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Note

Isolation and characterization of an unsubstituted 2,3-unsaturated sugar, *trans*-2,3-dideoxy-D-*glycero*-pent-2-enose, produced by thermal dehydration of 2-deoxy-D-*erythro*-pentose

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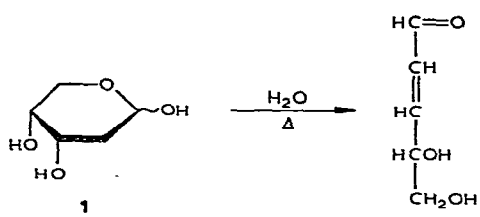
Heating dilute, buffer-free solutions (pH ~5.7) of 2-deoxy-D-*erythro*-pentose (**1**) in distilled water produces mainly a compound having intense, pH independent, u.v. absorption (λ_{max} 222 nm) and high cytotoxicity characteristic of α,β-unsaturated carbonyl compounds[1]. The resulting product, a completely unsubstituted, 2,3-unsaturated sugar, has been isolated and characterized as *aldehydo-trans*-2,3-dideoxy-D-*glycero*-pent-2-enose (**2**).



1 **2**

2,3-Unsaturated sugars have received much attention in recent years[2]. Most examples have been glycosides having furanoid or pyranoid ring-systems. A few reports have described non-glycosidic 2,3-unsaturated sugars, but all have had protecting groups present. Fraser-Reid[3] isolated 4,6-di-*O*-acetyl-*aldehydo-trans*-2,3-dideoxy-D-*erythro*-hex-2-enose and demonstrated that it exists as the free aldehyde rather than as the pyranose. Earlier, Carbon[4] found that 4-*O*-acetyl-*trans*-2,3-dideoxy-D-*glycero*-pent-2-enose, produced from di-*O*-acetyl-D-arabinal, exists entirely in the aldehydo form. He also showed that refluxing solutions of **1** with purines produced 3′-nucleosides, which were presumed to arise through nonstereospecific

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Michael addition of the purine to an intermediate pent-2-enose generated by loss of the C-3 hydroxyl group of the 2-deoxy sugar.

Freshly prepared solutions of **1** contained no detectable amounts of **2**. However, heating a 1% solution (pH = 5.7) for example, showed that the rate of formation of **2** and its equilibrium concentration increased rapidly with increasing temperature, with a maximum yield of 12.8% from the starting 2-deoxy-D-*erythro*-pentose (Table I). Measurable amounts of **2** were produced, even at 37°. The yield of **2** varied in proportion to the initial concentration of **1** in solution, indicating that the formation is first order. The pH of the solution and the presence of buffers had a marked effect on the formation of **2** (Table I). For example, when a 1% solution of **1** in 0.1M phosphate buffer at pH 7.0 was autoclaved for 2 h, the resulting solution was brown and showed a u.v. absorption maximum at 265 nm, but no characteristic maximum at 222 nm. The formation of the material absorbing at 265 nm was, to a minor degree, observed in phosphate buffer at pH 6.0, Tris buffer at pH 6.0, and in water adjusted with sodium hydroxide to pH 7.0.

TABLE I

EFFECT OF pH, TEMPERATURE, AND BUFFER ON THE FORMATION OF *aldehydo-trans*-2,3-DIDEOXY-D-*glycero*-PENT-2-ENOSE (**2**) UPON HEATING 1% SOLUTIONS OF 2-DEOXY-D-*erythro*-PENTOSE (**1**)[a]

Solution	*pH*	*Temp.*	*Time of heating h*	*Absorbance, A at* λ_{max} *222 nm*	*Yield*[b] *of 2 %*	*Absorbance, A at* λ_{265nm}
H_2O	5.7	20°	>300	0.20[c]	0	0
H_2O	5.7	37°	550(ext)[d]	1.0(ext)	0.09	0
H_2O	5.7	50°	500(ext)[d]	1.2(ext)[e]	1.0	0
H_2O	5.7	100°	8[d]	0.90[f]	7.7	0
H_2O	5.7	120°	4[d]	1.50[f]	12.8	0
H_2O	5.7	120°	2	1.35[f]	11.5	0
H_2O	6.0[g]	120°	2	1.25[f]	10.6	0
H_2O	7.0[g]	120°	2	1.12[f]	9.5	0.50
0.1M Na–K phosphate	6.0	120°	2	0.95[f]	8.1	0.80
0.1M Na–K phosphate	7.0	120°	2	1.20[f,h]	0[h]	1.75
0.1M Tris	6.0	120°	2	1.20[f]	10.2	0.22
0.1M Tris	7.0	120°	2	1.75[f]	14.9	0

[a]Concentrations measured by u.v. at λ_{max} 222 nm (ε 13 600). All pathlengths were 1 cm. [b]Expressed as % of the weight of the parent sugar. [c]No maximum or change in absorbance from 0 to >300 h. [d]Time at which maximum absorbance reached. [e]1:10 dilution. [f]1:100 dilution. [g]pH adjusted with 0.1M NaOH. [h]No maximum, yield estimated from t.l.c. and g.l.c.

