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STUDIES ON 3-EPIMERIC L-2-HEXULOSE PHENYLOSAZONES. STRUCTURE AND ANOMERIC CONFIGURATION OF THE 3,6-ANHYDRO-OSAZONE DERIVATIVES OBTAINED FROM L-*xylo*- AND L-*lyxo*-2-HEXULOSE PHENYLOSAZONE††

MOHAMMED A. E. SALLAM*, ESTRWAH I. A. HEGAZY,
Chemistry Department, Faculty of Science, Alexandria University, Alexandria (Egypt)
ROY L. WHISTLER**, JOHN L. MARKLEY, AND DAVID H. CROLL†
*Biochemistry Department** and Chemistry Department†, Purdue University, West Lafayette, Indiana 47907 (U.S.A.)*

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ABSTRACT

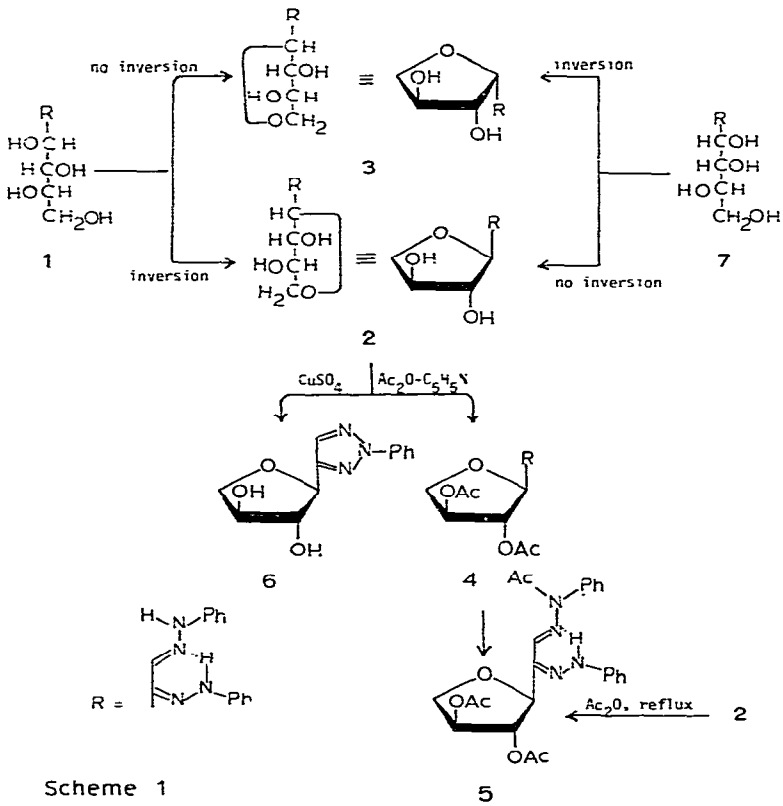
Dehydration of the 3-epimeric 2-hexulose phenylosazones L-*xylo*-hexulose phenylosazone and L-*lyxo*-hexulose phenylosazone afforded 3,6-anhydro-L-*lyxo*-2-hexulose phenylosazone (**2**) as the preponderant isomer from both. The identity of **2** was obtained by t.l.c., and by acetylation followed by comparison of the products. Acetylation of **2** with acetic anhydride–pyridine afforded the di-*O*-acetyl derivative **4**, and further acetylation gave the *N*-acetyldi-*O*-acetyl derivative **5**. Refluxing of **2** with copper sulfate afforded a *C*-nucleoside analog, namely, 2-phenyl-4-α-L-threo-furanosyl-1,2,3-osotriazole (**6**). The anomeric configuration was determined by n.m.r. spectroscopy. The stereochemical course of the dehydration process and the mass spectra of compounds **2**, **4**, **5**, and **6** are discussed.

