-wavelength absorption appeared at ~ 320 nm for the mixed methyl-phenylosazone as compared to 390 nm for the arylosazones. The hypsochromic shift was to be expected, as, in our compound, a phenyl group of the latter has been replaced by a methyl group.

From these results, it was concluded that the compound (2) that we have obtained is 3,6-anhydro-D-arabino-hexosulose 1-(phenylhydrazone) 2-(methyl-hydrazone); this compound is of special interest, as it is the only anhydro-osazone of the Diels type so far described that can yield a glycosulose capable of cyclization into a *C*-nucleoside having the α -D anomeric configuration.

B. Dianhydro-osazones. When D-arabino-hexosulose (D-glucosone) was treated with 2-hydrazinopyridine in the usual way, it afforded a mixture of D-arabinohexulose 2-pyridylosazone (3) and its dianhydro derivative (4). The mixture was separated by chromatography, and the structure of the latter compound (4) was elucidated by comparing its mass spectrum with that of the known anhydro-osazones. The mass spectrum (see Fig. 2) was quite similar to that of dianhydro-osazones of the Percival type. It showed a strong molecular peak at 324, two mass units higher than for the Percival dianhydro-osazones cf the hexose derivative (322). This was followed by a peak at 279, two units higher than corresponding peaks of the phenyl derivative (277), and a 264 peak, again two units higher than the 262 peak. Most of the remaining peaks had their corresponding peaks for the phenyl derivatives two mass units higher in the case of fragments having two rings, and one mass unit higher for those having one ring. This, together with the similarity of their u.v. spectra, led us to consider the bis-hydrazone to be a dianhydro-osazone of the Percival type, and to assign structure 4 to it.

2-Hydrazinoquinoline yielded with glycosuloses the corresponding 2-quinolylosazone (5); however, no crystalline anhydro derivative could be isolated. It seems that partial dehydration during osazone formation may not be uncommon, but that higher solubility and difficult crystallizability make the separation of anhydroosazones from the reaction mixture difficult. It would be interesting to verify this hypothesis by searching for anhydro-osazones in the reaction mixtures of phenyl-(and other substituted)osazones, using chromatography for detection of the anhydro derivatives, if any.



EXPERIMENTAL

N.m.r. spectra were recorded with a Varian HA-100 spectrometer, and mass spectra with a Varian M-60 instrument. I.r. spectra for KBr pellets were recorded with a Perkin–Elmer 621 spectrophotometer. Microanalyses were made by W. Spang, Ann Arbor, Michigan; melting points were measured on a Kofler block, and are uncorrected.

D-arabino-Hexosulose 1-(phenylhydrazone) 2-(methylhydrazone) (1). — Darabino-Hexosulose 1-(phenylhydrazone) was prepared by boiling a suspension of D-glucose phenylosazone (20 g) in ethanol (600 ml), water (1 liter), glacial acetic acid (12 ml), and benzaldehyde (32 ml) for 2 h under reflux. Benzaldehyde phenylhydrazone was removed by filtration, and the filtrate was washed with ether and evaporated to a thick syrup which crystallized on prolonged keeping in a refrigerator. Treatment of the glycosulose phenylhydrazone (2 g) with methylhydrazine (1.5 g) at room temperature yielded the mixed osazone 1 (1.1 g) as faint-yellow needles, m.p. 165°.

Anal. Calc. for C₁₃H₂₀N₄O₄: C, 52.69; H, 6.80; N, 18.91. Found: C, 52.53; H, 6.72; N, 19.25.

3,6-Anhydro-D-arabino-hexosulose 1-(phenylhydrazone) 2-(methylhydrazone) (2). — The syrupy glycosulose phenylhydrazone just described (6 g) was treated with methylhydrazine (5 ml), water (20 ml), and a few drops of acetic acid, and heated for 15 min on a boiling-water bath. 3,6-Anhydro-D-arabino-hexosulose 1-(phenylhydrazone) 2-(methylhydrazone) (0.6 g) separated as needles, m.p. 190–192°; v_{max}^{KBr} 1595 (C=N) and 3325 cm⁻¹ (OH); n.m.r. data (100 MHz; pyridine- d_6): δ 3.16 (Me), 4.05 (quadruplet, H-6a, $J_{5,6a}$ 4.0, $J_{6a,6b}$ 10.0), 4.35 (H-6b, $J_{5,6b}$ 5.5), 4.5–4.8 (H-4,5), 5.1 (H-3, $J_{4,3}$ 6.7), 6.7–7.2 (OH), 7.3–7.5 (Ph), 9.9–10.1 (NH), and 10.75 (NH).

Anal. Calc. for C₁₃H₁₈N₄O₃: C, 56.10; H, 6.52; N, 20.13. Found: C, 56.06; H, 6.40; N, 20.3.

D-arabino-Hexulose 2-pyridylosazone (3) and its dianhydro derivative (4). — A solution of D-arabino-hexosulose (D-glucosone) (2 g) and 2-hydrazinopyridine (1.4 g) in 1:1 ethanol-water (30 ml) and a few drops of acetic acid was heated gently on a hot-water bath for a few minutes, and allowed to cool overnight. The semi-crystalline mass was filtered, and the solid was separated into its constituents by preparative, thin-layer chromatography on silica gel plates with 1:1 ether-ethyl acetate saturated with water. The top two, yellow bands were scraped off, extracted with ethanol, and the two products recrystallized from ethanol-water.

The lower of these bands yielded D-arabino-hexulose 2-pyridylosazone (3), m.p. 217° (dec.).

Anal. Calc. for $C_{16}H_{20}N_6O_4 \cdot 0.5H_2O$: C, 52.03; H, 5.73; N, 22.75. Found: C, 52.51; H, 5.53; N, 22.63.

The upper band afforded, after recrystallization from ethanol, the dianhydrohexulose 2-pyridylosazone (4), m.p. 256° (dec.); λ_{max}^{EtOH} 247, 307, and 368 nm (log ε 4.04, 3.90, and 3.96); λ_{min}^{EtOH} 230, 272, and 333 nm (log ε 3.97, 3.75, and 3.74). Anal. Calc. for C₁₆H₁₆N₆O₂: C, 59.2; H, 4.95; N, 26.00. Found: C, 59.14; H, 4.95; N, 25.81.

D-arabino-Hexulose 2-quinolylosazone (5). — A solution of 2-hydrazinoquinoline (1 g) and syrupy D-arabino-hexosulose (D-glucosone) (2 g) in 1:1 ethanol-water (30 ml) was warmed on a hot-water bath for a few minutes, and allowed to cool overnight. The bis-hydrazone separated (yield 1.2 g), and was recrystallized from ethanol-water to give yellow needles, m.p. 246°.

Anal. Calc. for C₂₄H₂₄N₆O₄: C, 62.60; H, 5.25; N, 18.25. Found: C, 62.54; H, 5.31; N, 18.09.

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