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ENANTIOSPECIFIC SYNTHESIS OF POLYHYDROXYLATED INDOLIZIDINES RELATED TO CASTANOSPERMINE:[1] 1-DEOXY-CASTANOSPERMINE.

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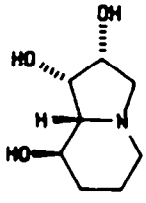
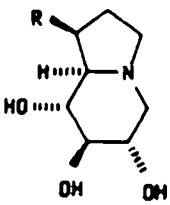
Enantiospecific synthesis of (6S,7R,8R,8aR)-6,7,8-trihydroxyindolizidine (1-deoxy-castanospermine) (3) is described from readily available D-glucose, where the key step involves oxidative bromination of a benzylidene acetal to afford 8-azido-3-O-benzoyl-5-bromo-5,6,7,8-tetradeoxy-1,2-O-isopropylidene-β-L-ido-octose (16). The synthetic indolizidine (3) was tested against a range of glycosidases.

Introduction

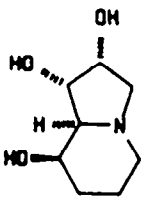
The isolation of the polyhydroxylated indolizidines swainsonine[2] (1) and castanospermine[3] (2) has generated much interest and continues to do so as a result of their ability to inhibit various glycosidases. Specifically, swainsonine (1) functions as a powerful inhibitor of lysosomal and jack bean α-mannosidase[4] and the glycoprotein processing enzyme Golgi mannosidase II;[5] it also inhibits the processing of asparagine-linked glycoproteins.[5] Castanospermine (2) is a potent inhibitor of various glucosidases including lysosomal α-glucosidase,[6] α- and β-glucosidase in fibroblast extracts,[6] the glycoprotein processing enzyme glucosidase I[7] as well as being a powerful inhibitor of β-xylosidase[6] and sucrase[8].



(1)



(2) R=OH
(3) R=H



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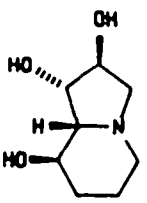

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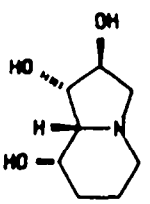

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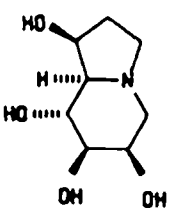


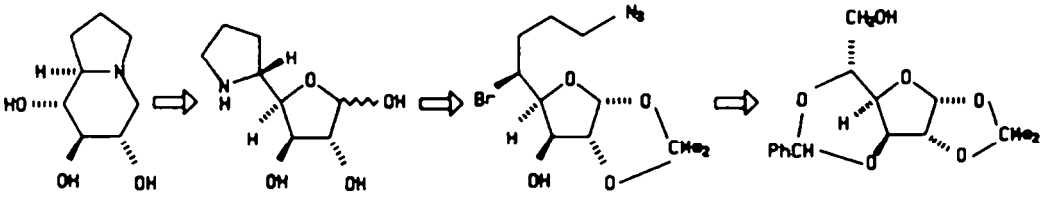
(7)

The ability of swainsonine (1) and castanospermine (2) to disrupt glycoprotein processing has resulted in the use of these compounds to modify glycoprotein biosynthesis and thus provide more insight into the role of oligosaccharides in glycoprotein function.[9] Recently, it has been observed that swainsonine[10] (1) and castanospermine[11] (2) are able to inhibit experimental metastasis of some cancers. Additionally, castanospermine (2) has been implicated as an inhibitor of human immunodeficiency virus (HIV)

syncytium formation and virus replication.[12]

Although a number of enantiospecific syntheses of swainsonine[13] (1) and castanospermine[14] (2) have been realised, few examples of attempts to synthesise analogues of (2) exist in the literature. A number of stereoisomers of swainsonine (1) have been synthesised[15] such as 2,8-di-*epi*-,[15a,b] 1,8-di-*epi*-,[15c] 2-*epi*-,[15b] 8-*epi*-,[15a,c,d] and 8a-*epi*-swainsonine.[15e] Notably, only 8a-*epi*-swainsonine (4) proved to be a potent glycosidase inhibitor, inhibiting human α-mannosidase[15e] to almost the same extent as swainsonine (1); 2-*epi*-swainsonine (5) was shown to inhibit fungal α-glucosidase[15b] and 2,8-di-*epi*-swainsonine (6) inhibited almond emulsin β-glucosidase[15b] to a lesser extent. Recently, a new α-glucosidase inhibitor, discovered during the isolation of castanospermine (2), was assigned the absolute configuration of (+)-6-*epi*-castanospermine (7).[16] However, comparison with synthetic material prepared by Ganem and workers[17] (D-*manno* configuration) revealed that the natural material was actually its enantiomer, namely (-)-6-*epi*-castanospermine, with the L-*manno* configuration. Both compounds proved to be weak inhibitors of α-mannosidase.

