THE REACTION OF GALACTOSE WITH HYDRAZINE AT ELEVATED TEMPERATURE[1]

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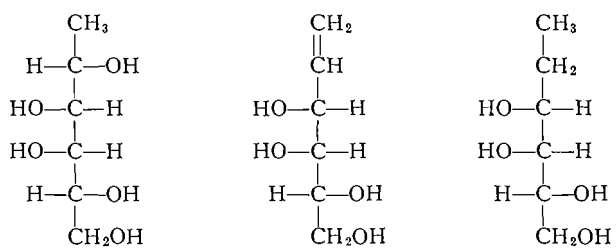
An investigation (1) of the hydrazinolysis of the α_1-acid glycoprotein of human plasma is in progress in this laboratory. We wished to study the effects of hydrazine on the carbohydrate moiety of the glycoprotein and therefore investigated the effect of hydrazine on some model compounds. It has already been shown (1) that the optimum results are obtained with α_1-glycoprotein using a reaction time of 48 h and this time therefore was adopted for the present study of the effect of hydrazine on galactose. Yosizawa and Sato (2) treated reducing sugars in 10% (w/v) solution in anhydrous hydrazine at 100° for 10 h (in sealed tubes). Under these conditions they reported that 20.9% of galactose was destroyed with production of a pentose, a 2,5-anhydro-hexose, and other unidentified products. In contrast, Kaverzneva and Tsi De-Fan (3) concluded that mannose was not decomposed (appreciably) when treated with anhydrous hydrazine for 4 h at 100°. The results obtained by us with D-galactose are reported here.

When a 2% (w/v) solution of galactose in 95+% hydrazine was boiled under reflux for 48 h, the products contained little unchanged galactose. Paper chromatography of the products and use of the silver nitrate spray (4) indicated the presence of three major components and traces of others. One of these major products, compound A, was obtained crystalline from the crude reaction mixture after removal of excess hydrazine. Paper chromatographic separation of the residual syrup yielded the other major products, B and C.

Compound A, which was obtained in 33% yield, was identified as L-fucitol (*syn*-1-deoxy-D-galactitol) on the basis of its optical rotation, and that of the derived pentabenzoate and on the basis of the melting point and mixed melting point with authentic specimens of the alcohol and its pentabenzoate.

Compound B was obtained as a syrup contaminated with traces of a pentose and compound C. It was chromatographically identical with 3,4,5,6-tetrahydroxy-L-*lyxo*-hex-1-ene, prepared by the acid hydrolysis and subsequent reduction with sodium borohydride of methyl 5,6-didehydroxy-α-L-*arabino*-hexoside (5). Ozonolysis of compound B yielded D-lyxose, whereas ozonolysis of the synthetic tetrahydroxy-L-*lyxo*-hex-1-ene yielded L-lyxose. Compound B is thus probably 3,4,5,6-tetrahydroxy-D-*lyxo*-hex-1-ene.



Compound A Compound B Compound C

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Catalytic hydrogenation of 3,4,5,6-tetrahydroxy-L-*lyxo*-hex-1-ene and of compound B gave products which were chromatographically identical with compound C. This is, therefore, tentatively identified as 1,2-dideoxy-D-*lyxo*-hexose (*syn* 5,6-dideoxy-D-*arabino*-hexose).

The reaction of hydrazine with galactose to give these three products is complex. Initial hydrazone formation and subsequent Wolff–Kishner reduction of the hydrazone under the slightly basic conditions of the reaction would account for compound A. Similarly, compound C could arise from a reduction of the osazone but compound B must arise from an elimination reaction, the mechanism of which is obscure.

EXPERIMENTAL

For paper chromatography the solvent systems were (*a*) 1-butanol, ethanol, water (3:1:1, v/v), (*b*) ethyl acetate, acetic acid, water (6:3:2, v/v). Rates of migration on paper chromatograms are quoted relative to the rate of movements of the solvent front (R_F), to that of fucose (R_{Fucose}), and to that of galactose (R_{Gal}). All solutions were concentrated under diminished pressure.

Optical rotations were measured at 20 ± 2° in water unless otherwise stated.

Hydrazinolysis of Galactose

D-Galactose (2.057 g) in 95+% hydrazine (Eastman; 100 ml) was boiled under reflux for 48 h using a "Drierite" guard tube. Excess of hydrazine was removed by distillation and the last traces removed by drying *in vacuo* over concentrated sulfuric acid. Chromatographic examination of the residual syrup in solvent (*a*) (silver nitrate spray) indicated the presence of three major components with R_{Gal} values of 2.62, 3.82, and 4.6, together with minor components, R_{Gal} 4.73, and a component streaking behind R_{Gal} 2.62. The *p*-anisidine hydrochloride spray reagent (6) showed only traces of material resembling a pentose.