Thin-layer chromatograms of autoclaved **1** showed, in addition to the major spot of unreacted **1**, two spots of medium intensity and two spots of very low intensity not present in the starting solution. When the t.l.c. plate was sprayed with 2,4-dinitrophenylhydrazine reagent, the spot of medium intensity having a higher R_F (compound **2**), was visible as an intense orange spot characteristic[5] of α,β-unsaturated

carbonyl compounds, whereas the remaining spots showed the characteristic pale-yellow color of saturated carbonyl derivatives. Evidence for the structure of the isolated compound was based on elemental analyses, and u.v., i.r., n.m.r., and mass spectrometry of the free aldehyde and its 2,4-dinitrophenylhydrazone.

The u.v. spectrum of **2** in aqueous solution showed an intense maximum at 222 nm and a weak one at 308 nm. These data are in good agreement with values expected for α,β-unsaturated aldehydes[6], and in particular for 4-hydroxy-2-alkenals[7]. The spectrum of the 2,4-dinitrophenylhydrazone in chloroform exhibited maximal absorption at 370 nm, which shifted to 455 nm in ethanolic potassium hydroxide, a behavior characteristic of 2,4-dinitrophenylhydrazones of 2-alkenals[8].

The i.r. spectrum (thin film in KBr) revealed the presence of a hydroxyl group, an aldehyde carbonyl group, and a double bond that displayed the characteristic *trans*-olefinic C–H out-of-plane deformation band at 980 cm^{-1}. No corresponding out-of-plane deformation characteristic of a *cis*-olefinic C–H was present in the spectrum.

N.m.r. spectra were recorded in dimethyl sulfoxide-d_6 and in D_2O (Fig. 1). The three protons of the CH=CH–CHO group gave signals at 9.55, 7.10, and 6.20 p.p.m. (Me_2SO-d_6) with relative intensities of 1:1:1. The value for the coupling constant of the olefinic protons, $J_{2,3} = 16$ Hz, confirmed the *trans* geometry of the double bond[9]. The spectrum in Me_2SO-d_6 clearly showed the secondary hydroxyl group resonating as a doublet at 5.25 p.p.m. and the primary hydroxyl group as a triplet at 4.75 p.p.m. The geminal protons (H-5a and H-5b) were not equivalent as they are adjacent to an asymmetric center. An analysis is possible from the 250-MHz spectrum in D_2O (Fig. 1), where these protons give an ABX spectrum. The value

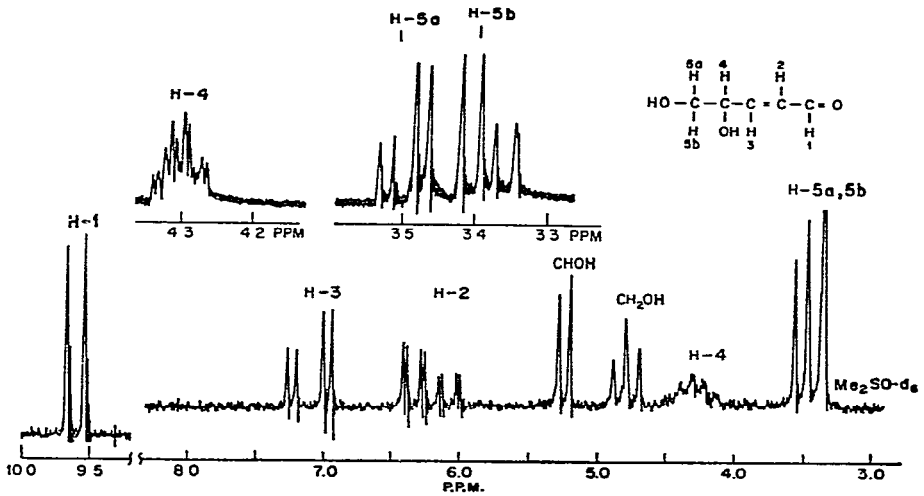

Fig. 1. Lower part represents the 60-MHz n.m.r. spectrum of *aldehydo-trans*-2,3-dideoxy-D-*glycero*-pent-2-enose (**1**) in Me_2SO-d_6. The upper part is the partial 250-MHz (Carnegie-Mellon University spectrometer) n.m.r. spectrum in D_2O.