INTRODUCTION

Monosaccharide 3,6-anhydro-osazones are useful precursors for the synthesis of *C*-nucleoside analogs having aldofuranosyl sugar moieties[2], especially those having rare configurations that cannot be readily obtained by other synthetic methods. They are prepared by dehydrative cyclization of saccharide osazones with methanolic sulfuric acid solution[3–7]. Recent studies on the dehydration of 3-epimeric D-2-hexulose phenylosazones[9] showed that, during the 3,6-anhydro-ring formation, inversion at C-3 of both 3-epimeric D-2-hexulose phenylosazones takes place with the formation of the same two isomeric 3,6-anhydro-osazones. The isomer having a *trans* relationship between the 2-hydroxyl group and the bis(phenylhydrazone) residue preponderates. The main problem militating against the extensive use of this reaction

††Studies on Anhydro-osazones, Part VI. For Part V, see ref. 1.
*To whom inquiries should be addressed.

in the field of *C*-nucleosides has been determination of the anomeric configuration of the products. Circular dichroism studies[10] have been used for this purpose, but n.m.r. spectroscopy has been shown[8] to be a more reliable tool for the assignment of the anomeric configuration, because of the mutarotation exhibited by the chelated-ring structure of osazones, which affects the chiroptical measurements.



Scheme 1

In the present work, we studied the dehydration of the 3-epimeric L-*xylo*- and L-*lyxo*-2-hexulose phenylosazones with methanolic sulfuric acid solution. Refluxing L-*xylo*-2-hexulose phenylosazone (**1**) with methanolic sulfuric acid solution, with monitoring of the reaction by t.l.c., afforded 3,6-anhydro-L-*lyxo*-hexulose phenylosazone (**2**) as the preponderant product. Compound **2** is formed from **1** with inversion in the configuration of C-3 (see Scheme 1). Its n.m.r. spectrum (see Fig. 1) showed the anomeric proton as a doublet at δ 4.38 ($J_{1',2'}$ 4.2 Hz); this value of the coupling constant is in close agreement[11] with the *trans* arrangement for H-1′ and H-2′ of the α-L-*threo* configuration of the glycosyl group. The minor isomer **3**, which is formed from **1** without inversion in the configuration of C-3, was detected by t.l.c. as a trace in the reaction products, and was tentatively assigned the β-L-*threo* configuration.

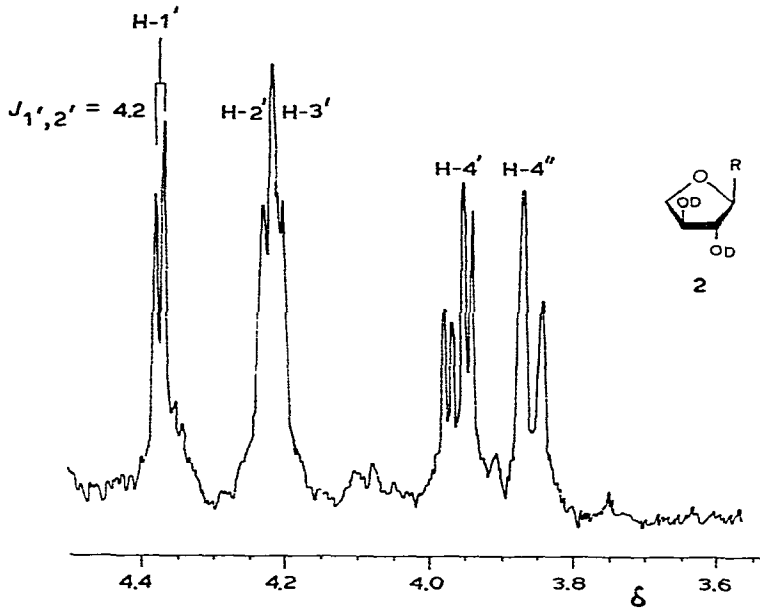


Fig. 1. N.m.r. spectrum, at 360 MHz, of 3,6-anhydro-L-*lyxo*-hexulose phenylosazone (2) + CD_3CO_2D [high resolution, for the sugar moiety; R = bis(phenylhydrazone) residue.]

