Structure Elucidation of Two Triterpenoid Tetrasaccharides from *Androsace saxifragifolia*

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Saxifragifolins A and B, two new triterpenoid tetrasaccharides isolated from the aerial part of *Androsace saxifragifolia*, were respectively shown to be androsacenol-3-*O*-{β-D-xylopyranosyl-(1 ⟶ 2)-β-D-glucopyranosyl-(1 ⟶ 4)-[β-D-glucopyranosyl-(1 ⟶ 2)]-α-L-arabinopyranoside} (**1**) and cyclamiretin A 3-*O*-{β-D-xylopyranosyl-(1 ⟶ 2)-β-D-glucopyranosyl-(1 ⟶ 4)-[β-D-glucopyranosyl-(1 ⟶ 2)]-α-L-arabinopyranoside} (**2**). The structural details were elucidated by a combination of fast-atom-bombardment mass spectrometry, chemical degradation, and one- and two-dimensional n.m.r. spectroscopy.

Androsace saxifragifolia (Syn. *Androsace rotundifolia*) is a small herb occurring wild in many parts of India. Members of this genus have been reported to be useful as contraceptive and abortifacient agents.[1,2] Isolation and characterisation of the triterpenoid sapogenols from the saponin mixture isolated from the plant have been reported.[3] This paper reports the isolation and characterisation of the two triterpenoid tetrasaccharides.

Results and Discussion

The ethanolic extract of the aerial part of *A. saxifragifolia*, on repeated chromatographic purification on a silica gel column, yielded two t.l.c.-distinguishable glycoside fractions, A and B. Fraction A liberated β-sitosterol and D-glucose on acid hydrolysis and was found to be identical with β-sitosterol β-D-glucoside. Fraction B gave a positive Liebermann–Burchard test for triterpenoids and Molisch test for sugars, and was shown to be a mixture of two glycosides, saxifragifolin A (**1**) and saxifragifolin B (**2**) (h.p.l.c.). These two glycosides were separated by preparative h.p.l.c. employing a Spherisorb S-10-ODS reversed-phase column, with MeOH–water (60:40) as the mobile phase. Acid hydrolysis of compound (**1**) resulted in the formation of an aglycone characterised as androsacenol[3] (**3**), and the monosaccharides D-glucose, D-xylose, and L-arabinose (identified by comparison with authentic samples).

The molecular weight of compound (**1**) was determined by fast-atom-bombardment mass spectrometry (FABMS) which has recently emerged as a powerful tool in molecular-weight determination and structural investigation of polar molecules.[4-6] This technique was applied to some naturally occurring oligoglycosides of known structure, *viz.* dioscin and gracillin,[7] tribulosin,[8] khasianine, solasonine, and solamargine,[9] to study the fragmentation pattern of such molecules.[10] The positive-ion FABMS spectrum of compound (**1**) exhibited ions at *m/z* 1 141 and 1 157, formed by the cationisation of the molecule with Na^+ and K^+ respectively. A molecular weight of 1 118 daltons was confirmed by an intense peak at *m/z* 1 117 in the compound's negative-ion spectrum, corresponding to the $(M - H)^-$ ion. Furthermore, negative fragment ions at *m/z* 985 and 955 may be ascribed respectively to the loss of a terminal pentose (xylose or arabinose) and a terminal glucose unit. A third negative fragment ion at *m/z* 823 may be attributed to the loss, from the $(M - H)^-$ ion, of a terminal glucose–pentose disaccharide

