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Synthesis of 1-deoxy-6-epicastanospermine and 1-deoxy-6,8a-diepicastanospermine*

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ABSTRACT

Extension of the carbon spine of 2,3;4,5-di-*O*-isopropylidene-β-D-fructopyranoside by oxidation at C-1 followed by a Wittig reaction using the phosphorane $Ph_3P=CHCO_2Et$ gave an oct-4-ulose derivative, which was then transformed into the key intermediate 1-azido-1,2,3-trideoxy-D-*arabino*-oct-4-ulose. Catalytic hydrogenolysis of this azide, followed by reductive amination between the resulting 1-amino substituent and the 4-keto-group then gave a mixture of pyrrolidines. After sulphonylation at the terminal 8-position, the pyrrolidines were then cyclised further between the nitrogen and C-8 to give 1-deoxy-6-epicastanospermine and 1-deoxy-6,8a-diepicastanospermine.

INTRODUCTION

Since 1950 there has been an enormous surge of activity in synthetic organic chemistry, but carbohydrate chemistry remained unexploited and the province of the specialist, with little impact on mainstream organic chemistry. However, in the last decade organic synthesis entered its "enantiospecific stage", in which it was important not only to manipulate and control relative stereochemistry of substituents, but to control their absolute stereochemistry. Towards this objective, the synthetic organic chemist scrutinised every possible source of chirality, and discovered that carbohydrate chemistry was a rich and economic source, once the intricacies of the seemingly complex stereochemical inter-relationships and diverse array of reactions had been mastered. The added influence of conformational effects which often led to highly regio- and stereo-selective reactions was an added bonus.

An important area which derived considerable stimulus from the application of carbohydrates is the synthesis of chiral hydroxylated piperidines, pyrrolidines and indolizidines. These compounds are widely distributed in many plants, wherein they probably function as insect repellents or anti-feedants[1]. They are remarkable inasmuch as they are often potent inhibitors of glycosidase enzymes, particularly those involved in the Golgi processing of glycoproteins in mammals[2]. The toxic effect of ingestion of locoweed by cattle in the arid parts of the southern United States has been documented

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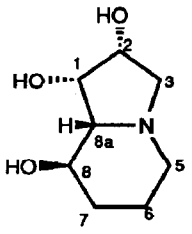
for over a hundred years, but only in 1982 was the toxin isolated and identified[3] as the indolizidine alkaloid swainsonine (**1**). It had been isolated earlier from a fungus *Rhizoctonia leguminicola* but its structure had been wrongly assigned[4]. Its structure was subsequently determined by Dorling *et al.*, who isolated it from an Australian plant *Swainsonia canescens*[5]. The toxic effect of the alkaloid was shown to be due to inhibition of the Golgi α-D-mannosidase[6], which brought about a state similar to mannosidosis, the genetic disorder in humans arising from the genetic deficiency of α-D-mannosidase[7].

Castanospermine (**2**) was first isolated in 1981 from the seeds of the Australian tree *Castanospermum australe*[8], but has since been detected in other species, notably those of the *Alexus* species[9]. Like swainsonine, it shows potent inhibitory activity against glycosidases, particularly glucosidases[10], and has been shown to affect the Golgi glycoprotein processing[11]. Futhermore, it has anti-viral properties, which includes activity against HIV and related viruses[12]. Inhibition of glucosidases is presumably attributable of its homo-stereochemical relationship to glucopyranosides in which the configuration of the hydroxyl groups at C-1, C-6, C-7, and C-8 exactly match those at C-6, C-2, C-3, and C-4 respectively on the glucopyranoside, but with the important difference of the "N-in-the-ring". Castanospermine is closely related to nojirimycin (5-amino-5-deoxy-D-glucopyranose) and 1-deoxynojirimycin, which were first isolated in the 1960s from *Streptomyces*[13] species, and which also have significant activity against glucosidases. As an extrapolation of this simple idea, it may, in certain cases, be possible to design inhibitors, which by virtue of a homochiral relationship would be specific for certain glycosidases. Thus, 1,5-dideoxy-1,5-imino-L-fucitol was designed as, and was found to be, a potent inhibitor of α-L-fucosidase[14]. However, the concept of homochiral recognition has led to too many failures for it to be a reliable aid to the rational design of glycosidase inhibitors. As a consequence, there has been much synthetic effort directed towards the synthesis of all possible hydroxylated pyrrolidines, piperidines, and indolizidines. We have previously been active in the synthesis of various indolizidines from hexoses[15,16], and here describe the synthesis of two further trihydroxylated indolizidines.

RESULTS AND DISCUSSION

The synthetic strategy that we and others have used for the synthesis of indolizidines, has been to add on the two-carbon unit to either end of the hexose sugar, and then to cyclise between the end of the two-carbon unit, through a nitrogen atom, to the terminal carbon (C-1 or C-6 of the original hexose) and either C-2, C-3 or C-4 of the original hexose. In the present work we start with D-fructose, extend onto C-1 with the two-carbon unit to give an octulose, and cyclise, through nitrogen, from C-1 to C-4 and C-8 (C-2 and C-6 of the fructose unit) as in **3**.