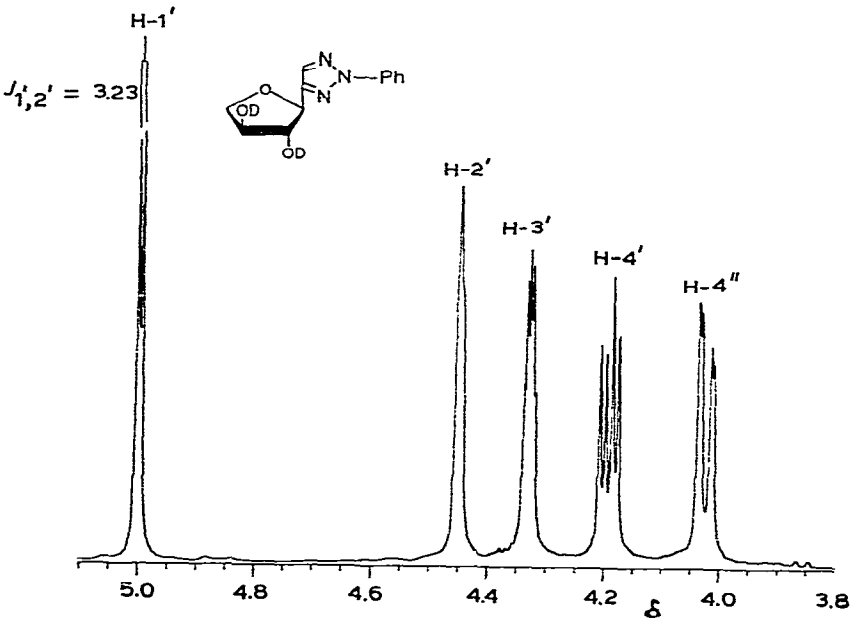


Fig. 2. N.m.r. spectrum, at 470 MHz, of 2-phenyl-4-α-L-threofuranosyl-1,2,3-osotriazole (6) after addition of CD_3CO_2D (high resolution, for the sugar moiety).

Acetylation of **2** with acetic anhydride–pyridine afforded the di-*O*-acetyl derivative **4**. Its infrared (i.r.) spectrum showed a band at 1730 cm^{-1} corresponding to the *O*-acetyl groups. Its n.m.r. spectrum showed the two acetyl groups as two singlets, each of three-proton intensity, at δ 2.11 and 2.08, and the signal of the anomeric proton was a doublet at δ 4.62 ($J_{1',2'}$ 4.57 Hz). Further acetylation of **2**, by prolonged treatment with acetic anhydride–pyridine, or by refluxing with acetic anhydride, gave the *N*-acetyldi-*O*-acetyl derivative **5**. Its i.r. spectrum showed the presence of both *O*- and *N*-acetyl groups, at 1760 and 1705 cm^{-1}, respectively. The n.m.r. spectrum of **5** showed the two *O*-acetyl groups as two singlets, each of three-proton intensity, at δ 2.06 and 1.97; the signal of the *N*-acetyl group was a broad singlet at δ 2.65, and that of the anomeric proton was a doublet at δ 4.44 ($J_{1',2'}$ 3.6 Hz).

On being refluxed with copper sulfate, compound **2** afforded the *C*-nucleoside derivative, namely, 2-phenyl-4-α-L-threofuranosyl-1,2,3-osotriazole (**6**). Its n.m.r. spectrum (see Fig. 2) showed the anomeric proton as a doublet at δ 4.86 ($J_{1',2'}$ 3.23 Hz), shifted to lower field, far from the glycosyl protons. The small coupling-constant observed for **6** is consistent[11] with the *trans* arrangement between H-1′ and H-2′ of the α-L-threofuranosyl group. Additional evidence supporting the α-L-*threo* configuration for compound **6** was obtained from the negative specific rotation ($[\alpha]_D^{20}$ −6.8°, in methanol) which is opposite in sign to the value ($[\alpha]_D^{20}$ +6.2°, in methanol) for the enantiomeric diastereoisomer, namely, 2-phenyl-4-α-D-threofuranosyl-1,2,3,-osotriazole[9].

The mass spectrum of **2** showed a fragmentation pattern identical to that of 3,6-anhydro-D-2-hexulose phenylosazones[12], with the molecular-ion peak at *m/z* 340 as a base peak. The mass spectrum of the di-*O*-acetyl derivative **4** (see Fig. 3a) showed the molecular-ion peaks M and (M + 1) at *m/z* 424 and 425, respectively; these were followed, at lower mass, by peaks at *m/z* 230, 213, and 212. Peaks characteristic of saccharide phenylosazones, at *m/z* 93, 92, and 77 (corresponding to $PhNH_2$, PhNH, and Ph), were abundant. The base peak occurred at *m/z* 43, corresponding to a CH_3CO group. The mass spectrum of the *N*-acetyldi-*O*-acetyl derivative **5** (see Fig. 3b) showed the molecular-ion peaks M and (M + 1) at *m/z* 466 and 467, respectively; these were followed, at lower mass, by peaks at *m/z* 230, 213, and 212. The peaks at *m/z* 136 and 135, respectively corresponding to $PhN^+H_2COCH_3$ and $PhNHCOCH_3$, which were absent for compound **4**, proved the presence of the *N*-acetyl group in **5**. The peaks characteristic of saccharide phenylosazones, at *m/z* 93, 92, and 77, were also abundant. The mass spectrum of **5** showed a remarkable difference from that of **4** as regards the base peak. Compound **5** showed the base peak at *m/z* 93, corresponding to $PhNH_2$, whereas that for compound **4** was at *m/z* 43. The mass spectrum of the *C*-nucleoside analog **6** showed the molecular-ion peaks, M and (M + 1), at *m/z* 247 and 248, respectively. The peak (B + 30) characteristic for *C*-nucleosides[9] was a base peak at *m/z* 174, as expected for 2-phenyl-1,2,3-osotriazole *C*-nucleosides[9], and this indicated the carbon–carbon linkage between the L-threofuranosyl group and the base moiety.

