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Note

Synthesis of the *E* and *Z* isomers of 1,3,4,5,6-penta-*O*-acetyl-*keto*-D-fructose (2,4-dinitrophenyl)hydrazone*

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Hydrazones of some saccharides exhibit significant biological activities. They inhibit nucleo- and proteo-synthesis in tumorous cells[2] and the growth of bacteria[3]. Hydrazones of monosaccharides generally crystallize well, and they are useful derivatives for the identification[4] and isolation of reducing carbohydrates[1].

Much attention has been paid to study of the structure of hydrazones, both cyclic and acyclic. The structure of hydrazones can be determined by several methods. For crystalline compounds, the most convenient method is X-ray crystal-structure analysis. By this method, the cyclic structure of D-arabinose (4-bromophenyl)hydrazone[5] and D-glucose (4-bromophenyl)hydrazone[6], as well as the acyclic structure of D-ribose (4-bromophenyl)hydrazone[7] and D-mannose (4-bromophenyl)hydrazone[8], have been determined. The best methods for structural analysis of hydrazones in solution seem to be ^{1}H- and ^{13}C-n.m.r. spectroscopy[9].

Per-*O*-acetylated hydrazones of saccharides can occur, like hydrazones, either in acyclic or cyclic forms. The respective forms of these compounds can be proved by ^{1}H-n.m.r. spectrometry[10,11] and mass spectrometry[12], as well as by synthesis from acyclic per-*O*-acetylated saccharides[13].

Several studies indicate that the predominant isomer in the crystalline form is not always that in solution, owing to ring–chain interconversion dependent on solvent, sugar configuration, and basicity of the hydrazine group[9,14].

Acyclic hydrazones, containing the C=N bond in the molecule, may exist in the forms of their *E* and *Z* isomers. Despite the fact that hydrazones were prepared for the first time at the end of the last century, the synthesis of their *E* and *Z* isomers had not been convincingly described until 1976, when Weclawowicz[15] described the

*Hydrazones and Their Derivatives, Part III. For Part II, see ref. 1.

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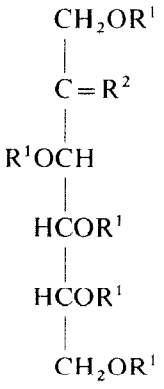
E and *Z* isomers of D-arabinose phenylhydrazone, but without experimental data concerning their synthesis.

The per-*O*-acetylated hydrazones of saccharides are usually prepared by acetylation of the corresponding hydrazone. By this method, we prepared[16] 1,3,4,5,6-penta-*O*-acetyl-*keto*-D-fructose (2,4-dinitrophenyl)hydrazone (**2**) from D-fructose (2,4-dinitrophenyl)hydrazone (**1**). The acyclic structure of **2** was proved by mass spectrometry[12] and ^{1}H-n.m.r. spectroscopy[11].



1 $R^1 = H$, $R^2 = NNHC_6H_3(NO_2)_2$-2,4
2 $R^1 = Ac$ $R^2 = NNHC_6H_3(NO_2)_2$-2,4
3 $R^1 = Ac$ $R^2 = O$

Proof of the acyclic structure of compound **2** was also provided by synthesis according to the procedure published by Wolfrom and Christman[13]. Unexpectedly, the reaction of 1,3,4,5,6-penta-*O*-acetyl-*keto*-D-fructose (**3**) with (2,4-dinitrophenyl)-hydrazine[17] gave a crystalline mixture, **4**, of two hydrazones, as revealed by t.l.c., which were separated by column chromatography, yielding the *E* (**2**) and *Z* (**5**) isomers of 1,3,4,5,6-penta-*O*-acetyl-*keto*-D-fructose (2,4-dinitrophenyl)hydrazone. The identity of each of the hydrazones, **2** and **5**, was proved by ^{1}H- and ^{13}C-n.m.r. spectroscopy (see Tables I and II). The structure of the *Z* isomer (**5**) was also confirmed by X-ray analysis[18].