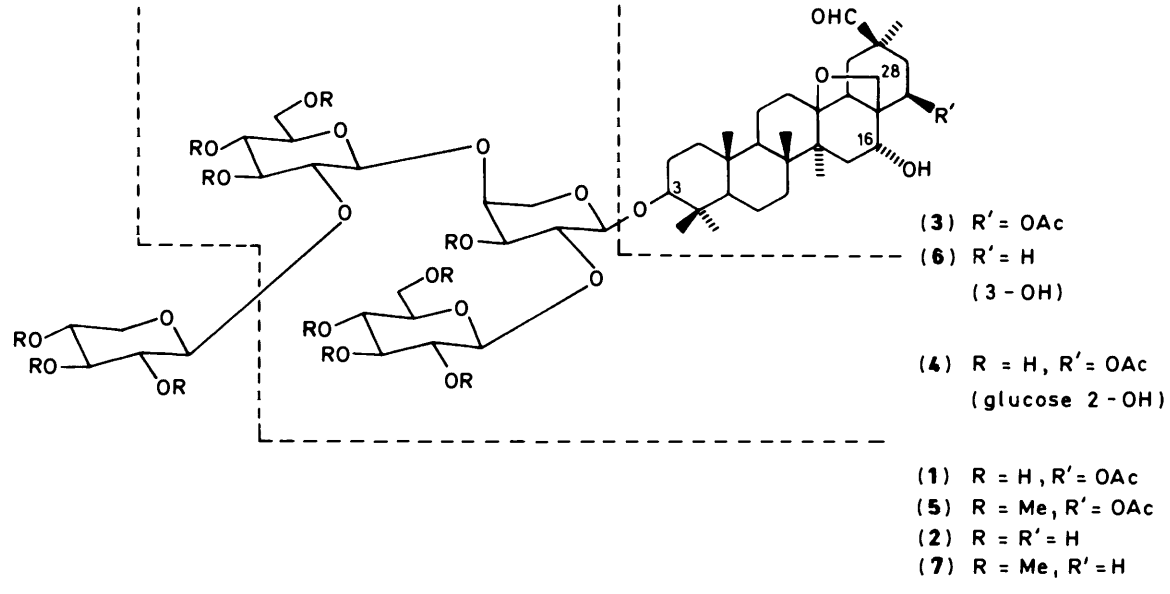


Table. Chemical shifts δ_C (±0.1) of androsacenol[3] (**3**) and cyclamiretin A (**6**) ($CDCl_3$) and saxifragifolin A (**1**) and saxifragifolin B (**2**) (C_5D_5N)

Carbon	(3)	(6)	(1)	(2)	Carbon	(1)	(2)
1	38.0	39.1	39.1	39.1	A-1	104.2[a]	104.1[a]
2	28.3	28.3	26.4	26.5	A-2	79.6	79.6
3	78.2	78.0	89.1	89.0	A-3	71.7	73.2
4	39.6	39.5	39.7	39.7	A-4	75.9	75.8
5	55.8	55.7	55.6	55.7	A-5	64.3	64.3
6	18.3[a]	18.6[a]	18.4	18.5	G-1	104.6[a]	104.6[a]
7	32.7	32.9	32.5[a]	32.5[a]	G-2	85.1	85.1
8	42.9	42.5	42.6	42.5	G-3	77.7	77.5
9	47.6	47.8	47.5	48.2	G-4	71.0	71.0
10	37.3	37.3	36.8	36.8	G-5	78.6	78.1
11	19.2[a]	19.3[a]	19.0	19.1	G-6	62.9	62.9
12	31.8[b]	31.8	31.6[a]	32.3[a]	X-1	107.5	107.4
13	86.1	86.4	86.0	86.3	X-2	75.9	75.8
14	44.6	44.7	44.5	44.5[b]	X-3	78.0	78.1
15	34.4	34.6	34.2	34.3	X-4	70.6	70.5
16	73.2	77.2	73.2	77.5	X-5	67.4	67.2
17	46.5[c]	44.7	46.5	44.0[b]	G′-1	104.6[a]	104.8[a]
18	50.6	50.6	50.3	50.4	G′-2	75.9	76.8
19	36.8	37.0	36.8	36.8	G′-3	78.2	78.5
20	47.6[c]	48.0	47.5	48.2	G′-4	71.0	71.7
21	30.1[b]	37.0	31.6	36.8	G′-5	78.0	78.1
22	74.1	33.8	73.9	33.4	G′-6	62.6	62.2
23	28.8	28.7	28.0	28.0			
24	16.6	16.6	16.3	16.4			
25	16.4	16.6	16.5	16.4			
26	18.7	18.2	18.0	18.4			
27	20.1	19.6	20.0	19.7			
28	70.3	78.0	20.0	78.4			
29	24.2	24.8	24.1	24.1			
30	205.3	207.2	205.4	207.4			
22-OAc	169.8		169.9				
	20.4		20.8				

G, G′ = glucose, X = xylose, A = arabinose [a,b,c] Assignments within a column may be reversed.

and/or, less likely, both a terminal pentose and terminal glucose unit accompanied by proton transfer. Fragmentions corresponding to a loss of either a pentose-pentose or a glucose-glucose disaccharide were not observed. The absence of a fragment ion, corresponding to the loss of a trisaccharide unit, at either *m/z* 677 or 661 suggests that a branch point in the glycone occurs on the monosaccharide directly attached to the aglycone. Thus, from the FABMS studies, it may be proposed that the glycone portion of compound (**1**) consists of either an aglycone-bound pentose or hexose with monosaccharide substituents on three of its oxygens, or, more likely, one of the sequences below.