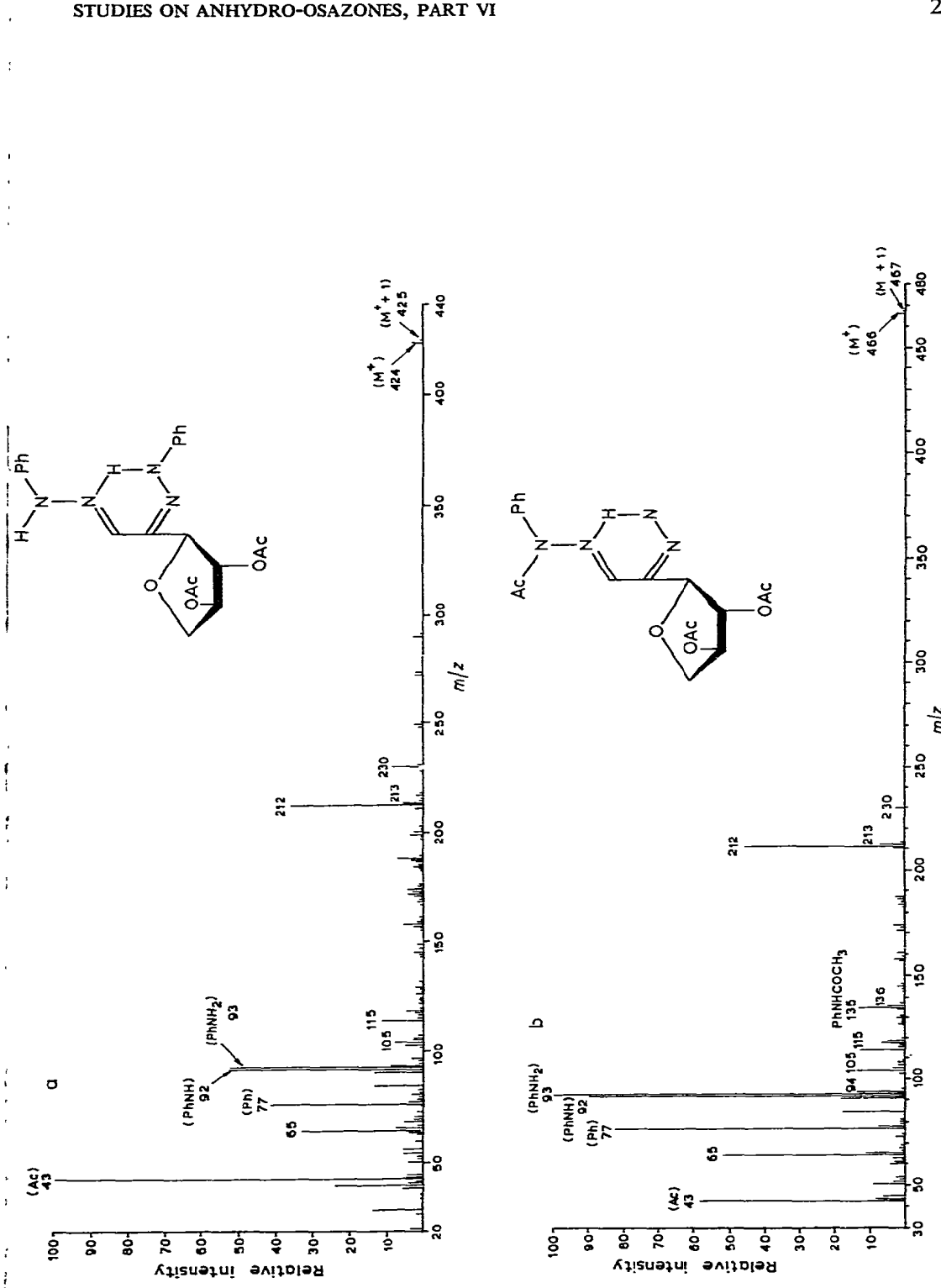
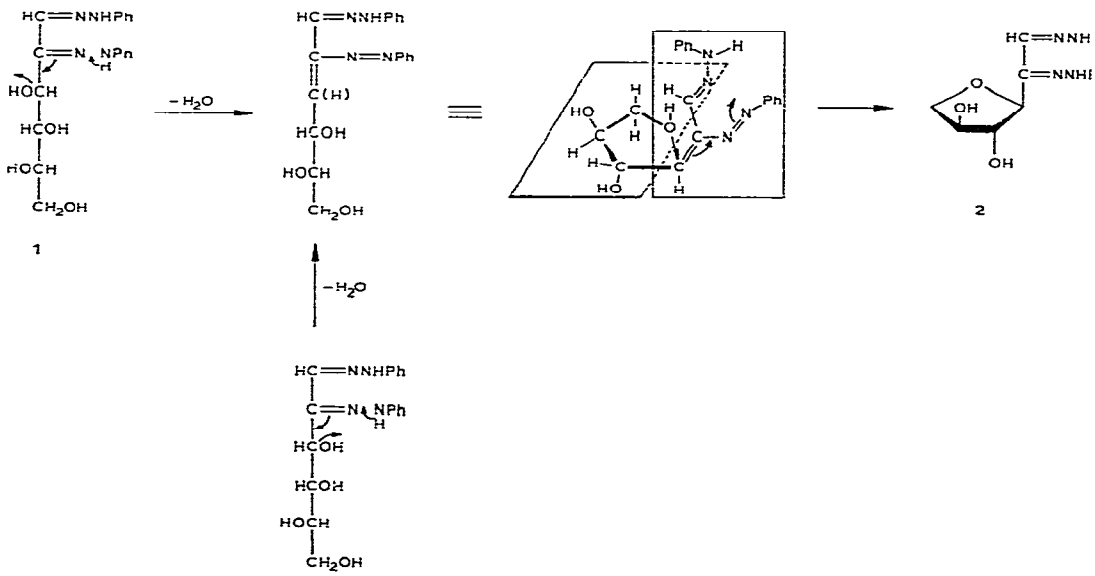