As part of an on-going programme in this laboratory to synthesise polyhydroxylated indolizidines such as swainsonine[18] (1), castanospermine (2) and their analogues[19,20] in an attempt to elucidate structure-activity relationships, we present full details of the synthesis of 1-deoxy-castanospermine (3). This compound has been prepared in order to establish if the 1-hydroxyl group of castanospermine (2) is necessary for it function as an effective glycosidase inhibitor; the absence of the 1-hydroxyl group leaves a compound that still contains four contiguous chiral centres of D-glucose. This work has been published in preliminary form.[19]



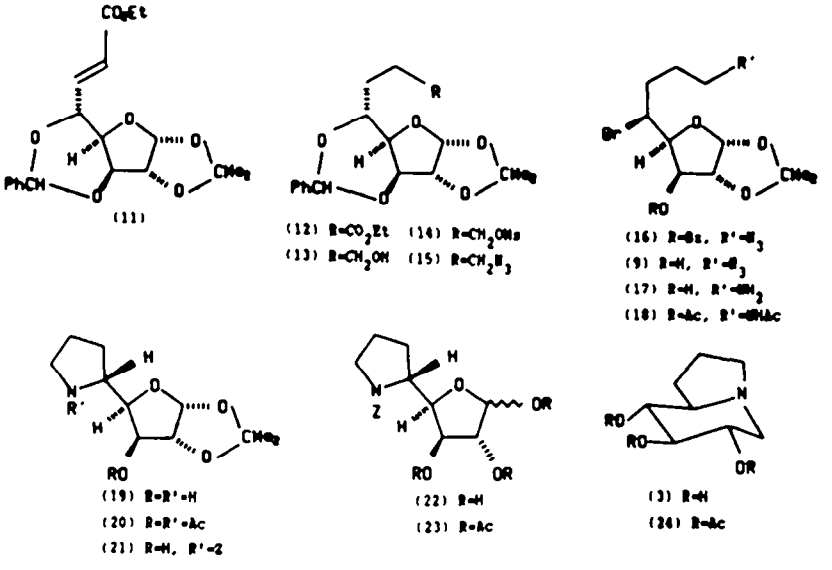
Scheme 1

Results and Discussion

Retrosynthetic analysis of 1-deoxy-castanospermine (3) suggested that one possible approach would require that pyrrolidine ring (8) be constructed first from the L-*ido* sugar (9) by displacement of a C-5 leaving group with an 8-amino function (Scheme 1), generating the correct stereochemistry at C-8a of the indolizidine (3). A convenient starting material was chosen as 3,5-*O*-benzylidene-1,2-*O*-isopropylidene-α-D-glucofuranose[21] (10), which is best synthesised by way of the 6-*O*-*p*-nitrobenzoate[22] to minimise formation of the unwanted 5,6-*O*-benzylidene acetal. Oxidation of the primary hydroxyl group of (10) was initially carried out with the Collins reagent, generated *in situ*,[23] but the resulting aldehyde proved to be unstable and could only be isolated in poor yield. An improved procedure involved oxidation of (10) with pyridinium

chlorochromate[24] in the presence of molecular sieves[25] followed, after five minutes, by addition of carboethoxymethylene triphenylphosphorane to the reaction mixture to afford the α,β-unsaturated ester (11) as the *E*-isomer ($J_{6,7}$ 16 Hz) [39-45% yield] (Scheme 2). Longer periods of oxidation led to diminished yields of the ester (11). Catalytic hydrogenation yielded the saturated ester (12) [96% yield], which was smoothly converted into 3,5-*O*-benzylidene-6,7-dideoxy-1,2-*O*-isopropylidene-α-D-*gluco*-octose (13) [93% yield] on treatment with lithium aluminium hydride.

In order to introduce an amino function into the 8-position the alcohol (13) was rapidly transformed into the mesylate (14) by reaction with methanesuphonyl chloride and triethylamine in dichloromethane,[26] followed by displacement with azide ion to furnish crystalline 8-azido-3,5-*O*-benzylidene-6,7,8-trideoxy-1,2-*O*-isopropylidene-α-D-*gluco*-octose (15) [88% yield from (13)]. The next step required the introduction of a leaving group into the C-5 position and subsequent S_N2 displacement with an 8-amino function to generate the desired pyrrolidine ring of castanospermine with the correct absolute stereochemistry at C-8a. Thus reaction of (15) with with *N*-bromosuccinimide according to the Hanessian-Hullar procedure[27] gave rise to 8-azido-3-*O*-benzoyl-5-bromo-5,6,7,8-tetradeoxy-1,2-*O*-isopropylidene-β-L-*ido*-octose (16) as



Scheme 2

a crystalline compound in 64% yield. The ^{1}H NMR spectrum of the L-idofuranose (16) displayed the usual furanose ring coupling constants with the exception of H-4 which now appeared as a double doublet ($J_{3,4}$ 2.9 Hz, $J_{4,5}$ 9.2 Hz), in comparison to the narrow multiplet observed in the spectra of the D-gluco-furanose series. Zemplen de-*O*-benzoylation furnished the syrupy alcohol (9) in 91% yield.

Difficulties were encountered when the azide (9) was subjected to catalytic hydrogenation or lithium aluminium hydride reduction due to loss of the 5-bromo group. However, the azide (9) was smoothly converted into the amine (17) on treatment with tin (II) chloride in methanol[28] and subsequently characterised as the amide (18) [64% from (9)]. Upon boiling the free amine (17) in ethanol containing sodium acetate intramolecular cyclisation occurred