(*a*) pentose–glc–S
|
glc–pentose

(*b*) glc–pentose–S
|
pentose–glc

S = androsacenol
pentose = xylose or arabinose

The glycoside (**1**), on partial hydrolysis with 2M-HCl in Bu^nOH at 70 °C for 3 h, furnished a prosapogenin (**4**) containing three monosaccharides (n.m.r. spectroscopy). Methanolic acid hydrolysis of compound (**4**) yielded androsacenol (**3**), L-arabinose, and D-glucose. Treatment of compound (**4**) with sodium metaperiodate followed by hydrolysis afforded L-arabinose as the only sugar constituent; therefore L-arabinose is directly linked to the aglycone in saxifragifolin A (**1**).

Permethylation of compound (**1**) using NaH–MeI in hexamethylphosphoric triamide (HMPA) afforded the permethylated product (**5**), which on acid hydrolysis liberated the sapogenin (**3**), and 2,3,4,6-tetra-*O*-methyl-D-glucose, 3,4,6-tri-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-xylose, and 3-*O*-methyl-L-arabinose. Thus the monosaccharide sequence of the glycone portion of saxifragifolin A (**1**) must be (b), which itself represents a mixture of two isomers, (b^1) and (b^2).

(b^1) Xyl*p*-(1 → 2)-Glc*p*-(1 → 4)-Ara*p* → S
2
↑
1
Glc*p*

(b^2) Xyl*p*-(1 → 2)-Glc*p*-(1 → 2)-Ara*p* → S
4
↑
1
Glc*p*

The attachment of the carbohydrate moiety at the C-3 position of androsacenol (**3**) was evident from the ^{13}C n.m.r. chemical shifts of saxifragifolin A (**1**) (Table). ^{13}C Assignments of compound (**1**) were made by comparison with those of the sapogenin[3] and the appropriate methyl glycosides by the application of known chemical-shift rules[11] and glycosylation shifts.[12–15] However, evidence to distinguish between the possible isomers (b^1) and (b^2) was not unambiguously available from ^{13}C data or from hydrolysis, since the glycosidic bond of the xyloside is cleaved faster than those of the glucosides in this system. This problem was overcome by the use of high-field two-dimensional ^1H correlation (COSY) and two-dimensional nuclear Overhauser effect (NOESY) spectroscopy. Severe spectral crowding in the region δ 2.5—4, characteristic of oligosaccharides, dictates the use of, and displays the benefit of, two-dimensional analysis.

Complete assignment of the carbon- and oxygen-bound protons of the glycone was possible from a COSY-45 spectrum (see Experimental section) of saxifragifolin A (**1**) in [$^2H_6$]-dimethyl sulphoxide ([$^2H_6$]DMSO). Assignment was commenced from the four anomeric signals, identified according to chemical shift after D_2O exchange of the hydroxy protons. Distinction between carbon- and oxygen-bound protons was also possible as a result of saturation transfer observed in a NOESY spectrum. An illustration of assignment is given in the Figure. The anomeric signal of arabinose, A1 *, (δ 4.33) exhibits a coupling to A2 (δ 3.63). A2 shows no cross-peak to a hydroxy proton, but is coupled to A3, which in turn *is* coupled to a hydroxy proton (A3OH). The connectivities may be continued to enable assignment of A4 (no A4OH), A5, and A5′.

Several important general points may be concluded from COSY-45 assignments. First, the substitution positions of the monosaccharide units may be determined by the presence or absence of a corresponding hydroxy proton. Secondly, the ring sizes of common aldopentoses (except those linked through their 4- or 5-position hydroxy group) and aldohexoses can be determined directly. Thirdly, the nature of the cross-peaks provides three useful pieces of information:

(a) the multiplicity of overlapping peaks is revealed, (b) the splitting of the peak yields a semi-quantitative estimate of coupling constants (sufficiently accurate in the cases examined to disclose the identity of each monosaccharide), (c) the cross-peaks due to vicinal couplings, where one proton is involved in a geminal coupling, have a 'tilted' appearance.

