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Structure Elucidation of Two Acylated Triterpenoid Bisglycosides from Acacia auriculiformis Cunn.

Shashi B. Mahato,* Bikas C. Pal and Ashoke K. Nandy

Indian Institute of Chemical Biology
4, Raja S.C. Mullick Road, Jadavpur
Calcutta 700 032, India.

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Key words: *Acacia auriculiformis;* Leguminosae; triterpenes; triterpenoid saponins.

Abstract: *Acaciasides A and B, two novel acylated triterpenoid bisglycosides isolated from the fruits of Acacia auriculiformis, were respectively defined to be 3-O-[β-D-glucopyranosyl (1→6){α-L-arabinopyranosyl (1→2)}-β-D-glucopyranosyl]-21-O-{(6'S)-2'-trans-2',6'-dimethyl-6'-O-β-D-glucopyranosyl-2',7'-octadienoyl} acacic acid 28-O-α-L-rhamnopyranosyl (1→6)[β-D-xylopyranosyl (1→2)]-β-D-glucopyranoside* **(1)** *and 3-O-[β-D-glucopyranosyl (1→6){α-L-arabinopyranosyl (1→2)}-β-D-glucopyranosyl]-21-O-[(6'S)-2'-trans-2',6'-dimethyl-6'-O-{β-D-xylopyranosyl (1→2)-β-D-glucopyranosyl}-2',7'-octadienoyl] acacic acid 28-O-α-L-rhamnopyranosyl (1→6)[β-D-xylopyranosyl (1→2)]-β-D-glucopyranoside* **(2)**. *The structural details were elucidated by a combination of fast-atom-bombardment mass spectrometry, ^1H-, and ^{13}C NMR spectroscopy, and some chemical transformations.*

Acacia auriculiformis A. Cunn (Leguminosae) is distributed throughout India on road sides and parks. This species is now widely cultivated for use mainly as fuel. However, the plant is reported to have central nervous system depressant activity.[1] The plant produces large amounts of fruits which give copious froth when shaken with water in powder form indicating the presence of saponins. Isolation and structure elucidation of acaciaside, a sparingly water soluble triterpenoid trisaccharide from the fruits have been reported.[2] The water soluble saponin fraction from the fruits exhibited strong spermicidal property[3] which made this saponin fraction an ideal target for isolation and structure determination of its saponin constituents. This paper reports the structure elucidation of two novel acylated triterpenoid bisglycosides isolated from this spermicidal saponin fraction.

RESULTS AND DISCUSSION

The methanolic extract of the defatted fruits of *A. auriculiformis* on chromatographic resolution on a silica gel column yielded the sparingly water-soluble saponin, acaciaside[2] and a mixture of two water-soluble saponins as revealed by froth and HPLC analysis. These two saponins were separated by repeated prepara-

tive HPLC employing Spherisorb S-10-ODS reversed phase column, with the solvent system MeOH-water (7:3) and flow rate 4 ml $min,^{-1}$ as white amorphous solids. The molecular weight of the less polar saponin, designated acaciaside A **(1)** could successfully be determined to be 1712 by the negative ion fast-atom-bombardment - mass spectrometry[4,5] (FAB MS) which showed a peak at m/z 1711 attributable to $[M-H]^-$. The spectrum also showed some fragment ions which provided information with regard to the sequence of the sugar units in the glycone part. The molecular weight of the saponin was also confirmed by positive ion FAB spectrum which exhibited a discernible peak at m/z 1735 ascribable to $[M+Na]^+$. Acaciaside A **(1)**, $C_{80}H_{128}O_{39}$, mp 240-242°C, $[\alpha]_D$-19.5° showed in its IR spectrum a strong absorption band at 1680 cm^{-1} and in the UV spectrum the λ_{max} at 218 nm suggesting the presence of an $\alpha:\beta$-unsaturated acyl function. The ^{13}C NMR spectrum showed two singlet signals at δ 174.7 and 164.6 assignable to C-28 and M-1 carbonyl carbons and four olefinic carbons at 145.8, 143.4, 127.9 and 114.9 in addition to the C-12 and C-13 signals at 123.1 and 143.4 respectively. The compound **(1)** on hydrolysis with 2M HCl in aqueous MeOH yielded two aglycones **(3)** and **(4)** and monosaccharides, D-glucose, D-xylose, L-arabinose and L-rhamnose. The sugar units were identified by paper chromatography and GLC by comparison with authentic samples. The aglycone **(3)** was identified as acacic acid lactone.[2] The aglycone **(4)** designated acaciagenin A, mp 192-193°C, $[\alpha]_D$+54° showed in its IR spectrum absorptions for hydroxy,$\alpha:\beta$ unsaturated ester and carboxy carbonyl functions. Its ^{13}C NMR spectrum exhibited four olefinic carbons at δ 134.9, 121.0, 137.1 and 129.9 besides the C-12 and C-13 carbon signals at δ 123.1 and 142.0 respectively. Moreover, the presence of a hydroxymethylene group was suggested by a triplet carbon signal at δ 64.9 in the spectrum. These changes in the chemical shifts of the olefinic carbons as well as the appearance of a hydroxymethylene carbon strongly indicated the occurrence of acid catalysed rearrangement during acid hydrolysis of the saponin. Hydrolysis of acaciagenin A **(4)** with 6% KOH in aqueous ethanol afforded acacic acid lactone **(3)** and a monoterpene acid (5). The acid **(5)** on esterification with ethereal solution of CH_2N_2 generated the methyl ester **(6)** as oil which was characterized as 2,6-trans-2,6-dimethyl-8-hydroxy-2,6-octadienoic acid methyl ester by its IR, ^1H, ^{13}C NMR and mass spectral analyses. Consequently, the structure of acaciagenin A **(4)** was suggested to be as shown.