to afford pyrrolidine (19) and subsequent acetylation furnished the amide (20). Confirmation that cyclisation had occurred was provided by the IR spectrum of (20) in which the amide II band was absent; it appeared at 1540 cm^{-1} in the IR spectrum of the uncyclised amide (18). Additional evidence was provided by the mass spectrum of (20) which displayed ions at m/z 112, arising from cleavage of the C-4 C-5 bond, and 70, due to loss of ketene from this ion. For the purposes of the synthesis the free pyrrolidine (19) was reacted with benzylchloroformate and sodium hydrogen carbonate in aqueous ethanol to yield the carbamate (21) [79% from (9)]. Hydrolysis of the acetal group (21) with 80% aqueous acetic acid proceeded slowly but cleanly to afford the free furanose (22) as an anomeric mixture [α:β, 4:1] in 65% yield. Analysis of the ^{1}H NMR spectrum of the acetylated furanose (23) was not possible due to the presence of a mixture of anomers and also the existence of rotamers. To complete the synthesis of 1-deoxy-castanospermine (3) the carbamate (22) was subjected to catalytic hydrogenation, a process that effected hydrogenolysis of the benzyloxycarbonyl group followed by intramolecular reductive amination of the C-1 aldehyde [70% yield]. Evidence for the structure of (3) was provided by the ^{1}H NMR spectrum which showed coupling constants $J_{5ax,6}$=10 Hz, $J_{6,7}$=$J_{7,8}$=$J_{8,8a}$=8.5 Hz indicating that the ring substituents were equatorial. The indolizidine (3) was further characterised as (6S,7R,8R,8aR)-6,7,8-triacetoxy-indolizidine (24).[29]

Glycosidase Inhibition[30,31]

The trihydroxyindolizidine (3) was tested against a wide range of enzymes originating from aqueous extract of human liver. Using 4-methyl umbelliferyl glycosides as substrates (3) showed 50% inhibition of β-*N*-acetylhexosaminidases at a concentration of 4 mM. At the same concentration (3) inhibited the following enzymes (% inhibition): β-glucosidase (31%), β-galactosidase (29%), α-arabinosidase (31%) and β-xylosidase (15%). Castanospermine (2) inhibited β-glucosidase (75%) and α-glucosidase (94%) at the same concentration. These results show that the 1-hydroxyl group of castanospermine (2) is important for it to function as a powerful glucosidase inhibitor, where it is probably involved in hydrogen-bonding interactions at the active site of the enzyme. It may also hold (2) in a more rigid conformation as a result of intramolecular hydrogen-bonding.

Experimental

Unless otherwise stated optical rotations were determined at room temperature (18-20°C) in 1 dm tubes on a Perkin Elmer 141 automatic polarimeter. ^{1}H NMR spectra were recorded either on a Bruker WH-400 (400 MHz), a Bruker WH-250 (250 MHz) or on a Nicolet NT-200 (200 MHz) spectrometer, and ^{13}C NMR spectra were recorded on a Bruker WP-60 (15.1 MHz) or WM-250 (61.3 MHz) spectrometer. All NMR spectra were run in deuteriochloroform unless stated otherwise. In all cases tetramethylsilane was used as internal standard. Mass spectra were determined on a Kratos MS-25 spectrometer by electron impact at 70 eV. Mass spectra of some of the more polar compounds were determined by the FAB technique. Melting points were measured on a Kofler hot-stage and are uncorrected. Microanalyses were obtained from either King's College, Kensington Campus or The London School of Pharmacy. Reactions were monitored by t.l.c. on silica gel ready-coated aluminium plates (Merck 5554).

Spots were visualised by spraying with 5% concentrated sulphuric acid in ethanol, followed by heating. Flash chromatography was performed on Merck Kieselgel 60 (230-400 mesh) at a pressure of 5-20 p.s.i. Acetylations were carried out by dissolving the compound in pyridine (5-10 mL per mmol) and adding excess acetic anhydride. The reaction was worked-up by pouring it into ice-water and extracting into chloroform. The organic layer was then washed with 10% hydrochloric acid, saturated sodium hydrogen carbonate solution and water, and finally dried over anhydrous magnesium sulphate. Diethyl ether was dried over sodium wire and tetrahydrofuran (THF) was freshly distilled from sodium benzophenone ketyl. Re-distilled light petroleum (b.p. 40-60°) was used throughout.

3,5-O-Benzylidene-1,2-O-isopropylidene-aldehydo-α-D-glucofuranose and subsequent reaction with carboethoxymethylene triphenylphosphorane. To a solution of the alcohol (10) (200 mg, 0.65 mmol) in dichloromethane (5 mL) containing molecular sieve (Type 3 A, 0.4 g) was added pyridinium chlorochromate (0.3 g, 1.4 mmol) and the mixture was stirred until it became dark brown (5 min.). Carboethoxymethylene triphenylphosphorane (0.45 g, 1.3 mmol) was added and the mixture stirred at room temperature for 2 h when t.l.c. (light petroleum-ether, 5:2) showed the presence of one major product together with some starting material. Addition of anhydrous ether precipitated a solid which was removed by filtration through Hyflo Supercell. The solid was washed with ether and the combined washings were concentrated to a pale yellow syrup. Purification by flash chromatography gave the Wittig adduct (11) as a solid [94 mg, 39% from the alcohol (10)], m.p. 96-98°, $[\alpha]_D$ +25.9° (c 1.1, chloroform). ν_{max} 1720, 1655 cm^{-1}. ^1H NMR δ 7.3-7.6 (m, 5H, ArH), 7.07 (dd, 1H, H-6, $J_{5,6}$ 3.9 Hz, $J_{6,7}$ 16 Hz), 6.19 (dd, 1H, H-7, $J_{5,7}$ 2.3 Hz), 6.06 (d, 1H, H-1, $J_{1,2}$ 3.6 Hz), 5.70 (s, 1H, ArC<u>H</u>), 5.07 (m, 1H, H-5), 4.66 (d, 1H, H-2, $J_{2,3}$ 0 Hz), 4.40 (d, 1H, H-3, $J_{3,4}$ 1.6 Hz), 4.24 (q, 2H, OEt), 4.21 (m, 1H, H-4), 1.54 (s, 3H, Me), 1.34 (s, 3H, Me), and 1.32 (t, 3H, OEt). ^{13}C NMR δ 165.6 (C=O), 142.6 (C-6), 124.4 (C-7), 112.1 (Me_2<u>C</u>), 94.1 (Ar<u>C</u>H), 105.2 (C-1), 83.7, 77.3, 74.2, 73.0, 26.7 (Me) and 26.1 (Me). Mass spectrum: m/z 377 (0.3%), 376 (0.3), 375 (0.8), 361 (2.3), 113 (100), 105 (59.8), 77 (31.5) and 43 (70). (Found: C, 63.82; H, 6.59. $C_{20}H_{24}O_7$ requires: C, 63.82; H, 6.43%).