for the $J_{5a,5b}$ coupling constant (12 Hz) is typical for geminal protons[9]. In Me_2SO-d_6 the C-5 protons give a triplet in the 60-MHz (Fig. 1) and 250-MHz spectra, presumably because they are isochronous in this solvent, thus showing coupling only with OH-5 (*J* 6 Hz) and H-4 (*J* 6 Hz). H-4 exhibits a complex multiplet at 4.3 p.p.m. in both solvents, and no attempt was made to assign coupling constants. The n.m.r. spectrum of the 2,4-dinitrophenylhydrazone of **2** was also in agreement with the proposed structure.

The mass spectrum of **2**, including the proposed mechanism of fragmentation, is shown in Fig. 2. As might be expected from the structure, the compound exhibited no molecular ion ($M^+ = 116$), but a distinct peak resulting from loss of water (*m/e* 98) and a weak signal resulting from loss of OH (*m/e* 99) were present. The main fragmentation-process is loss of one molecule of formaldehyde leading to a peak at *m/e* 86, followed by loss of a formyl radical and a second molecule of formaldehyde giving the base peak, *m/e* 57, and a peak at *m/e* 27. Evidence for the formation of *m/e* 57 from *m/e* 86 is given by the presence of a metastable peak at *m/e* 38. The compound also undergoes Type I cleavage[10], giving a peak at *m/e* 87. The cleavage of the C–C bond next to the primary hydroxyl group is of general occurrence[11], and leads to peaks at *m/e* 31 ($[CH_2OH]^+$) and *m/e* 85.

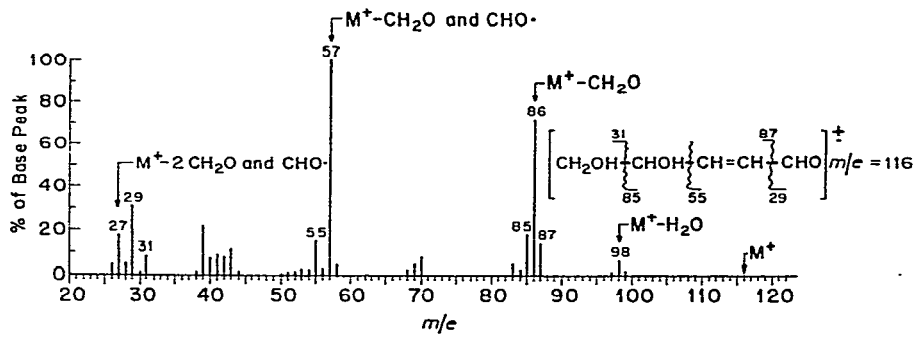

Fig. 2. Mass spectrum at 70 eV and fragmentation scheme of *trans*-2,3-dideoxy-D-*glycero*-pent-2-enose. The pure compound was introduced via a direct inlet into an LKB 9000 mass spectrometer (source temperature = 250°).

Direct analysis of the autoclaved mixture by gas chromatography–mass spectrometry gave additional information. The mass spectrum of the trimethylsilyl derivative[12] of **2** showed a weak molecular-ion peak at *m/e* 260 and an intense M − 15 peak at *m/e* 245, indicative of a dideoxypentenose having two *O*-trimethylsilyl groups. Hydrogenation of the aqueous solution of autoclaved **1** over palladium on charcoal, followed by trimethylsilylation, gave a chromatogram where only the peak assigned to compound **2** had disappeared and a new peak having longer retention time was present. All other peaks showed no change in retention time. The mass spectrum of the new peak exhibited an M − 15 peak at *m/e* 247, indicating the absorption of 1 mol of hydrogen. To provide further proof of structure, 4-*O*-acetyl-2,3-

dideoxy-D-*glycero*-pent-2-enose[4] was converted into its diacetate and analyzed by g.l.c.–mass spectrometry. The mass spectrum of this compound was identical to the mass spectrum of the diacetate prepared from **2**.

Compound **2** is most probably formed by β-elimination of water from the open-chain form of **1** and leads, as expected, principally to the *trans* form. The 2-deoxy sugars contain a greater proportion of the aldehyde form in aqueous solution than do completely oxygenated sugars[13], which do not form 2,3-unsaturated sugars upon autoclaving salt-free aqueous solutions. It is well known that 3-hydroxyaldehydes in solution readily eliminate water. Furthermore, dehydration of the 2-deoxy sugars does not occur in *N*,*N*-dimethylformamide after autoclaving, presumably because of the decreased stability of the *aldehydo* form in a non-hydroxylic solvent.