Hydrolysis of acaciaside A **(1)** with 6% KOH in ethanol (aq.) followed by usual work-up furnished two major products. One of these products was identified as acaciaside[2] and the other on further purification by preparative TLC was obtained as powder, mp 108-110°C. This compound **(7)** on esterification with CH_2N_2 yielded a methyl ester **(8)** as a colourless oily substance which showed in its ^{13}C NMR spectrum four olefinic carbons at δ 144.6, 143.2, 127.9 and 114.9 and one anomeric carbon at δ 104.7. The ^1H NMR spectrum of compound **(8)** exhibited the presence of terminal olefinic protons as double doublets of ABX type at 5.27 (J=11,

2 Hz), 5.40 (J=18, 2 Hz) and 6.25 (J=18, 11 Hz). These data along with the ^{13}C values when compared with those of (6S)-2-trans-6-hydroxy-2,6-dimethyl-2,7-octadienoic acid[6] led to the elucidation of the structure of the methyl ester **(8)** as shown. Acid hydrolysis of the ester **(8)** afforded the rearranged monoterpene acid **(5)** and D-glucose. The formation of the acid **(5)** from the compound **(8)** by acid - catalyzed rearrangement is a phenomenon of common occurrence.

Acaciaside A **(1)** on permethylation by Hakomori's method[7] gave the permethylate **(9)** which showed seven anomeric sugar protons besides the olefinic protons. Hydrolysis of the permethylate **(9)** with 2M HCl in aqueous MeOH furnished 16-O-methyl acacic acid lactone[2] **(10)** and 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,4-tri-O-methyl-L-arabinose, 2,3,4-tri-O-methyl-L-rhamnose, 2,3,4-tri-O-methyl-D-xylose and 3,4-di-O-methyl-D-glucose (identified by GLC of alditol acetates and comparison with authentic samples.[8])

On reduction with lithium aluminium hydride ($LiAlH_4$) the compound **(9)** yielded a mixture which could be separated into three products, a methylated monoterpene glycoside **(11)**, a methylated triterpene glycoside **(12)** and a methylated oligosaccharide **(13)**.

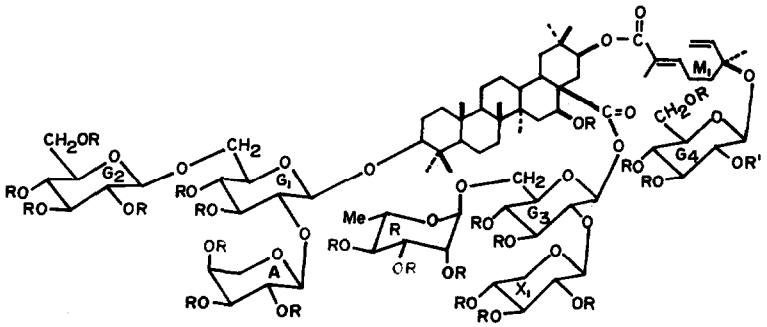
The ^1H NMR data of compound **(11)** as well as identification of the permethylated sugars obtained by acid hydrolysis led to its characterization as (6S)-2-trans-6-O-β-D-glucopyranosyl-2,6-dimethyl-2,7-octadienol.

The methylated triterpene glycoside **(12)** on hydrolysis with 2M HCl afforded 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,4-tri-O-methyl-L-arabinose, 3,4-di-O-methyl-D-glucose and a triterpene alcohol **(14)**. This triterpene alcohol **(14)** was found to be identical with an authentic sample of olean-12-en-16β-O-methyl-3β,21β,28-triol derived from acaciaside permethylate[2] **(15)** by $LiAlH_4$ reduction followed by acid hydrolysis. This result indicated the location of the glucosyl monoterpene carboxylate part in acaciaside A **(1)** to be at C-21.

The ^1H NMR spectrum of the methylated oligosaccharide **(13)** showed the presence of two anomeric protons at δ 5.02 (1H,br s) and 4.68(1H,d,J=8 Hz). On hydrolysis of the methylated oligosaccharide **(13)** with 2M HCl in aqueous methanol liberated 2,3,4-tri-O-methyl-L-rhamnose, 2,3,4-tri-O-methyl-D-xylose and 3,4-di-O-methyl-D-glucitol identified by GLC with authentic samples. Thus the oligosaccharide moiety at C-28 in acaciaside A **(1)** was characterized as α-L-rhamnopyranosyl (1→6)[β-D-xylopyranosyl (1→2)]-β-D-glucopyranoside.