Thus, the data in the Figure confirm the preliminary conclusions drawn from the FAB mass spectra and chemical degradation, and establish that arabinose is linked through its

* Atoms are designated by a capital letter abbreviation for the monosaccharide, and a number indicating the carbon atom to which the hydrogen (hydroxy) moiety is attached.

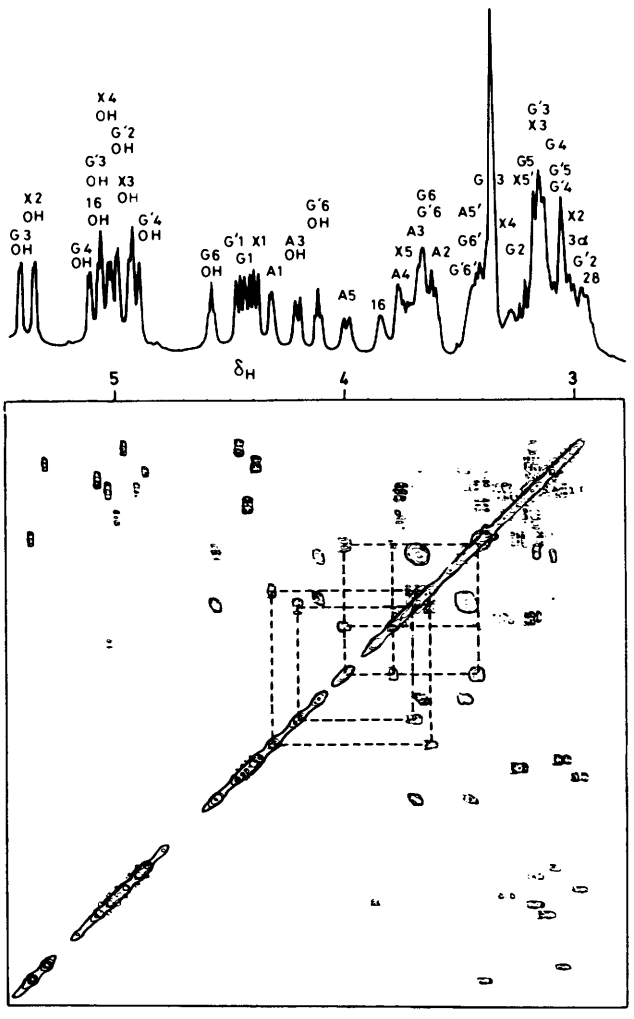


Figure. A section of a COSY-45 spectrum of saxifragifolin A (**1**) between 2.8—5.5 p.p.m. with a corresponding one-dimensional spectrum. The dotted lines represent the assignment of the protons of arabinose by *J*-connectivities.

2- and 4-position to a terminal glucopyranose (G'), and a second glucopyranose (G) linked in turn through its 2-position to a terminal xylopyranose. Assignment of each glucopyranose to its position on arabinose was achieved employing a NOESY spectrum. Diagnostic n.O.e.s were observed between A2 and G'1, and A4 and G1, confirming the structure as isomer (b^1) for saxifragifolin A (**1**). Further n.O.es, *e.g.* between X1 and G2, were consistent with the above assignments.

One possible ambiguity in the COSY-45 assignment (which in this case would contradict the FABMS results) arose because of the accidentally complete overlap of signals from G'3 and X3. However, n.O.e.s between G'1 and G'5, and X1 and X5, confirm the above assignment. In more demanding cases, temperature variation and/or T_1 measurements should overcome such difficulties.

