Note

Synthesis of gentianose

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In connection with previous papers on sucrose chemistry[1,2], we now report the chemical synthesis of gentianose undecaacetate (3) and gentianose (4).

Gentianose is found in gentians and is obtained by extraction of gentian root with aqueous ethanol[3,4]. Gentianose has the structure *O*-β-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl β-D-fructofuranoside, and has been synthesized by the action of levansucrase on sucrose and gentiobiose[5]. An anomeric isomer, *O*-α-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosyl β-D-fructofuranoside, was also synthesized with the aid of an enzyme[6], but an unequivocal chemical synthesis of gentianose has not been described. Gentiobiose, obtained by a partial hydrolysis of gentianose[3], has been synthesized chemically by several authors[7–11].

We have now synthesized gentianose undecaacetate (3) in 69% yield by the condensation of 2,3,4,1′,3′,4′,6′-hepta-*O*-acetylsucrose[2,12] (2) with tetra-*O*-acetyl-α-D-glucopyranosyl bromide (1). The product **3** was identified by p.m.r. spectroscopy. Deacetylation of **3** afforded gentianose (4), identical in all respects with the natural product.

RESULTS AND DISCUSSION

The condensation of the sucrose derivative **2** with the bromide **1** in the presence of mercuric cyanide afforded the acetylated trisaccharide **3**. Deacetylation of **3** in 0.05M sodium methoxide yielded gentianose (4) in 70% yield.

The p.m.r. spectrum of **3** showed eight sharp signals at high field (δ 2.02–2.17), attributed to eleven acetoxy groups, and two doublets at δ 5.70 and 4.57 due to the two anomeric protons. The doublet at δ 5.70 (*J* 3.7 Hz) was attributed to the equatorial anomeric proton of the D-glucosyl residue in the sucrose moiety of **3**, since the corresponding proton in the spectrum[13] of sucrose octaacetate resonated as a doublet at δ 5.68 (*J* 3.7 Hz). Another doublet, at δ 4.57 (*J* 7.6 Hz), was assigned to the axial anomeric proton of a β-D-glucosyl group. This assignment of the signal was consistent with the fact that the anomeric proton of the nonreducing residue of β-gentiobiose octaacetate shows its signal at δ 4.54 (*J* 7.5 Hz) as a doublet[14], and

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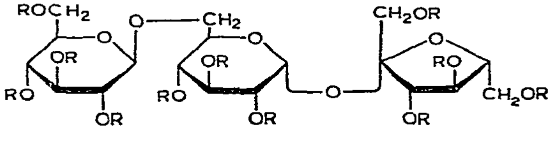

3 R = Ac
4 R = H

that of cyclohexyl tetra-*O*-acetyl-β-D-glucopyranoside appears at δ 4.58 (*J* 7.0 Hz) as a doublet.

The signal of a methylene bridge[13] was observed at δ 3.66 as a multiplet, strongly supporting the existence of a bond between O-6 of sucrose and a D-glucosyl group, because the methylene bridge signal of β-gentiobiose octaacetate* resonates at δ 3.68 and that of 1-kestose undecaacetate appears in the same region[13].

In the spectrum of **4**, there were two doublets, at δ 5.44 (*J* 3.2 Hz) and 4.49 (*J* 7.2 Hz), which were assigned to an equatorial and an axial anomeric proton of a D-glucosyl group, respectively; natural gentianose showed these doublets at the same positions. Furthermore, sucrose in D_2O showed the signal of an anomeric proton at δ 5.45 as a doublet (*J* 3.2 Hz), and gentiobiose in D_2O showed the signal of the anomeric proton of the nonreducing residue at δ 4.54 as a doublet (*J* 7.2 Hz). Thus the spectrum of **4** was consistent with that expected for the trisaccharide.

EXPERIMENTAL

General. — Solutions were evaporated below 50° under diminished pressure in a rotary evaporator. Melting points were determined in a liquid bath and are uncorrected. Optical rotations were measured with a Carl Zeiss photoelectric polarimeter. P.m.r. spectra were determined with Varian A-60D and Varian HA-100D instruments and peak positions are given in δ-values. I.r. spectra were recorded with a Hitachi EPI-2 spectrophotometer. T.l.c. was performed on silica gel (Wakogel B-10) plates.