The foregoing evidences led to the elucidation of the structure of acaciaside A as 3-O-[β-D-glucopyranosyl(1→6)[α-L-arabinopyranosyl (1→2)]-β-D-glucopyranosyl]-21-O-{6'S)-2'-trans-2',6'-dimethyl-6'-O-β-D-glucopyranosyl-2',7'-octadienoyl}acacic acid 28-O-α-L-rhamnopyranosyl (1→6)[β-D-xylopyranosyl (1→2)]-β-D-glucopyranoside **(1)**.

The ^{13}C NMR spectrum of acaciaside A **(1)** also supports the structure shown. ^{13}C chemical shifts of methyl-β-D-glucopyranoside, methyl-β-D-xylopyranoside,



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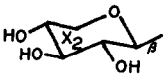
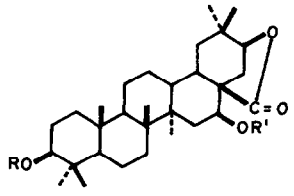
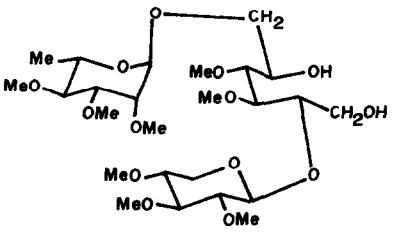
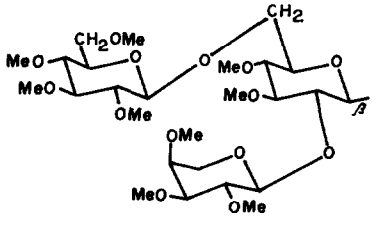
(2) R = H, R' = 
(9) R = R' = Me
(16) R = Me, R' = 

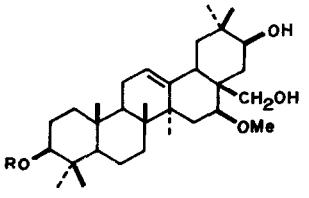


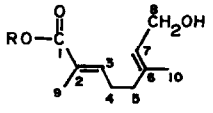
(3) R = R' = H (10) R = H, R' = Me



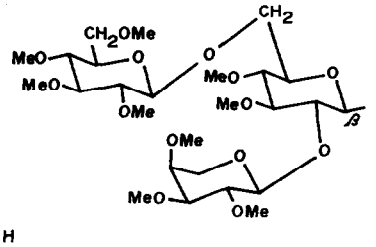
(13)

(15) R =  , R' = Me

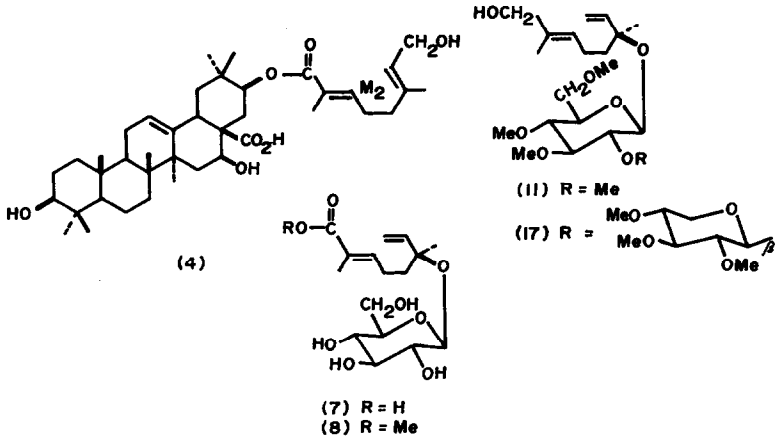




(5) R = H
(6) R = Me

(12) R = 

(14) R = H



methyl-α-L-rhamnopyranoside and methyl-α-L-arabinopyranoside in [$^2H_5$] pyridine are available.[9,10] Assignment of the signals of the saponin **(1)** (Table) were made by comparison with those of the sapogenin,[2] the monoterpene[6] and sugar moieties using known chemical shift rules[11] and glycosylation shifts.[10,12,13]

The more polar saponin, designated acaciaside B **(2)**, $C_{85}H_{136}O_{43}$, mp 255 - 257°C, $[\alpha]_D$-26.2° showed in its IR spectrum the absorption bands similar to those of acaciaside A **(1)**. The ^{13}C NMR spectrum of the compound **(2)** resembled to that of the compound **(1)**. Saponin **(2)** on hydrolysis with 2M HCl in aqueous MeOH yielded two aglycones **3** and **4** and monosaccharides, D-glucose, D-xylose, L-arabinose and L-rhamnose. However, the positive FAB MS of acaciaside B **(2)** exhibited a peak at m/z 1867 as the base peak which was assigned to $[M+Na]^+$. It was evident, therefore, that acaciaside B **(2)** contains an extra pentose unit (arabinose or xylose) with respect to acaciaside A **(1)**. The location of this pentose unit in acaciaside B **(2)** was determined as follows : Permethylation of compound **(2)** by Hakomori's method[7] furnished the permethylate **(16)** which displayed eight anomeric sugar protons. On reduction with lithium aluminium hydride the permethylate **(16)** yielded a mixture of three methylated products. Two of these products were characterized as the methylated triterpene glycoside **(12)** and the methylated oligosaccharide **(13)** which were also obtained from acaciaside A **(1)** by similar treatment. The ^1H NMR spectrum of the third methylated product **(17)** showed two anomeric protons at δ 4.56 (1H, d, J=7 Hz) and 4.68 (1H, d, J=7.5 Hz) as well as olefinic proton signals similar to those of compound **(11)**. Finally, the identification of the methylated sugars obtained by acid hydrolysis of compound **(17)** disclosed its structure as (6S)-2-trans-6-O-{β-D-xylopyranosyl (1→2)-β-D-glucopyranosyl}-2,6-dimethyl-2,7-octadienol.