Ethyl 3,5-O-benzylidene-6,7-dideoxy-1,2-O-isopropylidene-α-D-gluco-octuronate (12). The α,β-unsaturated ester (11) (0.6 g) was dissolved in ethanol and hydrogenated (45 p.s.i.) with 10% palladium on charcoal as catalyst for 1.5 h, when t.l.c. (light petroleum-ether, 5:2) showed a slower-moving product. The catalyst was removed by filtration through Hyflo Supercell and the filtrate was concentrated to a solid. Purification by flash chromatography using the same solvent system gave analytically pure (12) (579 mg, 96%), m.p. 98-100° (light petroleum), $[\alpha]_D$ +25.4° (c 1.1, chloroform). ν_{max} 1730 cm^{-1}. ^1H NMR (*inter alia*) δ 6.03 (d, 1H, H-1, $J_{1,2}$ 3.7 Hz), 4.63 (d, 1H, H-2, $J_{2,3}$ 0 Hz, 4.46 (d, 1H, H-3, $J_{3,4}$ 1.8 Hz), 4.32 (m, 1H, H-5), 3.96 (m, 1H, H-4), 2.32-2.54 (m, 3H) and 1.92 (m, 1H). ^{13}C NMR δ 172.9 (C=O), 111.8 (Me_2<u>C</u>), 105.2 (C-1), 92.4 (Ar<u>C</u>H), 83.7, 76.9, 75.1, 73.2, 30.6 (C-7), 26.7 (Me), 26.1 (Me) and 24.8 (C-6). Mass spectrum: m/z 378 (1.8%), 377 (0.5), 363 (1.0), 113 (100), 105 (50.1), 77 (24.4) and 43 (86.2). (Found: C, 63.44; H, 6.76. $C_{20}H_{26}O_7$ requires: C, 63.48; H, 6.93%).

3,5-O-Benzylidene-6,7-dideoxy-1,2-O-isopropylidene-α-D-gluco-octose (13). To a solution of the ester (12) (579 mg, 1.53 mmol) in dry ether was added lithium aluminium hydride (0.2 g, 5.3 mmol) and the mixture was heated under reflux for 1 h when t.l.c. (ether) showed the complete conversion of the ester into a slower-moving product. Sufficient water was added to precipitate inorganic material which was filtered and the filtrate concentrated to a solid (481 mg, 93%). Recrystallisation from ether afforded (13) as needles, which had m.p. 149-150°, $[\alpha]_D$ +26.6° (c 1.0, chloroform). ν_{max} 3420 (br) cm^{-1}. ^1H NMR (*inter alia*) δ 6.05 (d, 1H, H-1, $J_{1,2}$ 3.7 Hz), 4.64 (d, 1H, H-2, $J_{2,3}$ 0 Hz), 4.46 (d, 1H, H-3, $J_{3,4}$ 1.9 Hz), 4.34 (m, 1H, H-5), 3.97 (m, 1H, H-4), 3.72 (t, 2H, H-8a, H-8b), 2.10 (m, 1H), 1.58-1.88 (m, 3H). ^{13}C NMR δ 111.7 ($Me_2\underline{C}$), 105.1 (C-1), 92.3 (Ar$\underline{C}$H), 83.6, 76.8, 75.3, 73.4, 61.9 (C-8), 28.6 (C-6), 26.7 (Me), 26.1, (Me) and 26.0 (C-7). Mass spectrum: m/z 337 (0.3%), 336 (0.3), 321 (0.8), 113 (100), 105 (22.9), 77 (11.5) and 43 (29.2). (Found: C, 64.09; H, 7.11. $C_{18}H_{24}O_6$ requires: C, 64.27; H, 7.19%).

3,5-O-Benzylidene-6,7-dideoxy-1,2-O-isopropylidene-8-O-mesyl-α-D-gluco-octose (14). To a chilled solution of the alcohol (13) (481 mg, 1.43 mmol) in dichloromethane (7 mL) was added triethylamine (0.3 mL, 2.15 mmol) followed by methanesulphonyl chloride (0.12 mL, 1.55 mmol) and the resulting solution stirred for 45 min. After this time t.l.c. (ether-light petroleum, 4:1) showed the presence of a faster-moving product. Ice was added to the reaction mixture, which was extracted into chloroform and washed successively with 10% hydrochloric acid, saturated sodium bicarbonate solution, water and finally dried ($MgSO_4$). Evaporation of the solvent gave the mesylate (14) as a crystalline solid (543 mg, 92%), m.p. 126-128° (ethanol), $[\alpha]_D$ +20.7° (c 1.1, chloroform). ν_{max} 1350, 1160 cm^{-1}. ^1H NMR (*inter alia*) δ 6.05 (d, 1H, H-1, $J_{1,2}$ 3.7 Hz), 4.65 (d, 1H, H-2, $J_{2,3}$ 0 Hz), 4.46 (d, 1H, H-3, $J_{3,4}$ 1.7 Hz), 4.31 (m, 1H, H-5), 3.96 (m, 1H, H-4), 3.38 (m, 2H, H-8a, H-8b), 2.12 (m, 1H), 1.58-1.93 (m, 3H). ^{13}C NMR δ 111.8 ($Me_2\underline{C}$), 105.2 (C-1), 92.4 (Ar$\underline{C}$H), 83.6, 76.9, 75.2, 72.9, 69.2 (C-8), 26.7 (Me), 26.1 (Me), 25.6 (C-6) and 25.5 (C-7). Mass spectrum: m/z 415 (1.6%), 414 (0.5), 113 (100), 105 (31.4), 77 (19) and 43 (80.5). (Found: C, 55.05; H, 6.35. $C_{19}H_{26}O_8S$ requires C, 55.06; H, 6.32%).