2,3;4,5-Di-*O*-isopropylidene-β-D-fructopyranose (**4**), a readily available starting material[17], was investigated as an obvious precursor of the aldehyde **5** for chain extension. However, oxidation of **4** to the aldehyde **5**, with either the Collins reagent (CrO_3–dipyridine complex)[18], or pyridinium dichromate[19] was unsatisfactory. The latter



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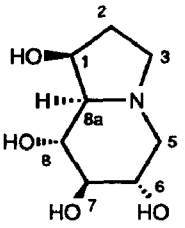

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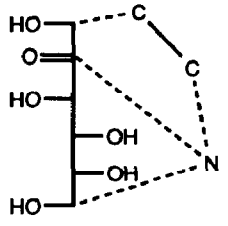

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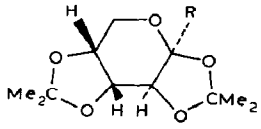
reaction was very slow and the former oxidant required a large excess of reagent which gave rise to solubility problems on a larger scale. Satisfactory oxidations were accomplished with the Corey reagent pyridinium chlorochromate[20] or Lemieux's modification[21] to the Collins reagent. The latter reagent was particularly efficient, giving yields of $>80\%$, when compared to the maximum yield of 60% using either pyridine–SO_3 or P_2O_5 in Me_2SO (ref. 22). At least 60% of the amorphous aldehyde **5** existed in its hydrated form, as revealed by n.m.r., consequently it was difficult to characterise fully, and so we proceeded to the next stage without any attempt at purification.



4 R = CH_2OH
5 R = CHO
6 R = $CH{=}CHCO_2Et$(*E*-isomer)*
7 R = $CH{=}CHCO_2Et$ (*Z*-isomer)*
8 R = $CH{=}CHCH_2OH$ (*E*-isomer)
9 R = $CH_2CH_2CO_2Et$*
10 R = $CH_2CH_2CH_2OH$
11 R = $CH_2CH_2CH_2OMs$
12 R = $CH_2CH_2CH_2N_3$

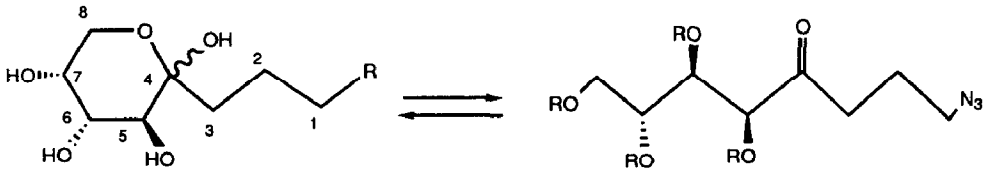
Condensation of the crude aldehyde **5** with ethoxycarbonylmethylene triphenylphosphorane in dichloromethane afforded mainly the syrupy *E*-isomer **6**, which was isolated in 60% yield after chromatography. As more-polar solvents such as ethanol favour the *Z*-isomer when a stabilised phosphorane is used, the reaction was repeated in ethanol and a 1:1 mixture of the *E*- and *Z*-isomers was obtained from which, in addition to **6**, the syrupy *Z*-isomer **7** was isolated in low yield. The stereochemistry of the two isomers was deduced from the values of alkenic couplings, 12.3 and 15.5 Hz, respectively.

Reduction of the double bond and the ethoxycarbonyl groups simultaneously with lithium aluminium hydride failed with mixtures of the *E*- and *Z*-isomers **6** and **7**

* These side chains are numbered with the ethoxycarbonyl carbon being C-1, whereas all the other derived side chains are numbered with the terminal carbon numbered C-3

and only reduction of the ethoxycarbonyl group was observed. When the pure *E*-isomer **6** was used, the allylic alcohol **8** was isolated as a syrup in 57% yield. Catalytic hydrogenation of a mixture of **6** and **7** over palladium-on-charcoal afforded the saturated ester **9** in quantitative yield. When this ester was reduced with lithium aluminium hydride, the desired alcohol **10** was obtained, and isolated in quantitative yield as the methanesulphonate **11**. Substitution of the methanesulphonyloxy group of **11** with sodium azide afforded the required azide **12** as a syrup in 76% overall yield from **6** and **7**.

Hydrolysis of the isopropylidene groups of **12** with aqueous acetic acid afforded the free octulose, 1-azido-1,2,3-trideoxy-D-*arabino*-oct-4-ulose (**13**), as a syrup. In order to characterise this product more fully, it was acetylated with acetic anhydride in pyridine. Only the acyclic azido-ketone **16** was formed, as revealed by the ^{1}H-n.m.r. spectrum, which was largely first-order. The presence of the ketone group was confirmed by the carbonyl resonance at 203 p.p.m. in the ^{13}C-n.m.r. spectrum of **15**, well to higher field of those of the four acetyl groups.



13 R = N_3
14 R = NH_2

15 R = H
16 R = Ac

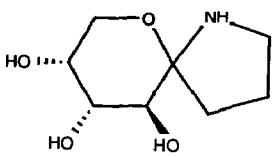
The acyclic structure of the octulose **16** was not entirely unexpected, since previous observations show that certain ketoses may undergo acetylation to give acyclic products[23]. Presumably any of the ketose existing in the pyranose or furanose forms would undergo acetylation of the primary and secondary OH groups whilst the tertiary 4-OH would resist acetylation. However, these cyclic forms would be in equilibrium with the open-chain form, which would contain a primary or secondary hydroxyl group, which would therefore undergo acetylation very readily to give the fully acetylated open-chain form. Moreover, Angyal *et al.*[24] have demonstrated with the aid of ^{13}C-n.m.r. spectroscopy that the amount of acyclic form present in free hexoses is related to the substituent attached to C-1. In aqueous solution, 1-deoxy-2-hexuloses exist in the acyclic form to the extent of 4–20%; a 50–80 fold increase by comparison with the parent hexulose. These workers concluded that the tendency of the carbonyl group to form a cyclic hemiacetal was governed by the electronegativity of the substituent at C-1. Such electron-withdrawing groups as OH increased the electrophilicity of the carbonyl group with a rise in the proportion of the hemiacetal, whereas the reverse was operative when the hydroxyl group was removed, as in the case of **13**.