Table 1. ^{13}C NMR chemical shifts ($\delta C \pm 0.1$) of acaciaside A **(1)**, acaciaside B **(2)** and acaciagenin A **(4)** measured in C_5D_5N.

Carbon No.	(1)	(2)	(4)	Carbon No.	(1)	(2)	Carbon No.	(1)	(2)	(4)
1	38.9	38.9	38.6[a]	G_1-1	105.3[c]	105.3[c]	R -5	69.7	69.7	
2	27.2	27.2	27.0[b]	G_1-2	83.3	83.3	R -6	18.7[b]	18.7[b]	
3	89.2	89.3	78.8	G_1-3	77.2	76.9	G_4-1	104.8[c]	103.8	
4	39.6	39.6	39.3	G_1-4	71.7[d]	71.8[d]	G_4-2	74.3	83.5	
5	56.0	56.1	55.2	G_1-5	78.2[e]	78.3[e]	G_4-3	78.2[e]	75.9	
6	18.4	18.7	18.7	G_1-6	69.7	69.8	G_4-4	71.4[d]	71.0[d]	
7	32.5	32.5	32.9	G_2-1	105.1[c]	105.1[c]	G_4-5	78.2[e]	78.6[e]	
8	40.1	40.2	39.8	G_2-2	76.3	76.4	G_4-6	62.8	62.9	
9	47.1	47.1	50.8	G_2-3	78.4[e]	78.1[e]	X_2-1		106.1	
10	37.0	37.0	36.9	G_2-4	71.8[d]	71.9[d]	X_2-2		75.5	
11	23.9	23.9	23.3	G_2-5	78.0[e]	78.0[e]	X_2-3		78.3[e]	
12	123.1	123.9	123.1	G_2-6	62.8	62.9	X_2-4		71.6[d]	
13	143.4	143.4	142.0	A -1	106.1	106.1	X_2-5		66.9	
14	42.0	42.1	41.0	A -2	72.3	72.3	M_1-1	167.6	167.5	
15	36.0	36.1	35.6	A -3	74.3	74.3	M_1-2	127.9	127.9	
16	73.4	73.4	73.7	A -4	69.0	69.1	M_1-3	143.4	143.5	
17	51.9	52.0	51.9	A -5	66.3	66.4	M_1-4	23.7	23.9	
18	40.9	40.9	39.3	G_3-1	95.6	95.6	M_1-5	40.7	40.8	
19	47.2	47.2	46.5	G_3-2	83.4	83.4	M_1-6	79.2	79.4	
20	35.4	35.5	34.6	G_3-3	77.9[e]	77.9[e]	M_1-7	145.8	145.7	
21	77.0	77.1	77.6	G_3-4	71.4[d]	71.3[d]	M_1-8	114.9	115.0	
22	36.1	36.2	36.2	G_3-5	76.1	76.2	M_1-9	12.5	12.6	
23	28.6	28.6	28.7	G_3-6	66.3	66.3	M_1-10	23.9	24.0	
24	15.8	15.9	15.3	X_1-1	106.5	106.5	M_2-1			165.0
25	16.4[a]	16.4[a]	16.7	X_1-2	75.6	75.6	M_2-2			129.9
26	16.9[a]	17.0[a]	16.7	X_1-3	78.1[e]	78.5[e]	M_2-3			137.1
27	27.4	27.4	28.0	X_1-4	71.3[d]	71.3[d]	M_2-4			26.6[b]
28	174.7	174.7	175.7	X_1-5	66.8	66.9	M_2-5			38.4[a]
29	29.2	29.1	29.6	R -1	101.6	101.6	M_2-6			134.9
30	19.1[b]	19.2[b]	18.2	R -2	72.1	71.8[d]	M_2-7			121.0
				R -3	73.2[f]	73.1[f]	M_2-8			64.9
				R -4	73.4[f]	73.4[f]	M_2-9			12.9
							M_2-10			15.9

a,b,c,d,e,f Assignments within the column of each compound may be interchanged

G = glucose, A = arbinose, R = rhamnose, X = xylose, M = monoterpene

Thus the structure of acaciaside B was defined as 3-O-[β-D-glucopyranosyl (1→6) {α-L-arabinopyranosyl (1→2)}-β-D-glucopyranosyl]-21-O-[(6'S)-2'-trans-2', 6'-dimethyl-6'-O-{β-D-xylopyranosyl (1→2)-β-D-glucopyranosyl}-2',7'-octadienoyl] acacic acid 28-O-α-L-rhamnopyranosyl (1→6) [β-D-xylopyranosyl (1→2)]-β-D-glucopyranoside **(2)**. The ^{13}C NMR chemical shifts of the saponin **(2)** (Table) also supports the structure as shown.