Fig. 3. Mass spectrum of (a) 4,5-di-*O*-acetyl-3,6-anhydro-L-*lyxo*-2-hexulose phenylosazone (**4**); (b) *N*-acetyl-4,5-di-*O*-acetyl-3,6-anhydro-L-*lyxo*-2-hexulose phenylosazone (**5**).

In order to investigate the steric course of the dehydration of **1**, the 3-epimeric L-*lyxo*-2-hexulose phenylosazone **7** was similarly dehydrated with methanolic sulfuric acid solution (with monitoring of the reaction by t.l.c.), and the products were compared with those from **1**. Dehydration of **7** afforded the same 3,6-anhydro-osazones, **2** and **3**, as had been obtained from **1**; they respectively had the same mobility in t.l.c., and compound **2** was also obtained as the preponderant isomer from **7**. Acetylation of **2** with acetic anhydride–pyridine afforded the di-*O*-acetyl derivative **4**, having the same melting and mixed melting point. Further acetylation afforded the same *N*-acetyldi-*O*-acetyl derivative **5**, having the same melting and mixed melting point.

The formation of **2** as the preponderant isomer from both 3-epimeric L-2-hexulose phenylosazones **1** and **7** (with inversion at *C*-3 from **1**, and without inversion from **7**) constitutes direct proof for the stereospecificity of the dehydration of L-2-hexulose phenylosazones. The formation of the 3,6-anhydro ring takes place in the sterically favored direction, with the production of the preponderant isomer **2**, having a *trans* relationship between the bis(phenylhydrazone) residue and the 4-hydroxyl group (C-2′ of the glycosyl group formed), as indicated by a study of models (see Scheme 2). The minor isomer **3**, having a *cis* relationship between the bis(phenylhydrazone) group and the 4-hydroxyl group, is formed by cyclization in the sterically disfavored direction.