The next stage of the synthesis was the reductive amination of the 4-keto group of **14** by the 1-amino function produced by reduction of the azido group. It was anticipated that the azide **13** would readily undergo hydrogenation to give the 1-amine **14**, which

would immediately ring close with the 4-keto group to give the stable spiro-*N*,*O*-acetal. However, such compounds normally undergo reductive amination; for example, the *N*,*O*-spiro-acetal in the alkaloid tomatidine is easily hydrogenated[25].

Initial attempts to hydrogenate the azide **13** using 20% w/w of palladium-on-charcoal, afforded, after acetylation, a mixture of two pyrrolidines, **19** and **23**, in low (20%) yield, together with what appeared to be a small amount of the *N*,*O*-spiroacetal **17**; its mass spectrum showed a $M^+ + 1$ ion at *m/z* 316. When the amount of catalyst was increased to ~50% w/w, and the reaction time increased, the two pyrrolidines **19** and **23** were isolated crystalline, after acetylation, in yields of 48 and 18%, respectively. The configurations of **19** and **23** could not be established on the basis of n.m.r. coupling constants, although the spectrum of the major isomer was almost first-order; rather unexpectedly this spectrum was not complicated by the existence of rotameric forms, which is usual for *N*-acylated pyrrolidines. In the case of the minor isomer, two rotameric forms were present in equal amounts, thus complicating the n.m.r. spectrum. *O*-deacetylation of small quantities (~10 mg) of each isomer, followed immediately by oxidation with sodium periodate, afforded solutions of each *N*-acetylprolinal. In the case of the major isomer, the calculated specific rotation of the prolinal was +30° and that for the minor isomer −30°. Unfortunately, optically pure *N*-acetylpyrrolidine-2-carboxaldehyde is unknown, but *N*-acetyl-D- and -L-proline[26] have $[\alpha]_D$ +114° and −114° respectively, suggesting that the major product was possibly the D-*manno* isomer and the minor constituent the D-*gluco* isomer. These assignments were later confirmed by the n.m.r.-spectral evidence of the derived indolizidines.

As the *N*-acetyl group may be difficult to remove, the synthesis was continued with the benzyloxycarbonyl (Cbz) protecting group on nitrogen. Therefore, the pyrrolidine mixture, **18** and **22**, was converted into a crystalline mixture of Cbz derivatives, **22**



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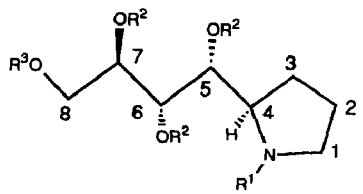
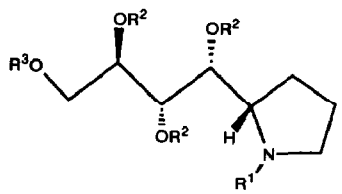
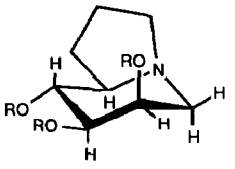


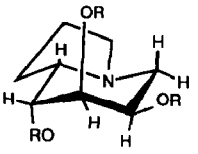


18 $R^1 = R^2 = R^3 = H$
19 $R^1 = R^2 = R^3 =$ Ac
20 $R^1 =$ Cbz, $R^3 = R^2 = H$
21 $R^1 =$ Cbz, $R^2 = H$, $R^3 =$ Mes

22 $R^1 = R^2 = R^3 = H$
23 $R^1 = R^2 = R^3 =$ Ac
24 $R^1 =$ Cbz, $R^3 = R^2 = H$
25 $R^1 =$ Cbz, $R^2 = H$, $R^3 =$ Mes

and **24** respectively, which could be separated neither by chromatography nor by fractional crystallisation. Reaction of the mixture of amides with mesitylenesulphonyl chloride afforded two major isomers, together with some minor faster-moving products and a trace of unreacted starting material. The two major mesitylenesulphonates were isolated by flash chromatography, to give **21** and **25** in 64 and 19% yields, respectively. The configurations of **21** and **25** were established only after their conversion into indolizidines, which was accomplished in each case by hydrogenation to remove the Cbz groups, and then cyclisation in the presence of sodium hydrogencarbonate. In each case the hydroxyl groups of the product were acetylated to aid isolation. The *manno* isomer **21** afforded the syrupy tri-*O*-acetylindolizidine **26** in 68% yield, the mass spectrum of which showed an $M^+ + 1$ ion at 300.1451. The ^{1}H-n.m.r. spectrum was assigned as far as possible by the NOESY technique. The resonances due to H-6, H-7, and H-8 were all readily recongnised at low field and the coupling constants (with indolizidine numbering, $J_{5ax,6} = J_{5e,6} = J_{6,7} = 2.7$ Hz, $J_{7,8} = 10.1$ Hz, $J_{8,8a} = 9$ Hz) indicated that the six-membered ring adopted a conformation close to the $^8C_5$ conformation, as in **26**. The large antiperiplanar coupling $J_{8,8a}$ indicated that this was the *manno* isomer; the *gluco* isomer **28** has H-8 and H-8a *cis*-disposed, and unlikely to give rise to such a large coupling constant.





26 R = Ac
27 R = H

28 R = Ac
29 R = H

Cbz = $PhCH_2OCO$
Mes = mesitylenesulphonyl

The *gluco* isomer **25** gave the *gluco* indolizidine **28** in 68% yield, with a mass spectrum that also showed an $M^+ + 1$ ion at *m/z* 300.1451. The ^{1}H-n.m.r. spectrum indicated that the compound adopted the $^5C_8$ conformation, as indicated by the coupling constants ($J_{5ax,6} = 8.5$, $J_{5e,6} = 5$, $J_{6,7} = 3.3$, $J_{7,8} = 3.3$, and $J_{8,8a} = 2.2$ Hz). Unlike the *manno* isomer **26**, in which H-8a was at about δ 1.7 (as indicated by NOESY), H-8a of **28** afforded a triplet of doublets at δ 2.37; the downfield shift being due to its 1,3-diaxial interaction with the 7-OAc group.