EXPERIMENTAL

All mps were measured on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer automatic polarimeter. ^{1}H NMR spectra were recorded on a JEOL FX-100 (99.6 MHz) instrument in $CDCl_3$ with $SiMe_4$ as internal standard. ^{13}C NMR spectra were recorded on a JEOL FX-100 Fourier - transform spectrometer operating at 25.05 MHz for solutions in C_5D_5N ($SiMe_4$ as internal standard). IR spectra were recorded in Nujol mulls on a Perkin - Elmer model 177 instrument. Electron-impact mass spectra were recorded on a Hitachi model RMU-6L mass spectrometer. GLC was performed on a Hewlett-Packard model 5730A instrument on a column of (i) ECNSS-M 3% on Gas chrom Q at 190°C for alditol acetates, or (ii) OV-225 on Gas chrom Q at 195°C for partially methylated alditol acetates. High-performance liquid chromatography (HPLC) was performed on a Spectra-Physics model SP 8000B instrument with a column of Spherisorb S-10-ODS and a Micromeritics 771 refractive - index detector in MeOH-water (7:3) as solvent. TLC was carried out on silica gel G (BDH) with the following solvent systems : (A) $CHCl_3$-MeOH (4:1), (B) benzene-acetone (3:2). Paper chromatography for sugars was performed on Whatman paper No.1 with the solvent system (C) Bu^nOH-C_5H_5N - water (6:4:3). The positive FAB MS were obtained on a VG ZAB-SE mass spectrometer equipped with a FAB source operating at an accelerating voltage of 8 kV. Samples were dissolved in [$^2H_6$]DMSO (2-10 $\mu g \mu l^{-1}$) and deposited on a FAB probe tip. A thin layer of either glycerol or thioglycerol was applied to the probe tip containing the samples and mixed thoroughly with a Pasteur pipette before insertion into the source. The primary atom (xenon) was produced using a saddle-field ion source operating at a tube current of 1-1.5 mA at an energy of 8 keV. The negative FAB MS were obtained using Kratos MS 9/50 TC mass spectrometer by bombardment of the sample with a fast atom beam of xenon produced by an Ion - Tech II NF atom gun operating at 9 kV.

Isolation of Acaciaside A (1) and Acaciaside B (2)

The air-dried and powdered fruits (2.5 kg) of *A. auriculiformis* were successively extracted with petroleum ether (boiling range 60-80°C), $CHCl_3$ and MeOH. A portion (60 g) of the total methanol extract (86 g) was chromatographed on silica gel (1 kg) with petroleum ether, petroleum ether - $CHCl_3$ (1:1), $CHCl_3$, $CHCl_3$-MeOH (9:1, 4:1, 7:3, 3:2, 1:1 and 2:3) as successive eluents. The $CHCl_3$-MeOH (7:3 and 3:2) eluates on further purification yielded acaciaside.[2] The $CHCl_3$-MeOH (1:1 and

2:3) eluates (5.0 g) were combined and subjected to rechromatography. Thus a TLC homogeneous glycoside fraction (4.6 g) was obtained. This glycoside fraction was found to be a mixture of mainly two compounds by HPLC analysis. Preparative HPLC of this glycoside mixture with reversed - phase Spherisorb S-10-ODS column with the solvent system MeOH-water (7:3) and flow rate 4 ml min^{-1} afforded acaciaside A **(1)** (1.8 g), mp 240-242°C; $[\alpha]_D$-19.5° (c 0.8 in MeOH) (Found: C,54.87; H, 7.55.$C_{80}H_{128}O_{39}\cdot 2H_2O$ requires C, 54,90: H, 7.60%) and acaciaside B **(2)** (1.5 g), mp 255-257°C; $[\alpha]_D$-26.2° (c 0.75 in MeOH) (Found: C, 54.32: H, 7.35. $C_{85}H_{136}O_{43}\cdot H_2O$ requires C, 54.30; H, 7.39%).

Hydrolysis of Acaciaside A (1)

Compound **(1)** (0.4 g) was hydrolysed with 2M HCl in aqueous MeOH under reflux for 4 h. The hydrolysate was worked up in the usual manner and the residue, on chromatographic purification over silica gel yielded two aglycones **(3)** and **(4)**. The aglycone **(3)** on crystallization in MeOH, yielded fine needles (130 mg) of acacic acid lactone,[2] mp 252-253°C, $[\alpha]_D$+54°C (c 0.25 in $CHCl_3$). The aglycone **(4)** on crystallization from EtOAC, afforded pure acaciagenin A (85 mg), mp 192-193°; $[\alpha]_D$+54° (c 0.2 in $CHCl_3$); ν_{max} 3500-3580, 1718, 1680 cm^{-1}; λ_{max} 218 (15000); ^{13}C NMR (Table) (Found: C, 73.30; H, 9.50. $C_{40}H_{62}O_7$ requires C, 73.35; H, 9.54%); methyl ester δ($CDCl_3$) 0.76 (3H, s), 0.86 (3H, s), 0.96 (3H, s), 1.04 (6H, s), 1.20 (3H, s), 1.36 (3H, s) (together 7xMe), 1.64 (6H, s) (2'-Me and 6'-Me), 3.56 (3H, s, COOMe), 5.28-5.64 (2H, m, 12-H, 7'-H), 7.04 (1H, m, 3'-H); m/z 484 (M^+-monoterpene acid, 100%), 425(M^+-monoterpene acid - COOMe, 24), 276 (b, 66), 258 (b-H_2O, 8), 217 (b-COOMe, 15), 207 (a, 12) and 189 (a-H_2O, 33) (Found: C, 73.55; H, 9.60.$C_{41}H_{64}O_7$ requires C, 73.61; H, 9.64%).