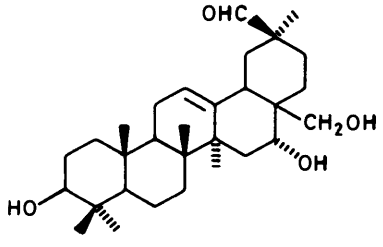
β-Configuration at the anomeric positions may be inferred from the values of the $^3J_{H1,H2}$ coupling constants for both glucopyranosyl units (G, 7.8 Hz, G', 7.7 Hz) and the xylopyranose (7.6 Hz). The configuration and ring size of the arabinosyl unit are less clear. The value of its $^3J_{H1,H2}$ coupling constant (5.5 Hz) is midway between that observed for methyl β-L-arabinofuranoside (4.0 Hz) and methyl α-L-arabinopyranoside (8.0 Hz) in [$^2H_5$]pyridine.[16] The value of $^3J_{H2,H3}$ (7.5 Hz) observed in a decoupling difference spectrum on irradiation of A1, is inconsistent with an arabinofuranoside and reflects an approximately *trans* diaxial arrangement in the pyranoside form. H1,H2 and H2,H3 Coupling constants of 7.2 Hz and 9.9 Hz respectively, observed for the α-D-arabinopyranoside in D_2O, were assigned as indicating virtually 100% of the $^1C_4$ conformation.[17] Variation in ^3J coupling constants has been employed to determine conformational equilibrium constants,[18] *e.g.* α-D-arabinopyranose tetra-acetate in $CDCl_3$ was shown to exist as 79:21 $^1C_4$:$^4C_1$ conformations at 31 °C, exhibiting H1,H2 and H2,H3 coupling constants of 6.4 and 9.0 Hz respectively. Indeed, $^3J_{H1,H2}$ values as low as 2.8 Hz have been assigned to α-L-arabinopyranosides.[19] In these latter cases, a $^1C_4$ conformation of the L-sugar was suggested to be in considerable excess over the $^4C_1$ conformer.

The coupling constants observed in saxifragifolin A (**1**) are therefore consistent with an α-L-arabinopyranoside moiety in a conformational equilibrium, with $^4C_1$ as the conformer in excess. The structure requires that in either conformer, one of the glucose units is glycosidically linked through an axial oxygen of the arabinose unit. The necessity of this is likely to bring about a shift in the conformational equilibrium with respect to unsubstituted methyl α-L-arabinopyranoside.

Further evidence supporting an α-L-arabinopyranoside configuration in rapid conformational exchange was obtained from NOESY and n.O.e. difference experiments. Nuclear Overhauser effects were observed from A1 to A2 *and* A3, as expected for $^1C_4$ and $^4C_1$ conformations respectively. The latter n.O.e. would not be expected for either a β-L-arabinopyranoside or a β-L-arabinofuranoside. The possibility of an α-L-arabinofuranoside was previously eliminated because of the value of $^3J_{H1,H2}$. An n.O.e. was also observed betwen A1 and A5', as expected for an α-L-arabinopyranoside in a $^4C_1$ conformation.

On the basis of the foregoing evidence, the structure of saxifragifolin A is proposed to be androsacenol 3-*O*-{β-D-xylopyranosyl-(1 ⟶ 2)-β-D-glucopyranosyl-(1 ⟶ 4)-[β-D-glucopyranosyl-(1 ⟶ 2)]-α-L-arabinopyranoside} (**1**).

Saxifragifolin B (**2**) on acid hydrolysis yielded two genins and D-glucose, L-arabinose, and D-xylose as the sugar components. The two genins were identified as cyclamiretins A (**6**) and D (**8**) by comparison of physical and spectral properties with authentic samples. Whilst cyclamiretin A is a genuine sapogenin, cyclamiretin D has been reported[20] to be an artifact formed by acid-catalysed rearrangement of compound (**6**). The ^{13}C n.m.r. data for saxifragifolin B (**2**) in the Table confirm that cyclamiretin A (**6**) is the genin present in (**2**), with the sugar moiety attached at the C-3 position of the genin.



(**8**)

Positive-ion FABMS of compound (**2**) exhibited peaks at *m/z* 1 099, 1 083, and 1 061, assignable to $[M + K]^+$, $[M + Na]^+$ and $[M-H]^+$ ions respectively. The negative-ion spectrum displayed an $[M + H]^-$ ion at *m/z* 1 059 and fragment ions, analogous to those in compound (**1**), at *m/z* 927, 897, and 765. The glycone moiety of saxifragifolin B (**2**) may be shown to be identical with that of compound (**1**) entirely by high-resolution 1-D and 2-D ^1H n.m.r. spectra using analogous experiments to those performed on compound (**1**). Confirmation of the identity

of saxifragifolin B (**2**) was obtained from ^{13}C shifts and permethylation studies. Thus saxifragifolin B was characterised as cyclamiretin A 3-*O*-{β-D-xylopyranosyl-(1 ⟶ 2)-β-D-glycopyranosyl-(1 ⟶ 4)-[β-D-glucopyranosyl-(1 ⟶ 2)]-α-L-arabinopyranoside} (**2**).