The reported dehydration appears general for 2-deoxyaldoses, as similar u.v.-absorbing products having high cytotoxicity are formed from 2-deoxy-D-*arabino*-hexose and 2-deoxy-D-*lyxo*-hexose under identical conditions[1]. However, each of these deoxy sugars give rise to three major compounds, which appear to consist of an equilibrium mixture of the open-chain aldehydes and cyclic pyranose and furanose forms.

EXPERIMENTAL

General methods. — The 60-MHz n.m.r. spectra were determined with a Varian A-60A spectrometer and 250-MHz spectra with a spectrometer at the Carnegie–Mellon University of Pittsburgh. I.r. spectra were recorded with a Beckman IR-12 spectrometer. Coupled g.l.c.–mass spectrometry was performed with an LKB 9000 instrument equipped with a 6 ft × 1/4 in. glass column, containing 3% OV-1 on Gas-Chrom Q, temperature-programmed from 90–140°, 2.5°/min, the temperature of the molecule separator was 220° and that of the ion source 250°, and the ionizing potential was 70 eV. Elementary analyses were made by Galbraith Laboratories, Inc., Knoxville, Tenn. 37921.

The t.l.c. and g.l.c. separations were made as follows: an autoclaved (2 h at 120°) 1% solution of **1** (3 ml) was freeze dried, the residue redissolved in methanol (1 ml), and resolved by t.l.c. on precoated plates of silica gel (Merck 5762, thickness 0.25 mm) with 50:50:1 ethyl acetate–methanol–acetic acid. After spraying with 2,4-dinitrophenylhydrazine reagent, 3 pale-yellow spots having R_F 0.18 (strong, 2-deoxy-D-*erythro*-pentose), 0.27 (moderate) and 0.63 (very weak), and one orange red spot having R_F 0.55 (moderate, from compound **2**) were visible. After heating at 120° (5 min) an additional spot of very weak intensity having R_F 0.67 appeared. For g.l.c., the autoclaved solution of **1** (1 ml) was freeze dried and trimethylsilated according to Sweeley *et al.*[12] and resolved with a Perkin–Elmer Model 900 chromatograph fitted with a flame-ionization detector and a 5 ft × 1/8 in. glass column packed with 3% OV-101 on Gas-Chrom Q. The helium flow-rate was 30 ml/min, the automatic temperature program was 100–120°, 5°/min, and the injection and manifold temperatures were 220 and 250°, respectively. G.l.c. showed 7 peaks having retention

times of 2.70 (1.2% of total peak area), 3.85 (9.0), 4.65 (0.6), 6.90, 7.75, 8.18 (78, 2-deoxy-D-*erythro*-pentose), and 8.5 min (9.5, compound **2**).

Isolation of aldehydo-trans-*2,3-dideoxy*-D-glycero-*pent-2-enose* (**2**). — A 1% aqueous solution of **1** (200 ml) was autoclaved for 2 h at 120° and water was then removed (rotary evaporator) at 20°. The viscous residue was dissolved in methanol (50 ml), 5 g of Unisil was added, and the methanol removed *in vacuo*. The Unisil, containing the adsorbed product, was placed on top of a glass column 1 cm in diameter filled with 15 g of Unisil previously equilibrated with 19:1 ethyl acetate–methanol. The column was eluted with 150 ml of 19:1 ethyl acetate–methanol followed by 100 ml of 1:1 ethyl acetate–methanol, and the separations were monitored by u.v. and t.l.c. The first fractions consisted mainly of **2** and the final fractions were mainly unaltered **1**. Further purification of **2** was performed by evaporation of the appropriate fractions in vacuo, redissolution of the residue in 2-3 ml of ethyl acetate, and resolution by column chromatography on Unisil (10 g) with pure ethyl acetate as eluant. The resultant solution of **2** was evaporated, the residue dissolved in 3 ml of ethyl acetate, and the mixture centrifuged to eliminate insoluble inorganic material derived from the column. The clear solution was diluted with ethyl acetate (30 ml) and kept at 4° with no detectable (u.v. or t.l.c.) decomposition, even after several weeks. However, storage of the pure compound *in vacuo* led to partial decomposition within 24 h. The net yield of **2** was 180 mg (10.4%).

