Carbohydrate Research, 26 (1973) 225–226

Note

An unsaturated disaccharide found in quenched acetolysates of chitin

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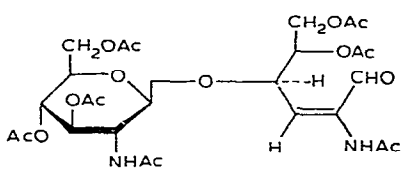
(Received July 7th, 1972; accepted for publication, August 18th, 1972)

Chitobiose octa-acetate, as prepared by acetolysis of chitin[1,2], is often contaminated by an acetylated amino-sugar of slightly greater R_F value (t.l.c., Silica Gel G; ethyl acetate–acetone, 2:1), which is readily distinguishable from the accompanying 2-amino-2-deoxy-D-glucose penta-acetate. Material for characterization was obtained by repeated fractionation on Kieselgel (Merck, 0.05–0.2 mm) of the chloroform extract of quenched acetolysates (acetolysis being terminated by pouring the reaction mixture into an excess of ice-cold 15% aqueous sodium acetate and storage overnight). Elution with ethyl acetate containing 0→30% of acetone gave good separation of the various acetates. The unknown (**1**) was eluted together with chitobiose octa-acetate at an acetone concentration of 20–25%, and the yield from chitin (10 g) varied between 20 and 150 mg in different runs. Compound **1** had m.p. 141–142° (from methanol–ether), $[\alpha]_D^{20}$ +40° (*c* 1.02, chloroform) (Found: C, 50.34, 50.47; H, 5.96, 5.07; N, 4.49. $C_{26}H_{36}N_2O_{15}$ calc.: C, 50.6; H, 5.84; N, 4.54%). Compound **1** decolorised bromine water, gave a positive Schiff test, and a positive Morgan–Elson reaction after deacetylation. Acid hydrolysis (5M HCl, 100°, 3 h) gave 2-amino-2-deoxyglucose, identified by t.l.c. The following data indicate that **1** has the structure 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-5,6-di-*O*-acetyl-2,3-dideoxy-*aldehydo*-D-*erythro*-hex-2-enose.



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An αβ-unsaturated aldehydic function was present in the molecule, as shown by the characteristic[3] u.v. absorption at $\lambda_{max}^{H_2O}$ 247 nm (ε 6.2×10^3), which was abolished on reduction with borohydride. The 2,4-dinitrophenylhydrazone had λ_{max}^{EtOH} 375 nm (ε 2.56×10^4). The 100-MHz p.m.r. spectrum ($CDCl_3$) of **1** showed signals at τ 0.7 (*s*, 1 proton, CHO), 2.3 (broad *s*, 1 proton, disappears on deuteration, NH), 3.9 (*d*, 1 proton, *J* 5.7 Hz, olefinic H), 4.2 (broad *d*, 1 proton, *J* 8.5 Hz, disappears on

deuteration, NH), 5.2 (*d*, 1 proton, *J* 8.5 Hz, *ax* anomeric H), 8.0 (overlapping signals, 21 protons, 6 OAc, NAc). The remaining multiplets in the spectrum accounted for 10 protons.

Reduction of **1** with sodium borohydride in aqueous boric acid gave an alcohol, which was isolated by extraction into ethyl acetate. Acetylation (pyridine–acetic anhydride) of this alcohol gave an acetate **2**, m.p. 118–119° (from ethyl acetate–hexane). (Found: C, 50.81; H, 5.95; N, 4.37. $C_{28}H_{40}N_2O_{16}$ calc.: C, 50.90; H, 6.06; N, 4.24%.) The 100-MHz p.m.r. spectrum of **2** ($CDCl_3$) showed signals at τ 2.3 (broad *s*, 1 proton, disappears on deuteration, NH), 3.9 (*d*, 1 proton, *J* 4.5 Hz, olefinic H), 8.0 (overlapping signals, 24 protons, 8 Ac). The remaining multiplets in the spectrum accounted for 14 protons.

The 2,4-dinitrophenylhydrazone, obtained by treating an ice-cold, aqueous solution of **1** with 2,4-dinitrophenylhydrazine in 0.1M hydrochloric acid, could not be obtained crystalline, although it was homogeneous by t.l.c. (Silica Gel G; ethyl acetate–acetone, 4:1). It gave the expected p.m.r. spectrum (100 MHz, $CDCl_3$) with signals at τ 1.1 (*s*, 1 proton, aromatic NH), 1.03 (unsymmetrical *d*, 1 proton, H-3 of aromatic ring), 1.76 (*d*, *J* 4 Hz, 1 proton, H-5), 2.2 (*s*, 1 proton, CH=N), 2.2 (*d*, 1 proton, H-6), 2.36 (*s*, 1 proton, NH), 3.8 (broad *d*, *J* 4.5 Hz, 1 proton, NH), 4.1 (*d*, *J* 4.5 Hz, 1 proton, olefinic H), 8.0 (overlapping signals, 21 protons, 7 Ac). The remaining multiplets accounted for 11 protons.

That **1** is an artifact of the work-up procedure was shown by subjecting small samples of di-*N*-acetylchitobiose, di-*N*-acetylchitobiose methyl glycoside, and tri-*N*-acetylchitotriose to the acetolysis conditions[2], followed by quenching. T.l.c. of chloroform extracts showed that significant amounts of **1** were formed from the glycosides of di-*N*-acetylchitobiose, but not from the disaccharide itself. In contrast, quenched acetolysates of di-*N*-acetylchitobiose contained an unsaturated aldehyde, presumably 2-acetamido-4,5,6-tri-*O*-acetyl-2,3-dideoxy-*aldehydo*-D-*erythro*-hex-2-enose, having an R_F value greater than that of 2-amino-2-deoxy-D-glucose penta-acetate.

Unsaturation is presumably the result of β-elimination undergone by a (possibly) acyclic molecular species formed in the acetolysis medium; such acyclic intermediates have been postulated to occur in acetolysis of glycosides[4,5]. The preparative significance of the above reactions is now being explored.

ACKNOWLEDGMENT

I thank Professor J. S. Mitchell for his support.

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