The filtrate from the hydrolysate of acaciaside A **(1)** was neutralized with Ag_2CO_3 and filtered. A portion of the filtrate was concentrated under reduced pressure and tested for carbohydrates by paper chromatography with solvent system C. D-glucose, D-xylose, L-arabinose and L-rhamnose were identified using authentic specimens. That the arabinose was the L-enantiomer was confirmed by its actual isolation by preparative paper chromatography and comparison of its specific rotation with that of authentic L-arabinose. The identification of the monosaccharides were also confirmed by GLC of the carbohydrate mixture on column (i) after preparation of their alditol acetates by reduction with $NaBH_4$ followed by acetylation in the usual way.

Alkaline Hydrolysis of Acaciagenin A (4)

Compound **(4)** (200 mg) was hydrolysed with 6% KOH-EtOH (aq) on water bath for 4 h. The reaction mixture was acidified with dil. HCl and extracted with Et_2O. The ethereal extract thus obtained was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue on chromatographic purification yielded acacic acid lactone **(3)** (85 mg) and monoterpene acid **(5)** (46 mg).

The acid **(5)** was treated with an ethereal solution of CH_2N_2, worked up as usual and purified by chromatography to yield the methyl ester **(6)** as colourless oil, ν_{max} 3550 (OH), 1740 (ester carbonyl), 1640, 1240, 960 cm^{-1}; $\delta(CDCl_3)$ 1.60 (3H, s, 6-Me), 1.67 (3H, s, 2-Me), 3.61 (3H, s, COOMe), 4.20 (2H, m, $C\underline{H}_2OH$), 5.48 (1H, m, 7-H), 7.0 (1H, t-like, 3-H); δ_C ($[^2H_5]$ pyridine) 166.1 (C-1), 137.0 (C-3, 134.8 (C-6), 129.4 (C-2), 121.1 (C-7), 64.8 (C-8), 51.5 (COO$\underline{Me}$), 38.2 (C-5), 26.4 (C-4), 16.0 (C-10) and 12.8 (C-9); $\underline{m/z}$ 198 (M^+, 1%), 180 (M^+-H_2O, 9), 139 (M^+-COOMe, 15), 138 (M^+-COOMe-H, 25), 121 (M^+-COOMe-H_2O, 47), 117 (50),97 (48) and 71 (100).

Hydrolysis of Acaciaside A (1) with 6% KOH

A solution of compound **(1)** (300 mg) and 6% KOH in EtOH (aq.) (45 ml) was refluxed for 5 h. The reaction mixture was neutralized with Dowex 50 Wx8 and concentrated to half the initial *in vacuo*. The Bu^nOH extract of the concentrated solution was purified on a silica gel column to yield two major fractions. The more polar fraction was identified as acaciaside from its physical and spectral characteristics.[2] The other fraction was further purified by preparative TLC with the solvent system A to give compound **(7)** (55 mg) as an amorphous powder, mp 108-110°C; ν_{max} 3450-3550, 1675, 1645 $cm.^{-1}$

The compound **(7)** (50 mg) on esterification with an ethereal solution of CH_2N_2 followed by chromatographic purification yielded the methyl ester **(8)** (38 mg) as a colourless oily substance which showed a single spot on TLC, $\delta(CDCl_3)$ 1.54 (3H, s, CH_3), 1.96 (3H, d, $\underline{J}$=1.5 Hz, CH_3), 3.65 (3H, s, $COOCH_3$), 4.32 (1H, d, $\underline{J}$=8 Hz, 1-H of glucose unit), 5.27 (1H, dd, $\underline{J}$=2, 11 Hz), 5.40 (1H, dd, $\underline{J}$=2, 18 Hz), 6.25 (1H, dd, $\underline{J}$=11, 18 Hz) and 7.06 (1H, t); δ_C($[^2H_5]$ pyridine) 168.5 (C-1), 144.6 (C-7), 143.2 (C-3), 127.9 (C-2), 114.9 (C-8), 104.7 (G-1), 79.2 (C-6), 78.3 (G-3), 78.2 (G-5), 74.2 (G-2), 71.4 (G-4), 62.8 (G-6), 51.6 ($COO\underline{CH}_3$), 40.8 (C-5), 24.0 (C-10), 23.6 (C-4) and 12.3 (C-9).

Acid Hydrolysis of the Ester (8)

Compound **(8)** (15 mg) was hydrolysed with 2M HCl in aqueous MeOH under reflux for 5 h and worked up in the usual way. The residue on chromatographic purification yielded the monoterpene acid **(5)** (3 mg). The filtrate was tested for sugar as usual by PC and GLC and D-Glucose was identified.