8-Azido-3,5-O-benzylidene-6,7,8-trideoxy-1,2-O-isopropylidene-α-D-gluco-octose (15). To a solution of the mesylate (14) (563 mg, 1.29 mmol) in *N,N*-dimethylformamide (10 mL) was added sodium azide (0.25 g, 3.9 mmol) and the reaction mixture was heated at 90° for 1 h when t.l.c. (ether) showed the presence of a faster-moving product. After cooling, the reaction mixture was poured into ice-water and the resulting solid filtered and washed well with water to yield the azide (15) (451 mg, 96%). Recrystallisation from ethanol gave the analytical sample, which had m.p. 116-117°, $[\alpha]_D$ +26.9° (c 0.8, chloroform). ν_{max} 2110 cm^{-1}. ^1H NMR (*inter alia*) δ 6.05 (d, 1H, H-1, $J_{1,2}$ 3.7 Hz), 4.65 (d, 1H, H-2, $J_{2,3}$ 0 Hz), 4.46 (d, 1H, H-3, $J_{3,4}$ 2.0 Hz), 4.32 (m, 1H, H-5), 4.31 (t, 2H, H-8a, H-8b), 3.97 (m, 1H, H-4), 1.72-2.22 (m, 4H). ^{13}C NMR δ 111.8 ($Me_2\underline{C}$), 105.2 (C-1), 92.4 (Ar$\underline{C}$H), 83.8, 76.9, 75.3, 73.0, 50.9 (C-8), 26.7 (C-6), 26.7 (Me), 26.1 (Me) and 25.1 (C-7). Mass spectrum: m/z 362 (0.9%), 361 (0.1), 360 (0.4), 346 (0.8), 113 (100), 105 (25.5), 77 (10.8) and 43 (32.5).(Found: C, 59.99; H, 6.46; N, 11.52. $C_{18}H_{23}N_3O_5$ requires: C, 59.82; H, 6.42; N, 11.63%).

8-Azido-3-O-benzoyl-5-bromo-5,6,7,8-tetradeoxy-1,2-O-isopropylidene-β-L-ido-octose (16). To a stirred solution of the azide (15) (73 mg, 0.20 mmol) in carbon tetrachloride (13 mL) was added *N*-bromosuccinimide (42 mg, 0.24 mmol) and barium carbonate (133 mg, 0.67 mmol) together with a few crystals of benzoyl peroxide. The resulting mixture was heated under reflux for 2 h when t.l.c. (light petroleum-ether, 3:1) showed the presence of a major faster-moving product as well as unreacted azide and a couple of minor slower-moving products. After cooling, the reaction mixture was filtered through Hyflo Supercell and concentrated. The residue was dissolved in ether and washed several times with water, dried ($MgSO_4$) and concentrated to a syrup. Flash chromatography using light petroleum-ether, 6:1 afforded the L-idofuranose (16) as a solid (57 mg, 64%). Recrystallisation from ethanol gave white crystals which had m.p. 90-91°, $[\alpha]_D$ -38.2° (c 0.6, chloroform). ν_{max} 2100, 1720 cm^{-1}. ^1H NMR δ 7.4-8.1 (m, 5H, ArH), 6.03 (d, 1H, H-1, $J_{1,2}$ 3.8 Hz), 5.52 (d, 1H, H-3, $J_{2,3}$ 0 Hz, $J_{3,4}$ 2.9 Hz), 4.68 (d, 1H, H-2), 4.51 (dd, 1H, H-4, $J_{4,5}$ 9.2 Hz), 4.20 (m, 1H, H-5), 3.23 (m, 2H, H-8a, H-8b), 1.68-1.91 (m, 4H), 1.61 (s, 3H, Me), and 1.35 (s, 3H, Me). ^{13}C NMR δ 165.1 (C=O), 112.5 ($Me_2\underline{C}$), 104.3 (C-1), 83.7, 82.7, 76.0, 50.8 (C-5), 50.2 (C-8), 31.2 (C-7), 26.8 (Me), 26.4 (C-6) and 26.3 (Me). Mass spectrum: m/z 426, 424 (1.3, 1.3%), 105 (100), 77 (26.7) and 43 (56.3). (Found: C, 49.18; H, 4.99; N, 9.31. $C_{18}H_{22}N_3O_5Br$ requires: C, 49.10; H, 5.04; N, 9.54%).