*2,3,4,1',3',4',6'-Hepta-*O*-acetylsucrose* (**2**). — The product was prepared by the method of Otake[12].

Gentianose undecaacetate (**3**). — A mixture of tetra-*O*-acetyl-α-D-glucopyranosyl bromide[15] (**1**) (776 mg, 1.83 mmoles), **2** (664 mg, 1.04 mmoles), "Drierite" (550 mg), mercuric cyanide (527 mg), and nitromethane (12 ml) was vigorously agitated for 28 h at room temperature, in the dark and with protection from atmospheric moisture. The mixture was filtered and the filtrate evaporated to a syrup, which was chromatographed on a column of silica gel (Wakogel C-200, 80 g, 2.8 × 26 cm) with benzene:ethyl acetate (1:1, v/v) as eluant, and each fraction was monitored by t.l.c. with the same solvent. Fractions having R_F 0.36 were combined and evaporated to a glassy solid (698 mg, 69%), m.p. 80–93°, $[\alpha]_D^{20}$ +32.2° (*c* 0.85, chloroform). The pro-

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*The p.m.r. spectrum of β-gentiobiose octaacetate was recorded at 100 MHz in $CDCl_3$ with a Japan Electron Optics JNM-4H spectrometer by Dr. Michio Matsui of Tokyo Biochemical Research Institute.

duct showed a single spot on t.l.c. with two solvent mixtures: R_F 0.17 with benzene:ethyl acetate (9:4, v/v), and R_F 0.51 with butanone:toluene (2:3, v/v). P.m.r. (100 MHz $CDCl_3$): δ 2.02 (s, 6H, OAc×2), 2.03 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.10 (s, 9H, OAc×3), 2.12 (s, 3H, OAc), 2.13 (s, 3H, OAc), 2.135 (s, 3H, OAc), 2.17 (s, 3H, OAc), 4.57 (d, 1H, *J* 7.6 Hz) and 5.70 (d, 1H, *J* 3.7 Hz).

Anal. Calc. for $C_{40}H_{54}O_{27}$: C, 49.68; H, 5.63. Found: C, 49.71; H, 5.76.

Gentianose (**4**). — Compound **3** (377 mg) was dissolved in 5 ml of 0.05M sodium methoxide in methanol and the solution was refrigerated for 2 h. The product that separated was filtered off, washed with methanol, dissolved in water, deionized with Amberlite IR-120 (H^+) and the solution evaporated to give amorphous **4**; yield 137 mg (70%), m.p. 212°, $[\alpha]_D^{25}$ +30.8° (*c* 0.50, water); p.m.r. (60 MHz, D_2O) δ 4.49 (d, 1 H, *J* 7.2 Hz) and 5.44 (d, 1 H, *J* 3.2 Hz).

Anal. Calc. for $C_{18}H_{32}O_{16}$: C, 42.86; H, 6.39. Found: C, 42.92; H, 6.51.

The product did not reduce Fehling's solution and its i.r. spectrum was superposable upon that of natural gentianose.

The product (140 mg) was hydrolyzed in 0.1M hydrochloric acid for 20 min at 100° and then neutralized with Amberlite IRA-400 (OH^-). The hydrolyzate was evaporated and the residue acetylated with acetic anhydride in pyridine. The acetylated product was recrystallized from 95% ethanol repeatedly to give β-gentiobiose octaacetate (26 mg), $[\alpha]_D^{25}$ −5.6° (*c* 0.72, chloroform), m.p. and mixed m.p. with an authentic sample, 189–191°. From the mother liquor, a mixture of α- and β- gentiobiose octaacetate (41 mg) was recovered.

Isolation of natural gentianose. — The procedure used was essentially that of Bridel and Desmarest[4], and powdered gentian root (Uchida Co. Ltd., Tokyo) was used as source material; m.p. 214°, $[\alpha]_D^{25}$ +30.8° (*c* 0.78, water) [lit.[4] m.p. 211°, $[\alpha]_D^{20}$ +31.5° (water)]; p.m.r. (60 MHz, D_2O) δ 4.49 (d, 1 H, *J* 7.2 Hz) and 5.44 (d, 1 H, *J* 3.2 Hz).
(Found: C, 42.76; H, 6.66).

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