O-Deacetylation of each triacetate afforded the *manno* indolizidine **27** and the *gluco* indolizidine **29** in good yields as syrups. They were found to inhibit the action of α-L-fucosidase, in particular the *gluco* isomer **29**, but neither was a good inhibitor of α-D-mannosidase. Full details of these studies will published separately.

EXPERIMENTAL

General methods. — Unless otherwise stated, optical rotations were determined at a concentration of ~1% in $CHCl_3$ solution at room temperature (18–20°) in 1-dm tubes on a Perkin–Elmer 141 automatic polarimeter. ^{1}H-N.m.r. spectra were recorded with either a Bruker WH-400 (400 MHz), a Bruker WM-250 (250 MHz), or a Nicolet NT-200 (200 MHz) and ^{13}C-n.m.r. spectra were recorded with either a Bruker WP-60 or WM-250 spectrometer. In all cases Me_4Si was the internal standard. Mass spectra were determined with a Kratos MS-25 spectrometer by electron impact at 70 eV. Melting points were measured on a Kofler hot-stage and are uncorrected. Reactions were monitored by t.l.c. on silica gel ready-coated on aluminium sheet (Merck 5554) and spots were made visible by spraying with 5% conc. H_2SO_4 in EtOH. Flash chromatography was performed on Silica Gel G (Merck 9385) at a pressure of 10–20 lb.in^{-2}. After use, the column was washed with either acetone or MeOH and used again after equilibration with the eluting solvent. Normally the same silica was used at least five times before being discarded.

*(1,2;3,4-Di-*O*-isopropylidene-β-*D*-arabinopyranosyl)carboxaldehyde* (**5**). — (*A*) Chromium trioxide (23 g, 0.23 mol) was freshly dried for 1 h at 100–150°, and added over 5 min to a stirred mixture of anhydrous CH_2Cl_2 (600 mL) and anhydrous pyridine (35 mL, 0.43 mol). The resulting solution was then stirred for a further 30 min and a solution of 2,3;4,5-di-*O*-isopropylidene-β-D-fructopyranose (**4**, 15 g, 58 mmol) in CH_2Cl_2 (40 mL) was added, followed immediately by Ac_2O (8.4 mL). The mixture immediately turned brown, but stirring was continued for a further 5 h, whereupon g.l.c. (130° + 6°.min^{-1}) indicated that the reaction was complete. Ethyl acetate (900 mL) was then added and the precipitated inorganic material filtered off. The filtrate was evaporated and the residue extracted with EtOAc (150 mL) and some residual inorganic material filtered off. The solution was then evaporated and last traces of pyridine and AcOH were removed by repetitively dissolving the residue in toluene and evaporating. The syrup was then partially decolourised by dissolving in ether (150 mL) and adding silica gel. The silica gel was the filtered off and the filtrate evaporated to give the crude aldehyde as a pale-yellow oil (13 g, ~85%) sufficiently pure for the next step.

(*B*). Pyridinium chlorochromate (1.0 g, 4.6 mmol), pre-dried *in vacuo* over P_2O_5, was added to a stirred solution of 2,3;4,5-di-*O*-isopropylidene-β-D-fructopyranose (**4**, 0.5 g, 1.9 mmol) in anhydrous CH_2Cl_2 (5 mL). The mixture rapidly turned black; it was heated under reflux for 2 h, cooled, diluted with ether, the precipitated inorganic material filtered off, and the filtrate evaporated. The residue was dissolved in light petroleum and decolourised with charcoal to give a clear syrup of the aldehyde **5** (0.38 g, 70%). Its ^{1}H-n.m.r. spectrum indicated that it existed as a mixture as the aldehyde and its hydrate.

Ethyl (E)-*3-(1,2:3,4-di-*O*-isopropylidene-β-*D*-arabinopyranosyl)-2-propenoate* (**6**) *and its* (Z) *isomer* (**7**). — A solution of the crude aldehyde (**5**, 15.07 g, 58 mmol) and ethoxycarbonylmethylenetriphenylphosphorane (26.1 g, 75 mmol) in CH_2Cl_2 (50 mL) was kept for 1 h at room temperature when t.l.c. (2:3 ether–light petroleum) indicated

that the reaction was complete and a faster-moving product had been formed. The mixture was evaporated and the residue extracted twice with ether (150 mL and 50 mL). The extract was concentrated to a purple-coloured syrup, which was then extracted twice with 5:3, ether–light petroleum (150 and 50 mL). The combined extracts were then decolourised by the addition of silica gel. Following filtration of the slurry, the filtrate was kept for 20 min at 0° to allow the last traces of Ph_3PO to crystallise out. The solid was then filtered off and the filtrate evaporated. The residue was flash chromatographed (2:3 ether–light petroleum) to give first the *E* product **6** (11.5 g, 60%) as a syrup; $[\alpha]_D$ −27° ($CHCl_3$) (Found: C, 58.13; H, 7.32. $C_{16}H_{24}O_7$ calc.: C, 58.53; H, 7.37%); *m/z* 313 (10%, $M^+ - CH_3$); ^{1}H-n.m.r. (250 MHz, C_6D_6): δ 5.81 (1 H, d, $J_{2',3'}$ 15.5 Hz, H-3′ or H-2′), 5.67 (1 H, d, H-2′ or H-3′), 4.24 (1 H, d, $J_{2,3}$ 2.6 Hz, H-2), 4.41 (1 H, dd, $J_{3,4}$ 7.8 Hz, H-3), 3.78 (1 H, ddd, $J_{4,5a}$ 2, $J_{4,5b}$ 1 Hz, H-4), 3.72 (1 H, dd, $J_{5a,5b}$ 13 Hz, H-5a), 3.65 (1 H, dd, H-5b), 1.49, 1.37, 1.28, 1.14 (3 H × 4, s, Me_2C groups), 4.07 (2 H, q, OCH_2–), and 1.07 (3 H, t, CH_3).