A glycoside of similar structure to compound (**2**) has been reported to have been isolated from *Cyclamen europaeum*.[21] However, owing to the non-availability of the authentic sample, or physical or spectral data (except m.p. and $[\alpha]_D$), the identity of compound (**2**) with the reported glycoside could not be confirmed.

Experimental

M.p.s are uncorrected. T.l.c. was carried out on silica gel G (B.D.H) using the following solvent systems: (A) benzene–$CHCl_3$–EtOAc–MeOH (1:7:1:1); (B) $CHCl_3$–MeOH–water (8:4:1). Paper chromatography for sugars was run on Whatman paper No. 1 using solvent system (C), Bu^nOH–C_5H_5N–water (6:4:3). A saturated solution of aniline oxalate in water was used as staining agent. H.p.l.c. analysis was performed with a Spectra-Physics model 8000B instrument using a Spherisorb S-10-ODS reversed-phase column (25 cm length and i.d. 10 mm) and a Micromeritics 771 refractive-index detector. G.l.c. was performed on a Hewlett-Packard model 5730A instrument using the columns (i) ECNSS-M, 3% on Gas Chrome Q at 190 °C for alditol acetates and (ii) OV-225 on Gas Chrome Q at 195 °C for partially methylated alditol acetates. Optical rotations were measured on a Perkin-Elmer automatic polarimeter; i.r. spectra were recorded in Nujol mulls on a Perkin-Elmer model 177 instrument.

Electron-impact mass spectra were recorded on a Hitachi RMU-6L mass spectrometer. FABMS was performed on a Kratos MS50 instrument fitted with a standard Kratos FAB source and a high-field magnet. The sample (*ca.* 10 mmol) was dispersed in thiodiglycol (1—2 μl) and bombarded with a 4—6 keV beam of Xe atoms. An accelerating potential of 8 kV was employed.

Low-field ^1H n.m.r. spectra were recorded on a JEOL FX-100 (99.6 MHz) instrument in $CDCl_3$ or C_5D_5N. ^{13}C N.m.r. spectra were also recorded on a JEOL FX-100 spectrometer, operating at 25.05 MHz, in C_5D_5N with tetramethylsilane as internal standard. High-field ^1H n.m.r. spectroscopy was performed on Bruker WM400 and AM500 instruments equipped with Aspect 2000 and 3000 computers respectively. All experiments (except D_2O exchange) were carried out on samples (*ca.* 20 mg) in [$^2H_6$]DMSO (0.5 ml). Two-dimensional COSY-45 experiments[22] were acquired at 400 MHz with sweep widths of 1 800 Hz (2 K data points in ω_2) and ±900 Hz (256 t_1 values zero-filled to 1 K) in ω_1. A 3 second relaxation delay was used and 112 transients were accumulated for each t_1 value. Two-dimensional NOESY experiments[23] were recorded at 500 MHz using a mixing time of 500 ms and a relaxation delay of 2 s. 64 Transients were performed for each t_1 value in a data matrix of 2 100 Hz (2 K data points) in ω_2 and ±1 050 Hz (256 t_1 values, zero-filled to 1 K) in ω_1. Important n.O.e.s, *e.g.* those involving A1, G1, G′1, and X1, were confirmed by 1-D n.O.e.-difference experiments with short irradiation times, thus minimising spin diffusion.

Isolation of the Glycosides.—The air-dried and powdered aerial part of *A. saxifragifolia* (1 kg) was successively extracted with light petroleum (b.p. 60—80 °C), chloroform, and 90% ethanol. The ethanolic extract, on removal of the solvent under reduced pressure, yielded a viscous dark brown mass (80 g). A portion of this extract (50 g) was chromatographed on silica gel (700 g). Graded elution was effected with light petroleum, followed by light petroleum–chloroform (1:1) and chloroform–methanol. (95:15, 90:10, 85:15, and 80:20). A total of 75 fractions (250 ml each) was collected and fractions giving similar spots on t.l.c. were combined.