Isolation and Characterization of Compound A

The hydrazine-free syrup crystallized on being moistened with methanol and cooled. The crystals were filtered, washed with methanol at −15°, and dried (0.559 g), m.p. 153–156°. After two recrystallizations from methanol and one from acetone the melting point was 155–157°, mixed melting point with authentic L-fucitol 156–157; $[\alpha]_D$ +185° (*c*, 0.216 in a 4:1 (v/v) mixture of 5% ammonium molybdate and 1 *N* sulfuric acid). Richtmyer and Hudson (7) give $[\alpha]_D$ +196 for this compound and $[\alpha]_D$ −195° for D-fucitol in acidified ammonium molybdate solution. The benzoate of compound A was prepared and, after recrystallization had m.p. 150–151°; mixed melting point with authentic L-fucitol benzoate (m.p. 148–150°) was 148–150°; $[\alpha]_D$ −4.1 (*c*, 4.42 in chloroform); lit. values (8), m.p. 149–150°; $[\alpha]_D$ −5.96° (in chloroform).

Anal. Calcd. for $C_{41}H_{34}O_{10}$: C, 71.7; H, 4.95; molecular weight, 686. Found: C, 71.68, 71.49; H, 5.12, 5.2; molecular weight, 657 (determined on a 0.5% solution in chloroform using a Mechtrolab osmometer).

Isolation and Characterization of Compounds B and C

The syrup (0.96 g) remaining after crystallization of compound A was separated on sheets of Whatman 3 MM paper using multiple development in solvent (*b*). The compounds were located by spraying guide strips with the silver nitrate spray (4) and three compounds recovered by extraction of the appropriate zones with water. Compound A (0.12 g) yielded a benzoate with melting point and mixed melting point with authentic L-fucitol benzoate of 147–149°.

Compound B (0.193 g) was a syrup with R_F 0.50, R_{Fucose} 1.82 in solvent (*a*) and was contaminated with traces of a pentose and compound C. It reduced neutral permanganate solution and decolorized bromine water. Subsequently, it was shown to be chromatographically indistinguishable from 3,4,5,6-tetrahydroxy-L-*lyxo*-hex-1-ene, in the two solvent systems.

Compound C (0.293 g) was a syrup with R_F 0.57, R_{Fucose} 2.08 in solvent (*a*). It had $[\alpha]_D$ +2.6°. It was chromatographically indistinguishable from the products obtained by catalytic reduction of compound B and of 3,4,5,6-tetrahydroxy-L-*lyxo*-hex-1-ene in the two solvent systems.

Synthesis of 3,4,5,6-Tetrahydroxy-L-lyxo-hex-1-ene

Methyl 5,6-didehydroxy-α-L-*arabino*-hexoside (0.8 g), prepared by the method of Ball, Flood, and Jones (5), was hydrolyzed with *N* sulfuric acid at 100° for 1 h. The solution was cooled, neutralized with barium hydroxide, filtered, and then concentrated to 10 ml. Sodium borohydride (1 g) was then added and the solution left for 16 h. Acetic acid was added to pH 6 and the solution passed through a column of Amberlite IR 120 (H^+ form). After concentration of the eluate to dryness, boric acid was removed as methyl borate by distilling off portions (10 ml) of methanol. The product was then purified by chromatography on sheets of Whatman 3 MM paper as described previously (yield, 0.18 g).

Ozonolysis of 3,4,5,6-Tetrahydroxy-L-lyxo-hex-1-ene

The above compound (0.15 g) in methanol (25 ml) was ozonolyzed for 15 min with external cooling. The

solution was then boiled under reflux for a few minutes with zinc powder and filtered, and the filtrate concentrated to a syrup. Chromatographic examination in solvents (*a*) and (*b*) indicated that the main product was lyxose. This product was separated from impurities by chromatography on filter paper as described above and yielded syrupy L-lyxose, $[\alpha]_D$ +7.0°. The *p*-tolylsulfonylhydrazone derivative had m.p. 141–142° (lit. 141° (9)).

Ozonolysis of Compound B

Compound B (0.15 g) was ozonolyzed and the product separated as described above to yield D-lyxose (89 mg), $(\alpha)_D$ −10.0°. The *p*-tolylsulfonylhydrazine derivative had m.p. 141–142°, mixed melting point with the *p*-tolylsulfonylhydrazine derivative of authentic D-lyxose (m.p. 142–143°) 142–143°.

Reduction of 3,4,5,6-Tetrahydroxy-L-lyxo-hex-1-ene

This compound (0.405 g) in ethanol (100 ml) was reduced with hydrogen (5 atm) over 5% palladium on charcoal for 10 h. After being filtered, the solution was evaporated to a syrup (0.400 g). Paper chromatography showed the absence of starting material and production of a compound identical in R_F value with compound C.

Reduction of Compound B

Compound B (5 mg) was reduced as above to give a compound identical in chromatographic mobility with compound C.

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THE DIHYDROXYSTRYCHNINES

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Edward (1) pointed out that reagents should attack the double bond of strychnine (I) (2) from the convex side of the molecule. He assigned structure II to 21,22-dihydroxydihydrostrychnine, which is obtained from strychnine by oxidation with potassium permanganate (3) or osmium tetroxide (4). Prelog and Kathriner (5) reported the preparation

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