The slower component could not be isolated pure in this experiment because it was present in such small amounts. However when the reaction was repeated with EtOH as a solvent the two components were present in equal amounts and consequently the *cis*isomer **7** was isolated as a syrup in low yield after careful chromatography; $[\alpha]_D$ −27° (Found: C, 58.14; H, 7.32. $C_{16}H_{24}O_7$ calc.: C, 58.53; H, 7.37%); *m/z* 313 (10%, $M^+ - CH_3$); ^{1}H-n.m.r. (250 MHz, C_6D_6): δ 7.31 (1 H, d, $J_{2',3'}$ 12.3 Hz, H-3′), 6.67 (1 H, d, H-2′), 4.14 (1 H, d, $J_{2,3}$ 2.6 Hz, H-2), 4.39 (1 H, dd, $J_{3,4}$ 8 Hz), 3.78 (1 H, dt, $J_{4,5a} = J_{4,5b}$ = 1 Hz, H-4), 3.78 (1 H, dd, $J_{5a,5b}$ 13 Hz, H-5a), 3.70 (1 H, dd, H-5b), 1.46, 1.34, 1.12, 1.02 (3 H × 4, s, Me_2C), 3.95 (2 H, q, OCH_2–), and 0.91 (3 H, t, CH_3)

(E)-*3-(1,2;3,4-di-*O*-isopropylidene-β-*D*-arabinopyranosyl)-2-propenol* (**8**). — The δ,β-unsaturated ester **6** was added to a solution of $LiAlH_4$ (0.15 g) in dry ether (20 mL). The mixture was heated under reflux for 1 h, when t.l.c. (5:1 ether–light petroleum) indicated that the reaction was complete and a slower-moving product had been formed. The excess of reagent was decomposed by the dropwise addition of water with vigorous stirring. The suspension was then filtered and the filtrate dried ($MgSO_4$) and concentrated to a syrup, which was purified by flash chromatography (10:1 ether–light petroleum) to give the allylic alcohol (**8**) as a syrup (0.35 g, 57%); $[\alpha]_D$ −12° (Found: C, 58.64; H, 7.20. $C_{14}H_{22}O_6$ calc.: C, 58.73; H, 7.74%); ^{1}H-n.m.r. (90 MHz, $CDCl_3$); δ 6.28 (1 H, dt, $J_{2',3'a} = J_{2',3'b}$ = 6 Hz, $J_{1',2'}$ 16 Hz, 2′-H), 5.86 (1 H, dt, $J_{1',3'a} = J_{1,3'b}$ = 1.5 Hz, 1′-H), 4.65 (1 H, dd, $J_{2,3}$ 2.5, $J_{3,4}$ 8 Hz, 3-H), 4.25 (4 H, m, H-3′a,3′b,2,4), 3.98 (1 H, dd, $J_{5a,5b}$ 11 Hz, $J_{5a,4}$ 2 Hz, H-5a), 3.84 (1 H, dd, $J_{5b,4}$ 1 Hz, 5b-H), 1.40 (3 H), 1.38 (3 H), and 1.28 (3 H), 1.15 2 × Me_2C)

*Ethyl 3-(1,2;3,4-di-*O*-isopropylidene-β-*D*-arabinopyranosyl)propanoate* (**9**). — A mixture of the α,β-unsaturated esters **6** and **7** (3.0 g) was hydrogenated over 5% palladium-on-charcoal (0.5 g) in EtOH for 2 h, when t.l.c. (2:3 ether–light petroleum) indicated that the reaction was complete, but the product had a similar mobility to the starting material. The mixture was filtered and evaporated to give **9** as a clear syrup (2.9 g, 98%); $[\alpha]_D$ −11° (Found C, 57.89; H, 7.86. $C_{16}H_{26}O_7$ calc.: C, 58.17; H, 7.39%); *m/z* 330 (1.3%, M^+), 315 (20%, $M^+ - CH_3$); ^{1}H-N.m.r. (250 MHz, C_6D_6): δ 4.09 (1 H, d, $J_{2,3}$

2.5 Hz, H-2), 4.41 (1 H, dd, $J_{3,4}$ 8 Hz, H-4), 3.85 (1 H, ddd, $J_{4,5a}$ 2, $J_{4,5b}$ 1 Hz, H-3), 3.72 (1 H, dd, $J_{5a,5b}$ 14 Hz, H-5a), 3.64 (1 H, dd, H-5b), 2.91 (1 H, ddd, $J_{3'a,3'b}$ 16.5, $J_{2'a,3'a}$ 9.5, $J_{2'b,3'a}$ 6.1 Hz, H-3'), 2.79 (1 H, ddd, $J_{2'a,3'b}$ 6.3, $J_{2'b,3'b}$ 9.8 Hz, H-3'b), 2.49 (1 H, $J_{2'a,2'b}$ 13.5 Hz, H-2'a), 2.26 (1 H, ddd, H-2'b), 1.54, 1.39, 1.19, 1.15 (3 H × 4, s, Me_2C), 4.01 (2 H, q, OCH_2–), and 0.99 (3 H, t, CH_3).

