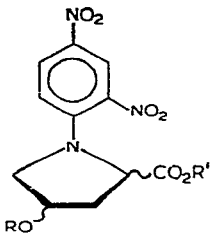
A 4-Hydroxy-L-proline glycoside of 2-amino-2-deoxy-D-glucose*

Previous papers from this laboratory described the synthesis and stability of *N*-2,4-dinitrophenylserine[1] and *N*-2,4-dinitrophenylthreonine[2] glycosides of 2-amino-2-deoxy-D-glucose. The present paper extends this series to 4-hydroxy-L-proline[3–6]. Glycosides of hydroxyproline are postulated as connective linkages in fibril proteins[7,8] and in the junction of glycoproteins to polysaccharide ground-substance[9–12].

A Koenigs–Knorr condensation[2,13,14] was used to couple 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[15] to the syrupy *N*-2,4-dinitrophenyl-4-hydroxy-L-proline methyl ester (**2**). A crystalline glycoside (**4**) was isolated by preparative thin-layer chromatography (t.l.c.). The structure of the glycoside was confirmed by elementary analysis, optical rotation, i.r. and n.m.r. spectra, and by acid hydrolysis and identification of the products by t.l.c. The H-1 signal of the hexosamine moiety (τ 5.20) showed a splitting ($J_{1,2}$ 9 Hz) indicative of the β-D anomeric configuration[16–18]. An orthoacetate structure for the glycoside[19,20] was considered improbable because no signal at *ca.* τ 8.3–8.5 (orthoacetate methyl group[21]) was observed. The acid stability of the glycoside, as well as steric considerations, also ruled out an orthoacetate structure.

Pure *trans*-4-hydroxy-L-proline was the starting material for synthesis of the *N*-dinitrophenyl derivative (**1**), the *N*-dinitrophenyl methyl ester (**2**), and the 4-*O*-*p*-tolylsulfonyl-*N*-dinitrophenyl methyl ester (**3**). Substances **1–3** gave n.m.r. spectra very similar to the hydroxyproline signals in the spectrum of the glycoside **4**. Nevertheless,



1 R=H, R'=H
2 R=H, R'=Me
3 R=Ts, R'=Me
4 R=2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl R'=Me

the geometric configuration of the pyrrolidine ring in **4** cannot be stated definitively, because the effects of esterification or glycosidation on the ring configuration are not known. The structure of the glycoside is simply assigned as 4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-2,4-dinitrophenyl-4-hydroxy-L-proline methyl ester (**4**).

Glycosides of *N*-2,4-dinitrophenylserine[1] and *N*-2,4-dinitrophenyl-threonine[2],

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and also substance 3, are unstable to base, but the glycoside 4 is quite stable to base. Glycosides of 4-hydroxy-L-proline in glycoprotein substances should likewise possess alkaline stability.

EXPERIMENTAL

Ascending t.l.c. was performed on silica gel G (E. Merck Co., Darmstadt, Germany; activated for 1 h at 110°), with 3:1:1 *n*-butyl alcohol–acetic acid–water (solvent A,) 1:1 ethyl acetate–ether (solvent B), 1:6 ethyl acetate–ether (solvent C), and 1:3 ethyl acetate–petroleum ether (b.p. 30–60°) (solvent D). M.ps. were determined in capillaries with a National Instruments Co. "Melt Meter" and are uncorrected. I.r. spectra were recorded with a Perkin–Elmer Model 137 "Infracord" spectrometer with potassium bromide pellets. N.m.r. spectra were recorded at 60 MHz with a Varian A-60 spectrometer with saturated solutions in chloroform-*d* or acetone-d_6, with tetramethylsilane as internal reference. Deuteration was effected by shaking the sample solutions with deuterium oxide. All n.m.r. spectra gave satisfactory integral ratios for protons. Microanalyses were performed by Weiler and Strauss Microanalytical Laboratories, Oxford, England.

N-*2,4-Dinitrophenyl*-trans-*4-hydroxy*-L-*proline* (**1**). — *trans*-4-Hydroxy-L-proline, $[\alpha]_D^{25}$ −76° (*c* 1, water), was 2,4-dinitrophenylated by the method of Sober and Rao[22]. The product, recrystallized from anhydrous ethyl ether–petroleum ether, had m.p. 174–176° (lit.[22] m.p. 175°); R_F 0.85 (solvent A); $[\alpha]_D^{25}$ −1142±10° (*c* 0.14, acetone). I.r. spectral data were in accord with those reported for **1** by Friedberg[23]. N.m.r. data: τ 1.37 (1-proton doublet, $J_{3',5'}$ and $J_{3',6'}$ 3 Hz, H-3′), τ 1.76 (1-proton quartet, $J_{5',3'}$ and $J_{5',6'}$ 9 Hz, H-5′), τ 2.94 (1-proton doublet, $J_{6',5'}$ and $J_{6',3'}$ 8 Hz, H-6′), τ 5.15 (1-proton triplet, $J_{2,3}$ 8 Hz, H-2), τ 5.35 (1-proton broad singlet, H-4), τ 6.16 (2-proton multiplet, H-5), τ 7.35 (2-proton multiplet, H-3), τ 3.11 (1-proton broad singlet, disappeared on deuteration, CO_2H), τ 7.40 (1-proton broad singlet, disappeared on deuteration, OH).