8-Azido-5-bromo-5,6,7,8-tetradeoxy-1,2-O-isopropylidene-β-L-ido-octose (9). To a solution of (16) (231 mg) dissolved in methanol was added a catalytic amount of methanolic sodium methoxide. The solution was kept at room temperature for 1 h when t.l.c. (ether) revealed that de-esterification was complete. After filtering the reaction mixture through a pad of silica gel, the filtrate was evaporated and the residue purified by flash chromatography (ether-light petroleum, 5:2) to give pure (9) as a syrup (161 mg, 91%), $[\alpha]_D$ -37.2° (c 0.2, chloroform). ν_{max} 2100 cm^{-1}. ^1H NMR δ 5.97 (d, 1H, H-1, $J_{1,2}$ 3.7 Hz), 4.56 (d, 1H, H-2, $J_{2,3}$ 0 Hz), 4.25-4.28 (m, 2H, H-3, H-4), 4.16 (m, 1H, H-5), 1.76-2.08 (m, 4H), 3.38 (m, 2H, H-8a, H-8b). Mass spectrum: m/z 322, 320 (1.7, 1.6%), 159 (4.6), 59 (100) and 43 (87.1). (Found: C, 39.55; H, 5.42; N, 12.15. $C_{11}H_{18}N_3O_4Br$ requires: C, 39.30; H, 5.40; N, 12.50%).

8-Acetamido-3-O-acetyl-5-bromo-5,6,7,8-tetradeoxy-1,2-O-isopropylidene-β-L-ido-octose (18). To a solution of the azide (9) (60 mg, 0.18 mmol) in methanol was added tin (II) chloride (51 mg, 0.27 mmol) and the resulting mixture was heated at 60° for 10 h, when t.l.c. (butanol-pyridine-water, 10:3:3) showed the presence of a slower-moving product. After concentrating to dryness water was added to the residue and this was made alkaline by the addition of potassium hydroxide solution. The solvent was removed *in vacuo* and the residue was extracted thrice with boiling ethyl acetate, filtered and the filtrate concentrated to afford syrupy amine (17).

Acetylation of the free amine (17) in the usual way gave the amide (18), which was purified by flash chromatography (ethyl acetate-acetone, 4:1) to afford (18) as a solid (45 mg, 64%), m.p. 113-115°, $[\alpha]_D$ -20.6° (c 1.1, chloroform). ν_{max} 1735, 1640, 1540 cm^{-1}. ^1H NMR δ 5.94 (d, 1H, H-1, $J_{1,2}$ 3.7 Hz), 5.60 (bs, 1H, NH), 5.26 (d, 1H, H-3, $J_{2,3}$ 0 Hz, $J_{3,4}$ 2.8 Hz), 4.54 (d, 1H, H-2), 4.36 (dd, 1H, H-4, $J_{4,5}$ 9.1 Hz), 4.04 (m, 1H, H-5), 3.26 (m, 2H, H-8a, H-8b), 2.1 (s, 3H, OAc), 2.0 (s, 3H, OAc), 1.60-1.91 (m, 4H), 1.54 (s, 3H, Me) and 1.34 (s, 3H, Me). Mass spectrum: m/z 396, 394 (0.5, 0.5%), 380,

378 (1.9, 1.8), 196 (13) and 43 (100). (Found: C, 45.84; H, 6.18; N, 3.29. $C_{15}H_{24}NO_6Br$ requires: C, 45.70; H, 6.14; N, 3.55%).

5,8-Benzyloxycarbonylimino-5,6,7,8-tetradeoxy-1,2-O-isopropylidene-α-D-gluco-octose (21). A solution of the free amine (17) in ethanol was boiled overnight in the presence of sodium acetate, when t.l.c. (butanol-pyridine-water, 10:3:3) revealed the presence of a slower-moving product. The solvent was evaporated and the residue extracted thrice with hot ethyl acetate, followed by filtration and concentration to afford the crude syrupy pyrrolidine (19).

Conventional acetylation of (19) yielded syrupy 3-O-acetyl-5,8-acetylimino-5,6,7,8-tetradeoxy-1,2-O-isopropylidene-α-D-gluco-octose (20), which was purified by flash chromatography (ethyl acetate-acetone, 4:1) to give (20) (33 mg, 58%). ν_{max} 1740, 1630 cm^{-1}. Mass spectrum: m/z 314 (1.5%), 298 (3.2) 112 (59.4) and 70 (100).

To an ice-cold solution of the free pyrrolidine (19) [from the azide (9), 200 mg] in aqueous ethanol was added solid sodium bicarbonate (0.1 g) followed by benzylchloroformate (0.1 mL). The reaction mixture was stirred for 3 h when t.l.c. (light petroleum-ether, 1:1) showed the presence of a faster-moving product. The reaction mixture was poured into water and extracted into chloroform, dried ($MgSO_4$) and finally evaporated to dryness. Flash chromatography of the crude syrup using light petroleum-ether (3:2) as solvent afforded the benzyloxycarbonyl derivative (21) [170 mg, 79% from azide (9)], $[\alpha]_D$ +28.3° (c 1.1, chloroform). ν_{max} 1665 cm^{-1}. ^1H NMR (*inter alia*) ($CDCl_3$ at 328 K) δ 5.86 (d, 1H, H-1, $J_{1,2}$ 3.7 Hz), 5.18 (d, 1H, H-3, $J_{2,3}$ 0 Hz, $J_{3,4}$ 2.3 Hz), 5.12 (s, 2H, $ArCH_2$), 4.44 (d, 1H, H-2), 4.41 (m, 1H, H-4), 4.19 (m, 1H, H-5), 3.44 (m, 2H, H-8a, H-8b), 1.7-2.3 (m, 4H). Mass spectrum: m/z 364 (19%), 348 (4.3), 204 (53.6), 160 (63.8), 91 (100) and 70 (12.9). (Found: C, 62.76; H, 6.76; N, 3.63. $C_{19}H_{25}NO_6$ requires: C, 62.80; H, 6.93; N, 3.85%).