*3-(1,2;3,4-di-*O*-isopropylidene-β-*D*-arabinopyranosyl)propanol* (**10**). — The ester **9** (9 g, 27 mmol) was dissolved in a minimum of dry ether and added to a stirred suspension of $LiAlH_4$ (2.1 g, 54 mmol) in dry ether (50 mL). The mixture was then heated under reflux for 1 h, whereupon the mixture was treated dropwise and very slowly with water. When the decomposition was complete, the mixture was filtered and the filtrate dried ($MgSO_4$) and evaporated to a syrup, which was purified by flash chromatography (10:1 ether–light petroleum) to give the alcohol as a thin syrup (7.1 g, 90%); $[\alpha]_D$ −11° (Found C, 57.16; H, 8.00. $C_{14}H_{24}O_6$ calc. C, 58.32; H, 8.39%); *m/z* 273 (16%, $M^+ - CH_3$); ^{1}H-n.m.r. (250 MHz, C_6D_6): δ 4.05 (1 H, d, $J_{2,3}$ 2.6 Hz, H-2), 4.45 (1 H, dd, $J_{3,4}$ 8 Hz, H-3), 3.82 (1 H, ddd, $J_{4,5a}$ 2, $J_{4,5b}$ 1 Hz, H-4), 3.75 (1 H, dd, $J_{5a,5b}$ 12.9 Hz, H-5a), 3.66 (1 H, dd, H-5b), 3.53 (2 H, t, *J*'s 6 Hz, H-1'a and H-1'b), 1.75–2.18 (4 H, m, H-2'a,2'b,3'a,3'b), 1.49, 1.36, 1.16, and 1.12 (4 × 3 H, 4 × s, Me_2C's).

*3-(1,2;3,4-di-*O*-isopropylidene-β-*D*-arabinopyranosyl)propyl azide* (**12**). — To an ice-cold stirred solution of the alcohol **10** (7.1 g, 25 mmol) in a mixture of CH_2Cl_2 (80 mL) and Et_3N (5.8 mL; 42 mmol) was added, dropwise over ~5 min, a solution of MsCl (3.3 mL, 42 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred for 5 min, when t.l.c. (10:1 ether–light petroleum) indicated that the reaction was complete. Ether was then added and the precipitated solid filtered off. The filtrate was then evaporated and the resulting syrup extracted with ether (100 mL), and the remaining isoluble solid filtered off. The filtrate was then washed with water (3 × 10 mL), dried ($MgSO_4$) and concentrated to afford the crude, syrupy mesylate **11** (9 g).

The mesylate **11** was then dissolved in *N,N*-dimethylformamide (25 mL), NaN_3 was then added, and the mixture was heated for 30 min at 90°, after which t.l.c. (1:1 ether–light petroleum) indicated that all the starting material had reacted to give a faster-moving product. Ether (150 mL) was then added to the cooled mixture, and the precipitated salts were filtered off. The filtrate was then washed with M NaOH solution (60 mL), then successively with 4 30-mL portions of water. The ethereal solution was then dried ($MgSO_4$) and evaporated to give a clear, colourless syrup of the azide **12** (5.9 g, 76%); $[\alpha]_D$ −12° (Found C, 54.38; H, 7.45. $C_{14}H_{23}N_3O_5$ calc.: C, 53.66; H, 7.40%); *m/z* 314 (1%, $M^+ + 1$), 298 (29%, $M^+ - CH_3$); ^{1}H-n.m.r. (250 MHz, C_6D_6): δ 3.98 (1 H, d, $J_{2,3}$ 2.5 Hz, H-2), 4.43 (1 H, dd, $J_{3,4}$ 7.9 Hz, H-3), 3.82 (1 H, ddd, $J_{4,5a}$ 1.5, $J_{4,5b}$ 1 Hz, H-4), 3.72 (1 H, dd, $J_{5a,5b}$ 12.9 Hz, H-5a), 3.65 (1 H, dd, H-5b), 2.90 (2 H, t, *J*'s 6 Hz, H-1'a and H-1'b), 1.62–2.00 (4 H, m, H-2'a,2'b,3'a,3'b), 1.49, 1.36, 1.16, and 1.12 (4 × 3 H, 4 × s, Me_2C').

*5,6,7,8-Tetra-*O*-acetyl-1-azido-1,2,3-trideoxy-*D*-*arabino*-oct-4-ulose* (**16**). — The azide **12** (2.0 g) was dissolved in AcOH (15 mL), water (30 mL) was added, and the cloudy suspension was then heated for 4 h at 100–105°, when t.l.c. (10:3 $CHCl_3$–MeOH) indicated that the reaction was complete with the conversion of starting material into a

slower-moving product. The pale-yellow solution was then evaporated and toluene evaporated several times from the residue to remove last traces of AcOH. The resulting syrup was then purified by flash chromatography (10:3 $CHCl_3$–MeOH), to give the free octulose **13** as a colourless syrup (1.4 g, 90%), suitable for the next step.