Permethylation of Acaciaside A (1) and Hydrolysis of the Product

Compound **(1)** (500 mg) was permethylated by Hakomori's method[7] with NaH (700 mg) in DMSO (65 ml) and CH_3I (30 ml) under N_2 atmosphere in the usual way. The reaction mixture was worked up as usual to yield a gummy residue which was chromatographed over silica gel with petroleum ether-EtOAC (2:3) as eluant to yield the permethylate **(9)** (450 mg) as powder, mp 118-120°C; $\delta(CDCl_3)$ 0.78 (3H, s), 0.86 (3H, s), 0.96 (3H, s), 1.02 (3H, s), 1.04 (3H, s), 1.20 (3H, s), 1.24 (3H, d, $\underline{J}$=6 Hz), 1.32 (3H, s), 1.53 (3H, s), 1.96 (3H, d, $\underline{J}$=1.5 Hz, CH_3), 3.32-3.65 (66H, all s, together 22xOCH_3), 4.13 (1H, d, $\underline{J}$=5 Hz, 1-H of arabinose unit), 4.20 (1H, d,

J=7, Hz, 1-H of glucose unit), 4.30 (2H, d, J=7 Hz, 1-H of two glucose units), 4.36 (1H, d, J=7 Hz, 1-H of glucose unit), 4.68 (1H, d, J=7 Hz, 1-H of xylose unit), 5.0 (1H, brs, 1-H of rhamnose unit), 5.27 (1H, dd, J=2, 11 Hz), 5.40 (2H, m), 6.28 (1H, dd, J=11, 18 Hz) and 7.03 (1H, m).

The permethylated product **(9)** (70 mg) was hydrolysed on being heated under reflux with 2M HCl in aqueous MeOH (25 ml) for 5 h. The reaction mixture was then cooled, evaporated to dryness under reduced pressure, diluted with water and filtered. The filtrate was neutralised with Ag_2CO_3 and filtered. The filtrate after work up as usual, was reduced with $NaBH_4$, acetylated with Ac_2O-pyridine (1:1) and then subjected to GLC analysis on column (ii). The peaks corresponding to 2,3,4,6-tetra-O-methyl-D-glucitol diacetate (R_t 1.00), 2,3,4-tri-O-methyl-L-arabinitol diacetate (R_t0.54), 2,3,4-tri-O-methyl-L-rhamnitol diacetate (R_t0.40), 2,3,4-tri-O-methyl-D-xylitol diacetate (R_t0.51) and 3,4-di-O-methyl-D-glucitol tetraacetate (R_t4.30) were identified by comparison of the R_t values with those of authentic samples.[8] The residue on chromatographic separation yielded 16-O-methyl acacic acid lactone **(10)**[2] (18 mg), mp 241-243°C; $[\alpha]_D$+47° (c 0.60 in $CHCl_3$) and the rearranged monoterpene **(5)** (7 mg).

Reductive Cleavage of Permethylate (9) with $LiAlH_4$

A solution of the permethylate **(9)** (250 mg) in anhydrous THF (25 ml) was treated with $LiAlH_4$ (260 mg) and the mixture was refluxed for 3 h. The excess $LiAlH_4$ was decomposed with moist ether and the mixture was extracted successively with ether and EtOAC. The ether extract was chromatographed on a silica gel column with the solvent system benzene - acetone (7:3) and purified further by preparative TLC with the solvent system B to give two chromatographically pure fractions. The first fraction was identified as the methylated monoterpene glycoside **(11)** (22 mg), $\delta(CDCl_3)$ 1.55 (3H, s, CH_3), 1.95 (3H, d, J=1.6 Hz, CH_3), 3.56 (3H, s), 3.60 (3H, s), 3.62 (3H, s), 3.64 (3H, s) (together 4xOCH_3), 4.30 (1H, d, J=7 Hz), 5.34 (2H, m), 6.21 (1H, dd, J=11, 18 Hz) and 7.0 (1H, m). On acid hydrolysis compound **(11)** yielded 2,3,4,6-tetra-O-methyl-D-glucose identified by GLC analysis of its alditol acetate.

From the second fraction the methylated triterpene glycoside **(12)** was obtained as a colourless amorphous powder (63 mg), $\delta(CDCl_3)$ 0.80 (3H, s), 0.85 (3H, s), 0.92 (3H, s), 1.04 (6H, s), 1.20 (3H, s), 1.28 (3H, s), 3.36-3.64 (30H, each s, together 10xOCH_3), 4.15 (1H, d, J=5 Hz, 1-H of arabinose unit) 4.30 (1H, d, J=8 Hz, 1-H of glucose unit) 4.58 (1H, d, J=8 Hz, 1-H of glucose unit) 5.42 (1H, br s, 12-H).

The methylated triterpene glycoside **(12)** (50 mg) was hydrolysed with 2M HCl in aqueous MeOH (15 ml) on a steam-bath for 3 h and then filtered. The filtrate was worked up as usual to prepare the alditol acetates which were identified by GLC analysis as 2,3,4,6-tetra-O-methyl-D-glucitol diacetate (R_t 1.00), 2,3,4-tri-O-

methyl-L-arabinitol diacetate (R_t 0.54) and 3,4-di-O-methyl-D-glucitol tetraacetate (R_t 4.32) by comparison of their R_t values with those of authentic samples.[8] The residue on chromatographic purification furnished the partially methylated aglycone **(14)** (15 mg) which was identified as olean-12-en-16β-O-methyl-3β,21β,28-triol by comparison with physical and spectral data of an authentic sample derived from acaciaside permethylate[2] **(15)**.