Compound **2** was a colorless, viscous liquid, insoluble in petroleum ether, of low solubility in chloroform and highly soluble in ethyl acetate, methanol, and water; $[\alpha]_D^{25}$ −10.2° (*c* 1.5, methanol); $\lambda_{max}^{H_2O}$ 222 (ε 13,600), 308 nm (51); λ_{max}^{MeOH} 220 (ε 10,800), 320 nm (25); ν_{max} 3400 (br), 2940 w, 2900 w, 2750 w, 1680 s, 1640 w, 1410 w, 1150 m, 1110 m, 1070 m, 980 m, 880 w; n.m.r. (250 MHz, D_2O, internal $Me_3SiCD_2CD_2CO_2Na$, Fig. 1): δ 9.00 ($J_{1,2}$ 8 Hz, H-1), 6.85 ($J_{2,3}$ 14 Hz, $J_{3,4}$ 3 Hz, H-3), 6.10 ($J_{2,4}$ 1.5 Hz, H-2), 4.30 (8 peaks, H-4), 3.48 ($J_{5a,5b}$ 12 Hz, $J_{4,5a}$ 4 Hz, H-5a), 3.38 ($J_{4,5b}$ 6 Hz, H-5b); n.m.r. (60 MHz, Me_2SO-d_6, internal tetramethylsilane, Fig. 1): δ 9.55 ($J_{1,2}$ 8 Hz, H-1), 7.10 ($J_{2,3}$ 16 Hz, $J_{3,4}$ 4 Hz, H-3), 6.20 ($J_{2,4}$ 2 Hz, H-2), 5.25 (*J* 5 Hz, CHOH), 4.75 (*J* 6 Hz, CH_2OH), 4.30 (approx. triplet, *J* 6 Hz, H-4), 3.45 (approx. triplet, *J* 6 Hz, H-5), 3.35 (traces of HOH in the solvent); mass spectrum: *m/e* 99 ($M^+ - 17$, 2%), 98 (6), 87 (14), 86 (72), 85 (18), 83 (5), 70 (8), 69 (5), 57 (100), 55 (15), 43 (12), 42 (8), 41 (9), 40 (7), 39 (22), 31 (8), 29 (31), 28 (5), and 27 (17). The origin of these peaks is shown in Fig. 2.

Anal. Calc. for $C_5H_8O_3$: C, 51.8; H, 6.89. Found: C, 51.9; H, 7.02.

The 2,4-dinitrophenylhydrazone of **2** was prepared from **2** (30 mg) by treatment with 2,4-dinitrophenylhydrazine (60 mg) dissolved in M HCl (25 ml) at room temperature (yield 65 mg, 85%). From ethyl acetate the product was obtained as an orange-red solid, m.p. 188.5°; $\lambda_{max}^{CHCl_3}$ 270 (ε 26,300); $\lambda_{max}^{0.25M\ alc.\ NaOH}$ 455 (ε 28,600); ν_{max}^{KBr} 3360 m (br), 3290 m, 3100 w, 2940 w, 1620 s, 1600 m, 1520 m, 1330 w, 1310 s, 1220 w, 1145 m, 1085 m, 1050 w, 835 w, 745 w, 730 w; n.m.r. (60 MHz, Me_2SO-d_6, internal tetramethylsilane): δ 11.5 (s, 1 proton, NH), 8.8 (d, 1 proton, arom. H-3′, $J_{3',5'}$ 2.5 Hz), 8.45 (m, 2 protons, arom. H-5′ and H-1, $J_{5',6'}$ 10 Hz), 7.86 (d, 1 proton,

arom. H-6'), 6.45 (m, 2 protons, H-2, H-3), 5.06 (d, 1 proton, OH-4, J 5 Hz), 4.71 (t, 1 proton, OH-5, J 5 Hz), 4.21 (m, 1 proton, H-4), and 3.46 (approx. t, 2 protons, $J_{4,5}$ 6 Hz).

Anal. Calc. for $C_{11}H_{12}N_4O_6$: C, 44.6; H, 9.08; N, 18.9. Found: C, 44.7; H, 9.06; N, 18.0.

*4,5-Di-O-acetyl-2,3-dideoxy-*D-glycero-*pent-2-enose* (3). — 4-*O*-Acetyl-2,3-dideoxy-D-*glycero*-pent-2-enose[4] and **2** were converted into **3** by treatment with excess acetic anhydride–pyridine and analyzed by coupled g.l.c.–mass spectrometry: *m/e* 170 (M^+-30, 0.3%), 168 (0.1), 167 (0.1), 161 (0.1), 160 (0.2), 128 (14), 98 (14), 86 (26), 81 (2.3), 73 (3.0), 57 (4.0), 50 (4.0), 43 (100).

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