β-Sitosterol Glucoside.—Fractions 15—20, eluted with $CHCl_3$–MeOH (90:10), were mixed (0.6 g), purified by re-chromatography, and crystallised from chloroform–methanol to afford crystals of β-sitosterol glucoside (0.15 g), m.p. 288—290 °C (decomp.), $[\alpha]_D$ −36° (*c* 0.5 in pyridine).

Isolation of Saxifragifolin A (**1**) *and Saxifragifolin B* (**2**).—Elution of the column with $CHCl_3$–MeOH (85:15) yielded a solid (1.5 g) which, on further purification by re-chromatography, afforded t.l.c.-homogeneous glycoside (0.8 g). This glycoside could not be crystallised from any solvent. It was found to be a mixture of two compounds by h.p.l.c. Preparative h.p.l.c. of glycoside B (0.75 g) with a Spherisorb S-10-ODS reversed-phase column with the solvent system MeOH–water (60:40) and flow rate 3 ml min^{-1} afforded two pure glycosides: (**1**) (0.35 g), m.p. 276—278 °C;$[\alpha]_D$ +17.40° (*c* 0.25) in MeOH) (Found: C, 57.8; H, 7.6. $C_{54}H_{86}O_{24}$ requires C, 57.96; H, 7.69%) and (**2**) (0.25 g), m.p. 258—260 °C; $[\alpha]_D$ +15.2° (*c* 0.19 in MeOH) (Found: C, 58.8; H, 7.9. $C_{52}H_{84}O_{22}$ requires C, 58.86; H, 7.92%).

Hydrolysis of Saxifragifolin A (**1**).—Compound (**1**) (100 mg) was hydrolysed with 2M-HCl in aqueous MeOH (30 ml) at water-bath temperature for 3 h. The usual work-up followed by chromatographic purification over silica gel gave androsacenol (**3**), which was crystallised from EtOAc as plates (20 mg), m.p. 261—263 °C; $[\alpha]_D$ +25° (*c* 0.35 $CHCl_3$). Its various spectral data and characterisation have been reported elsewhere.[3]

The filtrate from the hydrolysate was neutralised with Ag_2CO_3 and filtered. A portion of the neutral filtrate was concentrated under reduced pressure and tested for carbohydrates by paper chromatography using solvent system C. D-Glucose, D-xylose, and L-arabinose were identified using authentic specimens. L-Arabinose was isolated by preparative paper chromatography and its optical rotation was measured. The initial $[\alpha]_D^{25}$ value was observed to be +152° and the equilibrium value +103° (*c* 0.03 in water). The corresponding values for an authentic sample were observed to be +156° and +105°. The identities of the monosaccharides were also confirmed by g.l.c. of the carbohydrate mixture, after preparation of their alditol acetates, using column (i).

Partial Hydrolysis of Saxifragifolin A (**1**).—Compound (**1**) (100 mg) was heated in 2M-HCl (10 ml) and Bu^nOH (10 ml) at 70 °C for 3 h. Bu^nOH (50 ml) was added to the reaction mixture, which was then washed with water to remove acid and monosaccharides. Bu^nOH was removed under reduced pressure to give a gummy prosapogenin. This material was passed through a silica gel column, and elution with the solvent $CHCl_3$–MeOH (90:10) yielded t.l.c.-homogeneous prosapogenin (**4**) (20 mg) m.p. 234—236 °C; $[\alpha]_D$ +10.6° (*c* 0.24 in MeOH). Compound (**4**), on acid hydrolysis, produced androsacenol (**3**),[3] D-glucose, and L-arabinose.

Periodate Oxidation of Compound (**4**) *and Hydrolysis of the Product.*—To a solution of compound (**4**) (30 mg) in 90% EtOH (3 ml) was added dropwise a solution of sodium metaperiodate (30 mg) in water (2 ml) and the mixture was stirred at 15 °C for 3 h, and then kept at room temperature overnight. Work-up as usual afforded a residue, which was hydrolysed with 2M-HCl and the products were tested for carbohydrates by paper chromatography. Only L-arabinose was identified.