N-*2,4-Dinitrophenyl*-trans-*4-hydroxy*-L-*proline methyl ester* (**2**). — Substance **1** (5.4 g, 0.0181 mole) was dissolved in abs. methanol (100 ml). Following the esterification procedure of Fischer (*cf.* ref. 24) the solution was saturated with anhydrous hydrogen chloride and refluxed for 20 min (reaction completion discerned by t.l.c., product R_F 0.66, solvent B). The syrup was codistilled with isopropyl alcohol several times to remove hydrogen chloride. The syrupy product was dried *in vacuo* to a yellow, hromatographically homogeneous powder; $[\alpha]_D^{25}$ −884±10° (*c* 0.32, acetone); λ_{max}^{KBr} 2.77 (OH), 5.70 (CO_2Me), 6.21, 6.32 (aryl), 6.56, 6.65 (NO_2), 6.96, 7.53, 8.27, 8.54, 8.70, 8.86, 9.30, 10.04, 10.50, 10.92, 11.36, 12.01, 12.32, 13.16, 13.44, 13.71, 14.70 μm; n.m.r. data: τ 6.24 (3-proton singlet, OMe). Other chemical shifts were nearly identical with those of **1**.

Anal. Calc. for $C_{12}H_{13}N_3O_7$: C, 46.31; H, 4.21; N, 13.50. Found: C, 46.41; H, 4.44; N, 13.65.

N-*2,4-Dinitrophenyl-4-hydroxy-4*-O-p-*tolylsulfonyl*-L-*proline methyl ester* (**3**). —

Substance **2** (0.5 g, 1.6 mmole) was dissolved in pyridine (25 ml) containing *p*-toluenesulfonyl chloride (2 g, 15.7 mmoles) and the solution was kept in the dark at room temperature. Complete conversion into **3**, R_F 0.95 (solvent B), was observed after 7 days. After workup[25], the resulting syrup was dissolved in benzene (10 ml), petroleum ether (b.p. 30–60°) added to turbidity, and the solution was refrigerated for 2 days to give dense, yellow crystals, yield, 0.23 g (31%). Although t.l.c. showed more product to be present in solution, no further crop of crystals was isolable. The product, recrystallized from benzene–petroleum ether (b.p. 30–60°), had m.p. 112–113°; $[\alpha]_D^{25}$ −640 ±10° (*c* 0.34 acetone); λ_{max}^{KBr} 5.73 (CO_2Me), 6.23, 6.34 (aryl), 6.55, 6.67 (NO_2), 6.92, 7.35, 7.54, 8.20, 8.50, 8.88, 9.14, 9.49, 10.05, 10.26, 10.47, 11.12, 11.84, 12.35, 13.43, 13.75, 14.40 μm; n.m.r. data: τ 4.80 (1-proton broad singlet, H-4), τ 2.51 (4-proton quartet, aromatic A_2B_2 system, *p*-tolyl group), τ 7.51 (3-proton singlet, *p*-tolyl Me). With the exception of the OH and H-4 signals, the other n.m.r. signals were identical with those of **2**.

Anal. Calc. for $C_{19}H_{19}N_3O_9S$: C, 49.03; H, 4.11; N, 9.03; S, 6.89. Found: C, 49.19; H, 4.27; N, 9.20; S, 7.18.

In the presence of base, or on prolonged exposure to light, this compound underwent decomposition (t.l.c., solvent D).