For characterisation, it was acetylated in the usual way (Ac_2O–pyridine) to give the open-chain tetra-acetate **16**, $[\alpha]_D$ +45°; ^{1}H-n.m.r. (250 MHz, C_6D_6): δ 2.70 (2 H, t, J 6 Hz, H-1a,1b), 1.50–1.70 (2 H, m, H-2a,2b), 2.43 (1 H, dt, $J_{3a,3b}$ 10.0, $J_{3a,2a} = J_{3a,2b}$ = 7 Hz, H-3a), 2.26 (1 H, dt, $J_{3b,2a} = J_{3b,2b}$ = 7 Hz, H-3b), 5.83 (1 H, d, $J_{5,6}$ 2 Hz, H-5), 5.83 (1 H, dd, $J_{6,7}$ 9.2 Hz, H-6), 5.53 (1 H, ddd, $J_{7,8a}$ 2.4, $J_{7,8b}$ 4.6 Hz, H-7), 4.35 (1 H, dd, $J_{8a,8b}$ 12.5 Hz, H-8a), 4.34 (1 H, dd, H-8b), 1.85 (6 H), 1.74 (3 H), and 1.66 (3 H) (4 OAc)

*1,4-Acetimino-5,6,7,8-tetra-*O*-acetyl-1,2,3,4-tetradeoxy-*D-manno- *and* -D-gluco-octitol* (**19** and **23**). — A solution of the free octulose **13** (1.2 g) in EtOH was hydrogenated at 60 lb.in^{-2} for 15 h over 5% palladium-on-charcoal (0.6 g), after which t.l.c. (5:2 $CHCl_3$-MeOH) indicated the presence of a major non-migrating component together with three minor, faster-moving components. The catalyst was then filtered off and washed well with methanolic ammonia. The combined filtrate was then evaporated to a syrup, which was dissolved in a minimum of 10:1 $CHCl_3$–MeOH and applied to a short column of silica gel (~7 × 1.5 cm). Elution of the column with the same solvent mixture gave the three minor components (0.2 g), which were not amines and were therefore discarded. Subsequent elution of the column with methanolic ammonia (150 mL) then afforded a mixture of the pyrrolidines **18** and **22** as a white foam (0.88 g, 89%). A portion of the mixture (0.2 g) was then acetylated with a mixture of Ac_2O (2 mL) and pyridine (2 mL) for 15 h. This afforded two compounds by t.l.c. (5:2 ether–acetone). The components were not very well detected by the H_2SO_4 char reagents, but showed up well using the molybdate spray reagent or I_2. Ether was added to the mixture, and the resulting precipitate was filtered off and the filtrate evaporated, and the last traces of pyridine and Ac_2O were removed by several evaporations of toluene. The syrup was then fractionated by flash chromatography (5:2 ether–acetone).

First fractions eluted contained the *manno* isomer **19** (0.2 g, 48%); m.p. 121–124°, $[\alpha]_D$ +112° (Found C, 53.60; H, 6.79; N, 3.35, $C_{18}H_{27}NO_9$ calc.: C, 53.86; H, 6.78; N, 3.49%); *m/z* 402 (2.5%, $M^+ + 1$).

The next eluted component, the *gluco* isomer **23**, was obtained as a syrup which partly crystallised (0.08 g, 18%); m.p. 110–115° (softening from 105°), $[\alpha]_D$ +11° (Found C, 53.77; H, 6.68; N, 3.51. $C_{18}H_{27}NO_9$ calc.: C, 53.86; H, 6.78; N, 3.49%); *m/z* 402 (0.1%, $M^+ + 1$).

O-deacetylation and periodate oxidation of **19** *and* **23**. — About 10 mg of each of the two pyrrolidines was accurately weighed into 2-mL graduated flasks. To each was added 0.3 mL of MeOH; swirling completed solution. To each was added 1 drop of M methanolic NaOMe, and the flasks were stoppered and kept for 30 min to allow O-deacetylation. Each was then treated with 1 mL of $NaIO_4$ solution (20 mg $NaIO_4$ per mL) and the solution made up to 2 mL with water. Each solution was then transferred to a polarimeter tube and the rotation measured over the course of 2 h. In the case of the *gluco* isomer **23**, the rotation became constant within 1 h at α = −0.14° and the *manno*

isomer **18** changed to $a = +0.13°$. These rotations corresponded to $[a]_D$ values of about $-30°$ and $+30°$, respectively, for the 1-acetyl-2-formylpyrrolidines.

*1,4-Benzyloxycarbonylimino-1,2,3,4-tetradeoxy-8-O-mesitylenesulphonyl-*D-manno-*octitol* (**21**) *and its* D-gluco*isomer* (**25**). — The mixture of pyrrolidines **18** and **22** (0.78 g, 4.1 mmol) was dissolved in a little water (~3 mL) and then diluted with EtOH (10 mL). Sodium hydrogencarbonate (0.4 g) was then added to the mixture, and it was then cooled to 0°. Benzyl chloroformate (1.2 mL, 8.2 mmol) was then added to the stirred mixture, which was then stirred for 2 h, when t.l.c. (10:1 chloroform–methanol) indicated reaction to be complete. Ethanol (40 mL) was then added, and the mixture was filtered and the filtrate evaporated. The resulting syrup was then purified by flash chromatography (10:1 $CHCl_3$–MeOH). The first fractions contained benzyl alcohol, followed by an inseparable mixture of the two benzyloxycarbonylimines, **20** and **24** as an amorphous solid (0.8 g, 60%), m.p. 96–113° (Found C, 58.8; H, 6.80; N, 4.30. $C_{16}H_{23}NO_6$ calc.: C, 59.07; H, 7.13; N, 4.30%).

A portion of this solid (0.5 g, 1.5 mmol) was dissolved in anhydrous pyridine (20 mL), cooled to 0°, and freshly recrystallised mesitylenesulphonyl chloride (0.67 g, 3.1 mmol) added in small portions over ~5–10 min. The mixture was then allowed to warm slowly to room temperature and kept overnight. T.l.c. (5:2 EtOAc–light petroleum) then showed conversion of the starting material into two slower-moving products in the approximate ratio of 3:1. A minor, faster-moving product indicated that some higher substitution may have occurred. The mixture was then evaporated and freed from the last traces of pyridine by repeated evaporation with toluene. The product was then dissolved in $CHCl_3$ and washed successively with dilute HCl (20 mL), saturated $NaHCO_3$ solution (20 mL), and water (20 mL). The $CHCl_3$ solution was then evaporated and flash chromatographed (50:1 $CHCl_3$–MeOH) to give first the major isomer, 1,4-benzyloxycarbonylimino-1,2,3,4-tetradeoxy-8-*O*-mesitylenesulphonyl-D-*gluco*-octitol (**21**, 0.15 g, 19%); $[a]_D$ $-38°$.