Permethylation of Saxifragifolin A (**1**) *and Hydrolysis of the Product.*—A solution of compound (**1**) (30 mg) in HMPA (5 ml)

was treated with NaH (200 mg) and MeI (5 ml) at room temperature for 3 h. The reaction mixture was extracted with diethyl ether, and the extract was washed with water, dried over anhydrous Na_2SO_4, and evaporated to yield a gummy residue. This was chromatographed over silica gel with EtOAc–light petroleum (3:7) as eluant to give a permethylated product (**5**) (115 mg) as a powder, m.p. 118—120 °C (no hydroxy absorption in the i.r. spectrum); δ($CDCl_3$) 0.80 (3 H, s), 0.88 (3 H, s), 0.96 (3 H, s), 1.16 (3 H, s), 1.28 (3 H, s), 2.0 (3 H, s, 22-OAc), 4.40 (1 H, d, *J* 7 Hz, 1-H of a glucose unit), 4.44 (1 H, d, *J* 6 Hz, 1-H of other glucose unit), 4.68 (1 H, d, *J* 5 Hz, 1-H of arabinose unit), 4.76 (1 H, d, *J* 7 Hz, 1-H of xylose unit), 5.02 (1 H, t, *J* 4 Hz, 22-H), and 9.5 (1 H, s, 30-H).

The permethylated product (**5**) (10 mg) was hydrolysed in refluxing 2M-HCl in aqueous MeOH (5 ml) for 3 h. The reaction mixture was cooled, and evaporated to dryness under reduced pressure, the residue was dissolved in water, and the solution was filtered. The filtrate was neutralised with Ag_2CO_3 and filtered. The nuetral filtrate was concentrated, and reduced with $NaBH_4$. After work-up, the residue was acetylated with Ac_2O–pyridine (1:1) at water-bath temperature for 1 h, dried *in vacuo*, and subjected to g.l.c. analysis using column (ii). Four peaks were detected and identified according to their retention times (in minutes) relative to that of the alditol acetate of authentic 2,3,4,6-tetra-*O*-methyl-D-glucose (R_t values). The four peaks were identified as the alditol acetates of 2,3,4,6-tetra-*O*-methyl-D-glucose (R_t 1.0), 2,3,4-tri-*O*-methyl-D-xylose (R_t 0.53), 3,4,6-tri-*O*-methyl-D-glucose (R_t 1.83), and 3-*O*-methyl-L-arabinose (R_t 1.50) by comparison with the relative retention times of authentic samples.[24,25]

Hydrolysis of Saxifragifolin B (**2**).—Glycoside (**2**) (80 mg) was refluxed with 2M-HCl in aqueous MeOH (15 ml) for 4 h. After work-up, chromatographic separation of the sapogenols and crystallisation from EtOAc yielded two compounds, cyclamiretin A (**6**) (10 mg) and cyclamiretin D (**8**) (15 mg).

Permethylation of Saxifragifolin B (**2**) and *Hydrolysis of the Product*.—A solution of compound (**2**) (40 mg) in HMPA (6 ml) was treated with NaH (300 mg) and MeI (6 ml) at room temperature as before. The reaction mixture was worked up and purified by chromatography to yield a permethylate (**7**) as a powder (15 mg), m.p. 114—116 °C (no hydroxy absorption in the i.r. spectrum); δ($CDCl_3$) 0.76 (3 H, s), 0.88 (3 H, s), 0.92 (6 H, s), 1.24 (3 H, s), 4.33 (1 H, d, *J* 6 Hz, 1-H of a glucose unit), 4.33 (1 H, d, *J* 8 Hz, 1-H of other glucose unit), 4.64 (1 H, d, *J* 5 Hz, 1-H of arabinose unit), and 4.72 (1 H, d, *J* 8 Hz, 1-H of xylose unit).

The permethylated product (**7**) (10 mg) was hydrolysed with 2M-HCl in aqueous MeOH (8 ml) on a water-bath for 3 h. The reaction mixture was worked up and filtered. The filtrate was concentrated. neutralised with Ag_2CO_3, and converted into alditol acetates as above. G.l.c. analysis using column (ii) showed four peaks, again corresponding to the alditol acetates of 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-xylose, 3,4,6-tri-*O*-methyl-D-glucose, and 3-*O*-methyl-L-arabinose.[24,25]

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