TABLE I

^{1}H-N.M.R.-SPECTRAL DATA: CHEMICAL SHIFTS (δ) AND COUPLING CONSTANTS (Hz)

Isomer	*NH*	*H-1a, 1b*	*CH$_3$ (acetyl)*
E (**2**)	11.85s	4.75d, 5.03d J_{gem} 12.5	2.06s, 1.83s
Z (**5**)	10.95s	4.88t J_{gem} 12.5	2.06s

TABLE II

^{13}C-N.M.R.-SPECTRAL DATA: CHEMICAL SHIFTS (δ)

Aromatic moiety							
Isomer	*C=N*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
E (**2**)	144.66	144.40	139.08	123.23	130.25	130.23	116.22
Z (**5**)	144.63	146.32	139.17	123.19	130.59	130.20	116.14
Sugar moiety							
Isomer	*C-1*	*C-3*	*C-4*	*C-5*	*C-6*	*C=O (acetyl)*	
E (**2**)	65.32	71.81	68.82	68.56	61.43	170.37	
						169.59	
						168.82	
Z (**5**)	58.47	71.86	68.61	69.78	61.59	170.49	
						169.71	

The ^{1}H-n.m.r. chemical-shifts, important for identifying, or distinguishing between, the *E* and *Z* isomers, are given in Table I. The position of the signal due to the NH group of isomer *E* is shifted downfield, compared with that of isomer *Z*. Differences in the spectra are also seen with the signals of protons at C-1; the *E* isomer revealed 2 doublets, whereas the *Z* isomer gave a triplet. Similar differences are seen with the chemical shifts of the methyl groups of acetyl groups. These differences provided evidence that the NH group of the *E* isomer forms a hydrogen bond

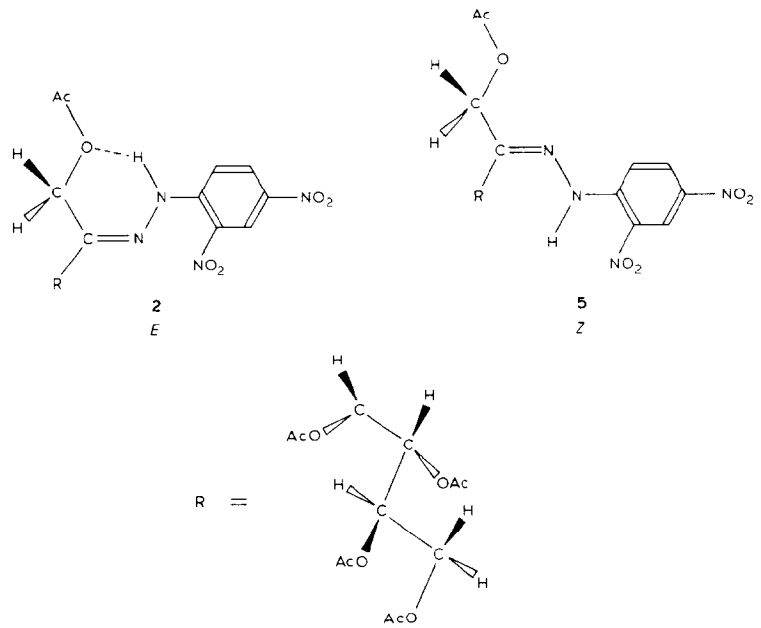


Fig. 1. *E* and *Z* isomers of penta-*O*-acetyl-*keto*-D-fructose (2,4-dinitrophenyl)hydrazone.

with O-1 (see Fig. 1): formation of a ring is associated with changes in shielding effects on the protons at C-1.

This suggestion is in accord with the ^{13}C-n.m.r.-spectral data (see Table II). The most significant, downfield shift was formed with C-1 of the *E* isomer, and alterations were also observed in the region of the carbonyl groups of the acetyl groups. The signal at δ 168.82 (*E* isomer) is that of the acetyl group forming the hydrogen bond. The conformation of the molecule of the *Z* isomer is in accord with the results of X-ray analysis, as illustrated in Fig. 1.

The *E* and *Z* isomers were further subjected to circular dichroism (c.d.) measurements. These two isomers can be unambiguously distinguished on the basis of the character of their c.d. spectra, and particularly, from the opposite sign of the long-wave, chiroptic band. Isomer *Z* shows a positive c.e. ($\Delta\varepsilon$) at 410 nm, whereas isomer *E* gives a negative one at ~355 nm.