Scheme 2

The inversion in the configuration at C-3 of both 3-epimeric 2-hexulose phenylosazones **1** and **7** cannot be explained as being of a simple, SN2 type; otherwise, **1** and **7** should produce different, preponderant isomers by the rear-side 6-oxygen attack on C-3 of the starting osazone (the α-L-threofuranosyl isomer, **2**, would be

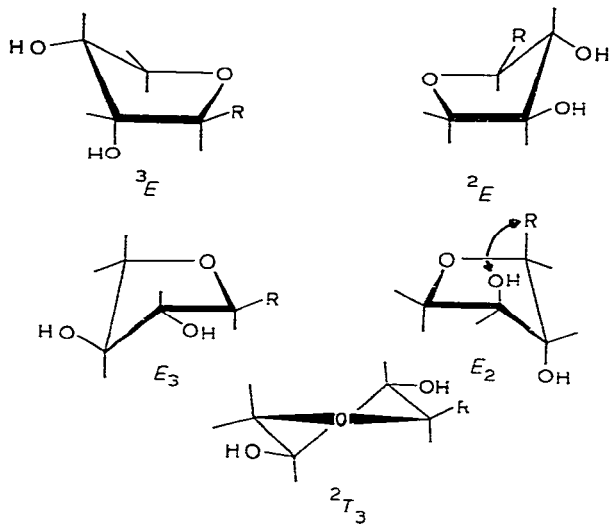


Fig. 4. Conformers of the β-L-threofuranosyl moiety of 3,6-anhydro-L-*lyxo*-2-hexulose phenylosazone (**2**).

obtained from **1** and the β-L-threofuranosyl isomer, **3**, from **7**). The possible explanation for the observation that the cyclization of **1** and **7** produces the same two 3,6-anhydro-osazones, **2** and **3**, is that racemization occurs at C-3 to afford the same 2-(phenylazo)-2-ene intermediate, as suggested by Simon and co-workers[13,14] and El Khadem[15]. Although such intermediates have not yet been isolated, several 2-ene intermediates have been shown to be formed in the course of osazone reactions[14]. The proposed mechanism is supported by the observation[4] that D-glucose alkylphenylosazones cannot be converted into anhydro-osazones. Recent studies on 3-epimeric hexulose phenylosazones[9] confirm the stereospecificity of the dehydration of hexulose phenylosazones[14], and support the racemization at C-3 of the starting osazone by the formation of the same 2-ene alkenic intermediate from each 3-epimeric pair of hexulose phenylosazones.

In connection with the conformation of the aldofuranosyl moiety of the *C*-nucleoside analog **6**, the puckered structures of the L-threofuranosyl ring are shown in Fig. 4. Conformers E_2 and ^3E are the least favored, due to the 1,3-*syn*-axial interaction between the base moiety and the 3-hydroxyl group for E_2 and the axial orientation of the 2-hydroxyl group for ^3E. It is expected that compound **6** will mostly be present as an equilibrium mixture of $^2E \rightleftarrows {}^2T_3 \rightleftarrows E_3$, having all substituents equatorially attached.

EXPERIMENTAL

General. — Melting points are uncorrected; evaporations were performed under diminished pressure below 60°. Thin-layer chromatography (t.l.c.) was conducted

on silica gel (Kiesel gel G, Merck) with 3:1 benzene–ethanol as the solvent. I.r. absorption spectra were recorded with a Unicam SP 1025 instrument. N.m.r. spectra were recorded with Nicolet 360-MHz and 470-MHz instruments, using internal tetramethylsilane as the reference. Mass spectra were recorded with Dupont MS 21-492 B and Finnigan 6100 Data System Gas-Chromatograph/EI-CI spectrometers. Combustion analyses were performed in the Department of Chemistry, Cairo University, Cairo, Egypt, and the Department of Chemistry, Purdue University, W. Lafayette, IN, U.S.A.