5,8-Benzyloxycarbonylimino-5,6,7,8-tetradeoxy-D-gluco-octose (22). A solution of (21) (170 mg) in 80% aqueous acetic acid was heated at 80-90° for 30 h, when t.l.c. (ethyl acetate) indicated that most of the starting material had been converted into a major slower-moving product. The solvent was evaporated and then co-evaporated with toluene to yield syrupy (22). Purification by flash chromatography furnished a syrup (99 mg, 65%). (Found: C, 59.13; H, 6.45; N, 4.22. $C_{16}H_{21}NO_6$ requires: C, 59.43; H, 6.55; N, 4.33%).

Acetylation of (22) afforded the syrupy triacetate (23) as an anomeric mixture, from which the α-anomer was purified by flash chromatography (ether-light petroleum, 3:1), $[\alpha]_D$ +84.2° (c 0.5, chloroform). ν_{max} 1740, 1685 cm^{-1}. Mass spectrum: m/z 204 (15.4%), 160 (26.6), 91 (100), 70 (5.6) and 43 (41.2).

(6S,7R,8R,8aR)-6,7,8-Trihydroxy-indolizidine (3). The free sugar (22) (64 mg), dissolved in methanol, was hydrogenated in the presence of 10% palladium on charcoal for 18 h at room temperature and pressure. After this time, t.l.c. (chloroform-methanol, 2:1) revealed the presence of a single slower-moving product. The catalyst was filtered off and the filtrate concentrated to a solid (3) (24 mg, 70%), m.p. 178-181° $[\alpha]_D$ +50.6° (c 0.2, methanol). ^1H NMR (pyridine-d_5 + D_2O) δ 4.34 (ddd, 1H, H-6, $J_{5eq,6}$ 5.2 Hz, $J_{5ax,6}$ 10 Hz, $J_{6,7}$ 8.5 Hz), 3.97 (t, 1H, H-7, $J_{7,8}$ 8.5 Hz), 3.88 (t, 1H, H-8, $J_{8,8a}$ 8.5 Hz), 3.53 (dd, 1H, H-5eq, $J_{5eq,5ax}$ 10.4 Hz), 3.03 (m, 1H, H-3a), and 2.45 (t, 1H, H-5ax).

A portion of the free indolizidine (3) was acetylated in the conventional

way to afford the triacetate (24), which was purified by flash chromatography (ether-light petroleum, 3:1) to give (6S,7R,8R,8aR)-6,7,8-triacetoxy-indolizidine (24) as a solid , m.p. 97-99° (ether-light petroleum), $[\alpha]_D$ +40.7° (c 0.3, chloroform). ^{1}H NMR (benzene-d_6) δ 5.37 (t, 1H, H-8, $J_{7,8}$ 9 Hz, $J_{8,8a}$ 9 Hz), 5.34 (dt, 1H, H-6, $J_{5eq,6}$ 5 Hz, $J_{5ax,6}$ 10 Hz), 5.17 (t, 1H, H-7, $J_{6,7}$ 9 Hz), 3.11 (dd, 1H, H-5eq, $J_{5eq,5ax}$ 10.5 Hz), 2.60 (dt, 1H, H-3a, $J_{2a,3a}$ 8.5 Hz, $J_{2b,3a}$ 6.5 Hz), 1.91 (dt, 1H, H-3b), 1.84 (t, 1H, H-5ax), 1.76 (s, 3H, OAc), 1.73 (s, 3H, OAc) and 1.67 (s, 3H, OAc). Mass spectrum: m/z 300 (34.7%), 240 (72.1), 239 (15.0), 180 (56.0), 138 (44.1), 120 (37.2) and 70 (29.5). High resolution mass spectrum: m/z found: 300.1436 (M + H). $C_{14}H_{21}NO_6$ requires: 300.1447 (M + H).