EXPERIMENTAL

General. – Melting points were determined on a Kofler micro hot-stage. Solutions were evaporated under diminished pressure at 30–40°. Compound **3** was prepared according to ref. 19, and hydrazone **1**, according to ref. 20. Thin-layer chromatography was conducted on plates of silica gel (Silufol; Kavalier) with (*a*) 7:3 benzene–ethyl acetate and (*b*) 7:2:1 heptane–chloroform–1,4-dioxane. On thin-layer plates, the components were located by spraying with 5% sulfuric acid in ethanol, and heating. Preparative chromatography was performed on columns (60 × 3 cm) of silica gel (0.04–0.1 mm) with solvents *a* and (*c*) 20:3:2 heptane–chloroform–1,4-dioxane. Mass spectra (70 eV) were recorded with an emission current of 300 μA by using a JMS-D 100 instrument and the direct-inlet technique. The temperature at the site of evaporation was 220°. The n.m.r. spectra were recorded for solutions in chloroform-*d* (50 mg/0.4 mL) containing Me_4Si as the internal standard. ^{1}H-N.m.r. spectra were recorded with an 80-MHz, Tesla BS 487B spectrometer, and ^{13}C-n.m.r. spectra with an Ft-n.m.r. spectrometer (JEOL FX-60) by using a repetition time of 0.5 s, a pulse width of 4 μs (45° flip-angle), 4,000 kHz sweep-width, and 8k real data-points in the proton-decoupled and off-resonance mode. The c.d. spectra were recorded with a Jobin Yvon Mark III instrument, for solutions in acetonitrile at a concentration of 0.5–1.0 mg/mL in 1-mm cells at 25°.

*1,3,4,5,6-Penta-*O*-acetyl-*keto-D-*fructose (2,4-dinitrophenyl)hydrazone, E isomer* (**2**). – To a mixture of acetic anhydride (3.6 mL) and dry pyridine (6.7 mL) was added compound **1** (1.5 g) at 0°, and the mixture was kept for 2 h at 0° with occasional shaking. After an additional 16 h at 3°, the solution was evaporated, and ethanol (150 mL) was added to the syrupy residue. The product (**2**; 1.8 g; 75.8%) crystallized slowly at 20°; pure product was obtained by two recrystallizations from ethanol; m.p. 109–110°, $[\alpha]_D^{20}$ −54.7° (*c* 0.5, ethyl acetate); ^{1}H-n.m.r. data are given in Table I and ^{13}C-n.m.r. data, in Table II; c.d. data, $\Delta\varepsilon$ (λ): −1.16 (355), −0.78 (263), −0.74 (232), and +0.41 (217).

Anal. Calc. for $C_{22}H_{26}N_4O_{14}$: C, 46.32; H, 4.59; N, 9.82. Found: C, 46.19; H, 4.70; N, 9.70.

Mixture of E *and* Z *isomers of 1,3,4,5,6-penta-*O*-acetyl-*keto-D*-fructose (2,4-dinitrophenyl)hydrazone* (**4**). — To a mixture of 1,4-dioxane (80 mL), acetic acid (50 mL), and water (50 mL) were added compound **3** (2 g) and (2,4-dinitrophenyl)-hydrazine (1.02 g), and the solution was stirred for 8 h at 20°. After an additional 16 h at 20°, the solution was evaporated, and the syrupy residue was analyzed by t.l.c. using solvent *a*. Three spots were detected [R_F 0.48, (2,4-dinitrophenyl)hydrazine; R_F 0.53, **3**; and R_F 0.67, **4**]. This mixture was separated on a column of silica gel using solvent *a*, to give **4** (0.82 g; 28.3%) as a syrup which crystallized from ethanol (50 mL). After two recrystallizations from ethanol, the product had m.p. 105–109°, $[\alpha]_D^{20}$ −24.0 ±3° (*c* 0.5, ethyl acetate). According to t.l.c. (solvent *b*), the hydrazone **4** consisted of a mixture of compound **2** (*E* isomer; R_F 0.26) and **5** (*Z* isomer; R_F 0.34). These isomers (0.94 g) were separated on a column of silica gel using solvent *c*, to give the *Z* isomer (**5**; 0.37 g; 39.4%) and *E* isomer (**2**; 0.51 g; 54.3%).

The *Z* isomer (**5**) crystallized from ethanol; after two recrystallizations (ethanol), it had m.p. 121–122°, $[\alpha]_D^{20}$ +47.2° (*c* 0.5, ethyl acetate); ^{1}H-n.m.r. data are given in Table I, and ^{13}C-n.m.r. data, in Table II; c.d. data, $\Delta\varepsilon$ (λ): +0.79 (410), −1.11 (347), +2.92 (279), −1.81 (250; shoulder), −2.13 (242), +2.37 (226), and −0.95 (212).

Anal. Calc. for $C_{22}H_{26}N_4O_{14}$: C, 46.32: H, 4.59; N, 9.82. Found: C, 46.22; H, 4.54; N, 9.85.

The *E* isomer prepared by this procedure was identical with compound **2** prepared by acetylation of hydrazone **1** (m.p., optical rotation, mass spectra, and ^{1}H- and ^{13}C-n.m.r. spectra).

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