3,6-Anhydro-L-lyxo-*2-hexulose phenylosazone* (**2**). — *Method 1. From* L-xylo-*hexulose phenylosazone* (L-*sorbose phenylosazone*, **1**). Compound **1** (5 g) was boiled under reflux with 0.05% methanolic sulfuric acid (250 mL) for 6 h, and the reaction was monitored by t.l.c.; after 6 h, t.l.c. revealed the absence of the starting osazone and formation of two more-mobile spots having R_F 0.49 and 0.32, respectively. The mixture was stirred with barium carbonate, filtered, and the filtrate concentrated to a small volume, whereupon yellow needles separated; yield 3 g; these were recrystallized from methanol, to give chromatographically (t.l.c.) pure, yellow needles of **2**; m.p. 225–226° (lit.[6] m.p. 220–221°): ν_{max}^{KBr} 3320 (OH) and 1605 cm^{-1} (C=N); n.m.r. data (360 MHz, acetone-d_6): δ 3.84–4.38 (m, 7 H, sugar protons), 6.88–7.54 (m, 10 H, aromatic protons), 7.87 (s, 1 H, aldimino proton), 9.87 (s, 1 H, nonchelated NH of C-1 hydrazone residue), and 12.41 (s, 1 H, chelated NH of C-2 hydrazone residue); after addition of CD_3CO_2D, the two NH protons and the two OH protons disappeared: δ 3.85–3.87 (bd, 1 H, H-4″, $J_{3',4''}$ zero), 3.94–3.98 (dd, 1 H, H-4′, $J_{3',4'}$ 3.9, $J_{4',4''}$ 9.5 Hz), 4.21–4.23 (m, 2 H, H-2′,3′, $J_{2',3'}$ 2.3 Hz), and 4.38 (d, 1 H, H-1′, $J_{1',2'}$ 4.2 Hz); mass-spectral data (selected ions): *m/z* 340 (100, M), 188 (13), 174 (6), 158 (5), 105 (4), 104 (4), 94 (8, PhN^+H_3), 93 (53, $PhNH_2$), 92 (49, PhNH), 91 (13, PhN), 77 (49, Ph), and 65 (51, cyclopentadienyl ion).

Method 2. From L-lyxo-*hexulose phenylosazone* (**7**). Compound **7** (4 g, prepared from the mixture of the 2-epimeric hexoses L-talose and L-galactose obtained from L-lyxose cyanohydrin)[16] was boiled for 4 h under reflux with methanolic sulfuric acid (300 mL), the reaction being monitored by t.l.c.; after 4 h, t.l.c. (solvent *A*) revealed the formation of two spots similar to those obtained by method 1, at the same R_F values (solvent *A*). The solution was processed as described for compound **1**; yield 3 g. It was purified by column chromatography with solvent *A* as the eluant; the fractions were collected, and evaporated to dryness, to give chromatographically pure (t.l.c.), yellow needles, m.p. and mixed m.p. (with **2**) 222–224°, and having the same R_F values.

The minor isomer **3** was detected in the mother liquor (methods 1 and 2), by t.l.c. using solvent mixture *A*, at R_F 0.32.

4,5-Di-O-*acetyl-3,6-anhydro*-L-lyxo-*2-hexulose phenylosazone* (**4**). — A solution of **2** (2 g) in pyridine (7 mL) was treated with acetic anhydride (7 mL) for 24 h at room temperature; it was then poured onto crushed ice, and the acetate was filtered off, washed with water, and dried; yield 2.4 g. The product was recrystallized from methanol, to give yellow needles, m.p. 121–122°; ν_{max}^{KBr} 1730 (OAc) and 1605 cm^{-1}

(C=N); n.m.r. data (470 MHz, $CDCl_3$): δ 2.083 and 2.113 (d, 6 H, 2 CH_3CO), 3.96–3.87 (dd, 1 H, H-4″, $J_{3',4''}$ 2.5, $J_{4',4''}$ 10.6 Hz), 4.187–4.221 (dd, 1 H, H-4′, $J_{3',4'}$ 5.4 Hz), 4.62 (d, 1 H, $J_{1',2'}$ 4.2 Hz), 5.215–5.230 (dd, 1 H, H-3′, $J_{2',3'}$ 4.9 Hz), 6.917–7.369 (m, 10 H, aromatic protons), 7.57 (s, 1 H, aldimino proton), 7.711 (s, 1 H, nonchelated NH of C-1 hydrazone residue), and 12.259 (s, 1 H, chelated NH of C-2 hydrazone residue); after addition of CD_3CO_2D the two NH protons disappeared.

Anal. Calc. for $C_{22}H_{24}N_4O_5$: C, 62.24; H, 5.70; N, 13.21. Found: C, 62.41; H, 5.58; N, 13.20.