References

1. Part 1. For Part 2 see: D. Hendry, L. Hough and A. C. Richardson, *Tetrahedron*, following paper.
2. S. M. Colegate, P. R. Dorling and C. R. Huxtable, *Austr. J. Chem.*, 1979, **32**, 2257.
3. L. D. Hohenschutz, E. A. Bell, P. J. Jewess, D. P. Leworthy, R. J. Pryce, E. Arnold and J. Clardy, *Phytochemistry*, 1981, **20**, 811.
4. (a) P. R. Dorling, C. R. Huxtable and S. M. Colegate, *Biochem J.*, 1980, **191**, 649, (b) R. J. Molyneux and L. F. James, *Science*, 1982, **216**, 190.
5. (a) D. R. P. Tulsiani, T. M. Harris and O. Touster, *J. Biol. Chem.*, 1982, **257**, 7936, (b) A. D Elbein, R. Solf, P. R. Dorling and K. Vosbeck, *Proc. Natl. Acad. Sci. USA.*, 1981, **78**, 7393, (c) A. D. Elbein, P. R. Dorling, K. Vosbeck and M. Horisberger, *J. Biol. Chem.*, 1982, **257**, 1573.
6. R. Saul, J. P. Chambers, R. J. Molyneux and A. D Elbein, *Arch. Biochem. Biophys.*, 1983, **221**, 593.
7. (a) Y. T. Pan, H. Hori, R. Saul, B. A. Sanford, R. J. Molyneux and A. D. Elbein, *Biochem.*, 1983, **22**, 3975, (b) H. Hori, Y. T. Pan, R. J. Molyneux and A. D. Elbein, *Arch. Biochem. Biophys.*, 1984, **228**, 525, (c) V. W. Sasak, J. M. Ordovas, A. D. Elbein and R. W. Berninger, *Biochem. J.*, 1985, **232**, 759.
8. G. Trugnan, M. Rousset and A. Zweibaum, *FEBS Lett.*, 1986, **198**, 28.
9. (a) A. D. Elbein, *CRC Crit. Rev. Biochem.*, 1984, **16**, 21, (b) P. M. Schwarz and A. D. Elbein, *J. Biol. Chem.*, 1985, **260**, 14452.
10. M. J. Humphries, K. Matsumoto, S. L. White and K. Olden, *Proc. Natl. Acad. Sci. USA.*, 1986, **83**, 1752.
11. M. J. Humphries, K. Matsumoto, S. L. White and K. L. Olden, *Cancer Research*, 1986, **46**, 5215.
12. (a) R. A. Gruters, J. J. Neefjes, M. Tersmette, R. E. Y. de Goede, A. Tulp, H. G. Huisman, F. Miedema and H. L. Ploegh, *Nature*, 1987, **330**, 74, (b) R. Dagani, *Chem. Eng. News*, 1987, June 29, 25, (c) D. B. Walker, M. Kowalski, W. C. Goh, K. Kozarsky, M. Krieger, C. Rosen, L. Rohrschneider, W. A. Haseltine and J. Sodroski, *Proc. Natl. Acad. Sci. USA.*, 1987, **84**, 8120.
13. (a) M. H. Ali, L. Hough and A. C. Richardson, *J. Chem. Soc., Chem. Commun.*, 1984, 447, (b) G. W. J. Fleet, M. J. Gough and P. W. Smith, *Tetrahedron Lett.*, 1984, **25**, 1853; B. P. Bashyal, G. W. J. Fleet, M. J. Gough and P. W. Smith, *Tetrahedron*, 1987, **43**, 3083, (c) T. Suami, K.-I. Tadano and Y. Iimura, *Chem. Lett.*, 1984, 513; T. Suami, K.-I. Tadano and Y. Iimura, *Carbohydr. Res.*, 1985, **136**, 67, (d) N. Yasuda, N. Tsutsumi

and T. Takaya, *Chem. Lett.*, 1984, 1201, (e) C. E. Adams, F. J. Walker and K. B. Sharpless, *J. Org. Chem.*, 1985, **50**, 420, (f) H. Setoi, H. Takeno and M. Hashimoto, *J. Org. Chem.*, 1985, **50**, 3948.

14. (a) R. C. Bernotas and B. Ganem, *Tetrahedron Lett.*, 1984, **25**, 165, (b) H. Setoi, H. Takeno and M. Hashimoto, *Tetrahedron Lett.*, 1985, **26**, 4617.
15. (a) N. Yasuda, M. Tsutsumi and T. Takaya, *Chem. Lett.*, 1985, 31, (b) A. D. Elbein, T. Szumilo, B. A. Sanford, K. B. Sharpless and C. Adams, *Biochemistry*, 1987, **26**, 2502, (c) Y. Iimura, Y. Hotta, C. Fukabori, K. Tadano and T. Suami, *J. Carbohydr. Chem.*, 1986, **5**, 147; K.-I. Tadano, Y. Iimura, Y. Hotta, C. Fukabori and T. Suami, *Bull. Chem. Soc. Jpn.*, 1986, **59**, 3885, (d) G. N. Austin, P. D. Baird, G. W. J. Fleet, J. M. Peach, P. W. Smith and D. J. Watkin, *Tetrahedron*, 1987, **43**, 3095, (e) K.-I. Tadano, Y. Hotta, M. Morita, T. Suami, B. Winchester and I. C. di Bello, *Chem. Lett.*, 1986, 2105; K.-I. Tadano, Y. Hotta, M. Morita, T. Suami, B. Winchester and I. C. di Bello, *Bull. Chem. Soc. Jpn.*, 1987, **60**, 3667.
16. R. J. Molyneux, Y. N. Roitman, G, Dunnheim, T. Szumilo and A. D. Elbein, *Arch. Biochem. Biophys.*, 1986, **251**, 450.
17. H. Hamana, N. Ikota and B. Ganem, *J. Org. Chem.*, 1987, **52**, 5492.
18. M. H. Ali, L. Hough and A. C. Richardson, *Carbohydr. Res.*, 1985, **138**, 225.
19. D. Hendry, L. Hough and A. C. Richardson, *Tetrahedron Lett.*, 1987, **28**, 4597.
20. D. Hendry, L. Hough and A. C. Richardson, *Tetrahedron Lett.*, 1987, **28**, 4601.
21. O. T. Schmidt, *Methods Carbohydr. Chem.*, 1963, Vol. II., 318.
22. J. W. Van Cleve, G. E. Inglett and L. W. Tjarks, *Carbohydr. Res.*, 1985, **137**, 259.
23. R. E. Arrick, D. C. Baker and D. Horton, *Carbohydr. Res.*, 1973, **26**, 441.
24. E. J. Corey and J. W. Suggs, *Tetrahedron Lett.*, 1975, 2647.
25. J. Hersovici and K. Antonakis, *J. Chem. Soc., Chem. Commun.*, 1980, 56.
26. R. K. Crossland and K. L. Servis, *J. Org. Chem.*, 1970, **35**, 3195.
27. S. Hanessian and N. R. Plessas, *J. Org. Chem.*, 1969, **34**, 1036.
28. S. N. Maiti, M. P. Singh and R. G. Micetich, *Tetrahedron Lett.*, 1986, **27**, 1423.
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