4-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-N-2,4-dinitrophenyl-4-hydroxy-L-proline methyl ester (**4**). — A Koenigs–Knorr coupling reaction was carried out according to Helferich's[13,14] procedure, as modified by Hardy[26], and utilized in a previously reported synthesis of a threonine glycoside[2]. 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride was prepared according to Horton[15]. Reaction progress was monitored by t.l.c. (solvent C, sulfuric acid detection). Maximum formation of product (at 2.5 h) coincided with the appearance of a decomposition product, R_F 0.00. After workup the mixture was resolved by preparative t.l.c.[2]. Six isolable zones were separated in solvent C; R_F 0.78 (yellow), 0.56 (yellow), 0.45 (yellow) mixed with 0.43 (colorless, H_2SO_4 detection), 0.31 (yellow), and 0.00 (brown). Upon concentration of the eluates (ethyl acetate) from these zones, each was preparatively rechromatographed. The purified eluates were evaporated, dissolved in benzene, and treated with decolorizing carbon. The filtrates were concentrated to syrups which were again dissolved in benzene (5 ml). The benzene solutions were diluted with petroleum ether (b.p. 30–60°) until turbid and refrigerated overnight. The zone having R_F 0.31 crystallized, but the product was not characterized. The zone having R_F 0.45 did not crystallize. The zone having R_F 0.56 crystallized giving **4**, yield 0.22 g (7%), m.p. 119–120°; $[\alpha]_D^{25}$ −400 ± 10° (*c* 0.12, chloroform); λ_{max}^{KBr} 2.90 (NH), 3.32 (CH), 5.70 (CO_2Me, OAc), 5.94 ($CONH_2$), 6.20 (aryl), 6.30 (NO_2), 6.65, 6.94, 7.28, 7.49, 8.09, 8.49, 8.69, 8,96, 9.55, 10.94, 11.99, 13.12, 13.39, 13.70, 14.61 μm; n.m.r. data: τ 3.41 (1-proton doublet, $J_{NH,2}$ 10 Hz, disappears on deuteration, NH of hexosamine), τ 5.20 (1-proton doublet, $J_{1,2}$ 9 Hz, H-1), τ 4.01-4.97 (6-proton multiplet, H-2, H-3, H-4, H-5, H-6), τ 7.83, 7.91, 7.97 (9 protons, OCOMe), τ 8.15 (3-proton singlet, NAc). Other chemical shifts and coupling constants were similar to those for **2** (OH of **2** absent).

Anal. Calc. for $C_{26}H_{32}N_4O_{15}$: C, 48.75; H, 5.04; N, 8.75. Found: C, 49.09; H, 5.21; N, 8.43.

To a solution of **4** in methanol (5 ml) was added 1 drop of 10% hydrochloric acid and 1 drop of water. The solution was refluxed for 6 h, at the end of which time, t.l.c. (solvent A, sulfuric acid as well as ninhydrin locating reagent) indicated complete disappearance of starting material. Only two zones formed, R_F 0.14 and R_F 0.74 (mobilities identical to those of 2-amino-2-deoxy-D-glucose and *N*-2,4-dinitrophenyl-hydroxyproline, respectively).

The glycoside **4** was treated with base[2] (up to 10 equivalents of sodium carbonate or sodium methoxide in methanol). T.l.c. indicated no scission to hexosamine and an elimination product of the aglycon. This stability of **4** in the presence of base was contrasted (t.l.c.) to that of **3** above and other glycosides of *N*-2,4-dinitrophenyl-hydroxyamino acid esters reported[1,2].

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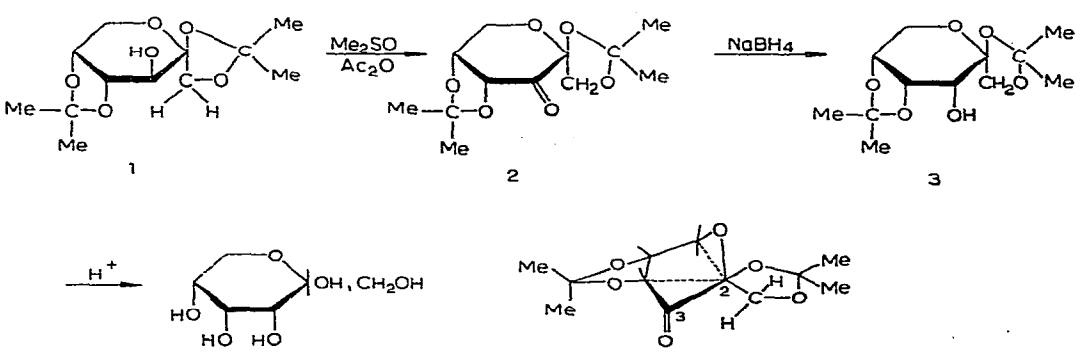
A new synthesis of D-psicose (D-*ribo*-hexulose)

D-Psicose has previously been prepared by the epimerization of D-altrose[1,2], and by the action of diazomethane on tetra-*O*-acetyl-D-ribonyl chloride with subsequent deacetylation of the resulting *keto*-D-psicose pentaacetate[3]. Recent work[4,5] in which methyl sulfoxide–acetic anhydride was used to oxidize secondary alcoholic groups to ketonic groups suggested that 1,2:4,5-di-*O*-isopropylidene-β-D-*erythro*-2,3-hexodiulose (**2**) might be preparable by similar oxidation of 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose (**1**).

Reduction of this diulose acetal (**2**) should then afford a mixture of 1,2:4,5-di-*O*-isopropylidene-β-D-*ribo*-hexulopyranose (**3**) and the corresponding D-fructose acetal (**1**). Study of a molecular model of **2** suggested that the favored conformer is that skew form (S^{O}_{3}) in which both dioxolane rings are *exo* (**2a**), and indicated that borohydride reduction would favor formation of **3**. From seven of the eleven conformations of **2** considered, the D-*ribo* isomer (**3**) alone would be expected, whereas, for the four others,



1 2 3 4 2a