The EtOAc extract of the reduction products of the permethylate **(9)** was purified by chromatography to yield a methylated oligosaccharide **(13)** as colourless oily substance (30 mg), $\delta(CDCl_3)$ 1.24 (3H, d, J=6 Hz), 3.37-3.63 (24H, each s, together 8xOCH_3), 4.68 (1H, d, J=8 Hz, 1-H of xylose unit) and 5.02 (1H, brs, 1-H of rhamnose unit).

The methylated oligosaccharide **(13)** (25 mg) was refluxed with 2M HCl for 4h. The reaction mixture was worked up usual to prepare alditol acetates and subjected to GLC analysis on column (ii). Three peaks were detected and identified as 2,3,4-tri-O-methyl-L-rhmnitol diacetate (R_t 0.40), 2,3,4-tri-O-methyl-D-xylitol diacetate (R_t 0.50) and 3,4-di-O-methyl-D-glucitol tetraacetate (R_t 4.30) according to their retention times relative to that of alditol acetate of authentic 2,3,4,6-tetra-O-methyl-D-glucose.

Hydrolysis of Acaciaside B (2)

Compound **(2)** (0.4g) was refluxed with 2M HCl in aqueous MeOH (50 ml) for 5 h and the reaction mixture was worked up in the usual manner. Chromatographic separation of the residue and crystallization of the products from MeOH afforded two aglycones, acacic acid lactone **(3)** (115 mg), mp 252-253°C and acaciagenin A **(4)** (78 mg), mp 192-193°C. From the filtrate four monosaccharides, D-glucose, D-xylose, L-arabinose and L-rhamnose were identified by PC and GLC. The presence of L-arabinose was also confirmed by its actual isolation and comparison of its $[\alpha]_D$ value with an authentic sample.

Permethylation of Acaciaside B (2)

Compound **(2)** (400 mg) was completely methylated by Hakomori's method.[7] Usual work up followed by purification by silica gel column chromatography furnished the permethylate **(16)** (320 mg) as amorphous powder, mp 126-127°C; $\delta(CDCl_3)$ 0.76 (3H, s), 0.85 (3H, s), 0.95 (3H, s), 1.03 (6H, s), 1.20 (3H, s), 1.25 (3H, d, J=6 Hz), 1.30 (3H, s), 1.57 (3H, s), 1.92 (3H, d, J=1.5 Hz), 3.34-3.66 (72H, all s, together 24xOCH_3), 4.15 (1H, d, J=5 Hz, 1-H of arabinose unit), 4.22 (1H, d, J=7 Hz, 1-H of glucose unit), 4.32 (2H, d, J=7 Hz, 1-H of two glucose units), 4.48 (1H, d, J=7 Hz, 1-H of glucose unit), 4.69 (2H, d, J=7.5 Hz, 1-H of two xylose units), 5.02 (1H, brs, 1-H of rhamnose unit), 5.28 (1H, dd, J=2, 11 Hz), 5.4 (2H, m), 6.28 (1H, dd, J=11, 18 Hz) and 7.02 (1H, m).

Reductive Cleavage of the Permethylate (16) with $LiAlH_4$

Compound **(16)** (200 mg) in THF (20 ml) was reduced with $LiAlH_4$ (210 mg) under reflux for 4h and worked up as described above in the reductive cleavage of

the permethylate **(9)**. Two of the isolated products were identified as the methylated triterpene glycoside **(12)** (45 mg) and the methylated oligosaccharide **(13)** (22 mg) by comparison of their ^{1}H NMR spectra with those of corresponding compounds obtained from the permethylated derivative **(9)** of acaciaside A **(1)** by similar treatment. The third isolated product **(17)** was obtained as colourless gummy solid (15 mg), $\delta(CDCl_3)$ 1.58 (3H, s, CH_3), 1.94 (3H, d, J=1.5 Hz, CH_3), 3.52-3.65 (18H, all s, together 6xOCH_3), 4.56 (1H, d, J=7 Hz, 1-H of glucose unit), 4.68 (1H, d, J=7.5 Hz, 1-H of xylose unit) 5.27 (1H, dd, J=2, 11 Hz), 5.4 (1H, dd, J=2, 18 Hz), 6.26 (1H, dd, J=11, 18 Hz) and 7.0 (1H, t).

Hydrolysis of Compound (17)

Compound **(17)** (10 mg) was hydrolysed with 2M HCl in aqueous MeOH (7 ml) under reflux for 3h, work up in the usual way afforded a mixture of alditol acetates obtained from the carbohydrate fraction, which was subjected to GLC analysis on column (ii). The peaks corresponding to 3,4,6-tri-O-methyl-D-glucitol triacetate (R_t 1.84) and 2,3,4-tri-O-methyl-D-xylitol-diacetate (R_t 0.53) were identified by comparison with authentic samples.[8]

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