The major *manno* isomer **25** was eluted next and was obtained as a syrup (0.5 g, 64%), $[a]_D$ 0°, $[a]_{328}$ $+10°$.

Neither isomer could be characterised at this stage as their n.m.r. spectra were complex because of rotational isomerism about the N–Cbz bond.

(6R,7R,8R,8aR)-*6,7,8-Triacetoxyindolizidine* (**26**). — A solution of the *manno* isomer **21** (0.5 g) in EtOH (20 mL) containing suspended $NaHCO_3$ (~1 g), was hydrogenated with 5% palladium-on-charcoal (~0.5 g) over H_2 at 1 atm until the Cbz group had been removed (~1–2 h). The mixture then appeared by t.l.c. (5:2 $CHCl_3$–MeOH) to be composed of three partly overlapping spots. The mixture was then heated for 3 h at 60° during which time the three components coalesced into one, together with a small amount of faster-moving material which could be detected under u.v. light. The mixture was then filtered and evaporated to a syrup, which was acetylated with Ac_2O and pyridine. The mixture was then evaporated and toluene evaporated from the residue to remove the last traces of pyridine, and the residue extracted with ether. The ethereal extract was evaporated and the product purified by flash chromatography (5:2 EtOAc–light petroleum) to give the indolizidine **26** as a syrup (200 mg, 68%), $[a]_D$ $-43°$;

m/z 300.1451 ($M^+ + 1$, 1%, calc.: $C_{14}H_{22}NO_6$ 300.1447), 240.1238 (31%, $M^+ - OAc$, calc.: $C_{12}H_{18}NO_4$ 240.1236); ^{1}H-n.m.r. (400 MHz, C_6D_6): δ 5.56 (1 H, dd, $J_{8,8a}$ 9.0, $J_{7,8}$ 10.1 Hz, H-8), 5.50 (1 H, q, $J_{5ax,6} = J_{5e,6} = J_{6,7}$ 2.7 Hz, H-6), 5.06 (1 H, dd, H-7), 2.83 (1 H, dd, $J_{5ax,5e}$ 12.8 Hz, H-5*e* or H-5*ax*), 2.70 (H-1, dt, $J \sim 2.5$, 7.5 and 7.5, H-3), 1.5–2.00 (cm, remaining ring protons), 1.77, 1.69, and 1.71 (Ac singlets)

*(6*R,*7*R,*8*R,*8a*R)-*6,7,8-Hydroxyindolizidine* (**27**). — The triacetate **26** (20 mg) was dissolved in a small quantity of dry MeOH and treated with one drop of M methanolic NaOMe. After ~30 min the mixture was decolourised with a little charcoal and then evaporated to give a crystalline solid (10 mg, 86%); $[\alpha]_D$ −25.4° (MeOH); *m/z* 174 (7%, $M^+ + 1$), 173.1057 (10%, M^+, $C_8H_{15}NO_3$ calc.: 173.1052), 156 (21%, $M^+ - OH$), 138 (6%, $M^+ - OH - H_2O$), and 70 (100%).

*(6*R,*7*R,*8*R,*8a*S)-*6,7,8-Triacetoxyindolizidine* (**28**). — The minor mesitylenesulphonate **25** (0.15 g) was hydrogenated and ring-closed as described for the major isomer **21** and obtained as a syrup (60 mg, 68%); $[\alpha]_D$ −31°; *m/z* 300.1451 (0.6%, $M^+ + 1$, $C_{14}H_{22}NO_6$ calc. 300.1447), 200.1234 (26%, $M^+ - OAc$, $C_{12}H_{18}NO_4$ calc. 200.1236), 198 (3.4%), 180 (21%), 138 (31%), 120 (19%), and 43 (100%); ^{1}H-n.m.r. (250 MHz, C_6D_6): δ 5.26 (1 H, dd, $J_{8,8a}$ 2.2, $J_{7,8}$ 3.4 Hz, H-8), 5.57 (1 H, ddd, $J_{5ax,6}$ 8.5, $J_{5e,6}$ 5, $J_{6,7}$ 3.3 Hz, H-6), 5.69 (1 H, t, H-7), 2.97 (1 H, dd, $J_{5ax,5e}$ 9.9 Hz, H-5*e*), 2.79 (1 H, dt, *J*'s 2.5, 10.5 and 10.5 Hz, H-3), 2.40 (1 H, t, H-5*ax*), 2.37 (1 H, $J_{1,8a} = J_{1',8a}$ = 5 Hz, H-8a), 1.90 (1 H, m, H-3'), 1.25–1.75 (cm, remaining ring protons), 1.70, 1.68, and 1.58 (Ac singlets).

*(6*R,*7*R,*8*R,*8a*S)-*6,7,8-Trihydroxyindolizidine* (**29**). — The foregoing triacetate **28** (20 mg) was deacetylated as before to give the free indolizidine as a syrup (10 mg, 86%); $[\alpha]_D$ +23° (MeOH); *m/z* 174 (10%, $M^+ + 1$), 173.1051 (9%, M^+, $C_8H_{15}NO_3$ calc. 173.1052), 156 (19%, M − OH), 136 ($M^+ - OH - H_2O$), and 70 (100%).

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