N-*Acetyl-4,5-di*-O-*acetyl-3,6-anhydro*-L-lyxo-*hexulose phenylosazone* (**5**). — Compound **2** (2 g) was acetylated with acetic anhydride–pyridine for 7 days at room temperature, the mixture processed as described for **4**, and the solid product recrystallized from methanol, to give yellow needles, m.p. 178–180°; ν_{max}^{KBr} 1760 (OAc), 1705 (NAc), and 1590 cm^{-1} (C=N); n.m.r. data (470 MHz, $CDCl_3$): δ 1.966 and 2.059 (d, 6 H, 2 CH_3CO), 2.648 (bs, 3 H, N-CH_3CO), 3.782–3.809 (dd, 1 H, H-4″, $J_{3',4''}$ 2.0 Hz), 4.030–4.063 (dd, 1 H, H-4′, $J_{3',4'}$ 5.0 Hz), 4.440 (d, 1 H, H-1′, $J_{1',2'}$ 3.6 Hz), 5.108–5.117 (m, 1 H, H-3′), 5.529–5.536 (bs, 1 H, H-2′, $J_{2',3'}$ 2.9 Hz), 6.963–7.593 (m, 10 H, aromatic protons), and 12.22 (bs, 1 H, chelated NH of C-2 hydrazone residue).

Anal. Calc. for $C_{24}H_{26}N_4O_6$: C, 61.80; H, 5.62; N, 12.01. Found: C, 62.02; H, 5.76; N, 11.72.

Refluxing of **2** *with acetic anhydride.* — Compound **2** (2 g) was refluxed with acetic anhydride (20 mL) for 1.5 h, the solution was poured into ice, and the precipitate obtained was filtered off, washed with water, and dried; yield 0.6 g. It was recrystallized from methanol, to give yellow needles, m.p. and mixed m.p. (with **5**), 180–182°.

*2-Phenyl-4-*α-L-*threofuranosyl-1,2,3-osotriazole* (**6**). — A solution of the crude osazone mixture of **2** and **3** (10 g) in methanol (300 mL) was boiled under reflux, with stirring, and a solution of copper(II) sulfate (10 g) in water (100 mL) was added; then, 1-propanol (5 mL) was added, and the mixture was boiled for 8 h. It was cooled, filtered, the filtrate evaporated to dryness, the residue extracted with hot methanol, and the extract filtered. The filtrate was freed of copper ions by bubbling H_2S gas through it, filtering the suspension, and then stirring the filtrate with Amberlite IR-MB3 cation-anion-exchange resin. The resin was filtered off, and washed thoroughly with methanol, and the filtrate and washings were combined, and evaporated to a syrup which was purified by column chromatography on Dowex-1 X-8 (OH^-) ion-exchange resin[17], with gradient elution with aqueous methanol (30, 60, and 90% methanol). Compound **6** was eluted with 90% methanol, and the eluate evaporated to dryness, giving a syrup which crystallized from ether–hexane as colorless needles, m.p. 97–99° (lit.[6] m.p. 102–103°), $[\alpha]_D^{20}$ −6.8° (*c* 1.6, methanol); its enantiomer, 2-phenyl-4-α-D-threofuranosyl-1,2,3-osotriazole[9a] has $[\alpha]_D^{20}$ +6.2° (*c* 2, methanol); n.m.r. data (470 MHz, $CDCl_3$): δ 3.815 (d, 1 H, OH, *J* 3.3 Hz), 3.911 (d, 1 H, OH, *J* 6.6 Hz), 3.961–3.987 (dd, 1 H, H-4″, $J_{3',4''}$ 2.0, $J_{4',4''}$ 9.9 Hz), 4.129–4.160 (dd, 1 H, H-4′, $J_{3',4'}$ 4.6 Hz), 4.253–4.261 (m, 1 H, H-3′), 4.395 (bs, 1 H, H-2′), 4.940 (d, 1 H, H-1′, $J_{1',2'}$ 3.23 Hz), 7.264–7.312 (q, 1 H, *p*-proton of the

phenyl group), 7.386–7.419 (t, 2 H, *m*-protons of the phenyl group), 7.777 (s, 1 H, H-5), and 7.898–7.915 (d, 2 H, *o*-protons of the phenyl group); after addition of CD_3CO_2D, the two OH protons disappeared; mass-spectral data: *m/z* 247 (24, M), 188 (28, B + 44, where B = 2-phenyl-1,2,3-osotriazole-4-yl moiety), 187 (12, BCH_2CHO), 175 (19, B + 31), 174 (100, B + 30), 158 (7), 92 (18, PhNH), 91 (42, PhN), 77 (31, Ph), 65 (10, cyclopentadiene ion), 64 (12), 57 (6), 56 (7), and 55 (10).

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