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STRUCTURE OF ACACIASIDE, A TRITERPENOID TRISACCHARIDE FROM *ACACIA AURICULIFORMIS*

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Key Word Index—*Acacia auriculiformis*; Leguminosae; acacic acid lactone; acaciaside; triterpenes, triterpenoid saponin.

Abstract—The structure of a new triterpenoid trisaccharide isolated from the seeds of *Acacia auriculiformis* has been elucidated as acacic acid lactone-3-*O*-*β*-D-glucopyranosyl (1→6)-[α-L-arabinopyranosyl (1→2)]-*β*-D-glucopyranoside based on its spectral properties and some chemical transformations.

INTRODUCTION

Acacia auriculiformis A. Cunn. is widely distributed in India on road sides and in parks. It is reported to have central nervous system depressant activity [1]. Previous phytochemical work on the plant reported the isolation of a flavan glycoside [2], three isomeric flavan-3,4-diols, a dihydroflavanol, flavanone, flavonol, chalcone and polymeric polyphenols consisting of prodelphinidins and procinidins [3]. The powdered seeds of the plant were found to produce copious froth when shaken with water indicating the presence of saponins. In continuation of our work on the isolation and characterization of the pharmaceutically important naturally occurring saponins [4–8] this paper reports the isolation and structure elucidation of a new triterpenoid trisaccharide from the seeds of this plant.

RESULTS AND DISCUSSION

The ethanol extract of the defatted seeds of *A. auriculiformis* on chromatographic purification on a silica gel column yielded a glycosidic fraction. This fraction on reversed phase silica gel column chromatography afforded a pure triterpenoid glycoside designated as acaciaside and two other unresolved fractions. Acaciaside (**1**) showed positive Liebermann–Burchard and Molisch tests. Acid hydrolysis of **1** generated the aglycone (**2**). The physical and spectroscopic data of **2** were found to be comparable with those reported for acacic acid lactone [9]. The aqueous portion after acid hydrolysis was examined for monosaccharide constituents by PC and GLC. Thus D-glucose and L-arabinose were identified by comparison with authentic samples. The identification of L-arabinose was also confirmed by its isolation and a comparison of its $[\alpha]_D$ with that of an authentic sample.

Fast atom bombardment mass spectrometry (FABMS) [10, 11] was employed for the determination of the M_r of **1** and the monosaccharide sequence in its glycone portion. The negative ion FABMS of **1** exhibited ions at *m/z* (relative intensity) 1851 (9), 925 (100), 793 (46), 763 (46), 631 (5) and 437 (6) assigned to $[2M-H]^-$, $[M-H]^-$, $[M-H-p]^-$, $[M-H-h]^-$, $[M-H-h-p]^-$ and $[M-H-2h-p-2w]^-$, respectively, where p, h and w denote pentose, hexose and water. The positive ion FABMS of **1** displayed ion peaks at 949 (2), 927 (7), 765 (2), 633 (2), 471 (50), 453 (100) and 435 (92) assigned to $[M+Na]^+$, $[M+H]^+$, $[M+H-h]^+$, $[M+H-h-p]^+$, $[M+H-2h-p]^+$, $[M+H-2h-p-w]^+$ and $[M+H-2h-p-2w]^+$, respectively. Thus the identification of the sugar constituents coupled with positive and negative ion FABMS studies demonstrated that one glucose unit and the arabinose of the glycone part of acaciaside (**1**) are located at terminal positions and are linked to the other glucose unit which is bonded to the aglycone.

Treatment of acaciaside (**1**) with sodium metaperiodate followed by acid hydrolysis and examination for sugars revealed that degradation of all the three sugar units had occurred. This observation suggested the presence of free vicinal diol even in the inner glucose unit.

Permethylation of compound (**1**) by Hakomori's method [12] afforded the permethylate (**3**) which on acid hydrolysis liberated 2,3,4,6-tetra-*O*-methyl-D-glucose, 3,4-di-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-L-arabinose identified by GLC of their alditol acetates and the partially methylated aglycone, 16-*O*-methyl acacic acid lactone (**4**). The compound (**4**) on acetylation yielded an acetate (**5**) which showed in its ^{1}H NMR spectrum a signal at δ4.82 (1H, *dd*, $J=10.5$ and 6 Hz) assignable to the proton at C-3 bearing the acetoxy group [13]. The results demonstrated that the terminal glucose and arabinose units of the glycone part are linked through their anomeric hydroxyls to the 2- or 6-positions of the inner glucose which is connected to the C-3 hydroxyl of the aglycone.

Acaciaside (**1**) on partial hydrolysis with 2 M hydrochloric acid in *n*-butanol afforded a mixture of prosapogenins which was permethylated and subjected to PLC to yield permethylates assigned structures **6** and **7** containing two and one monosaccharide, respectively, as revealed by ^{1}H NMR spectroscopy. The permethylate **6** on acid hydrolysis yielded 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-glucose. The permethylate **7** on hydrolysis liberated 2,3,4,6-tetra-*O*-methyl-D-glu-

cose. Thus the formation of the permethylate **6** was helpful in the unambiguous determination of the linkages of the terminal arabinose and glucose at the 2- and 6-positions, respectively, of the aglycone bound glucose.

A β-configuration ($^4C_1$ conformation) for the two glucopyranosyl units and an α-configuration ($^1C_4$ conformation) for the arabinopyranoside were inferred from the J values of the respective anomeric protons in the ^{1}H NMR spectrum of the permethylate **3** (see Experimental). The attachment of the carbohydrate moiety at the C-3 position of the aglycone **2** was revealed from the ^{13}C NMR chemical shifts of **1** and **2** (Table). Assignment of the ^{13}C NMR δ-values of compound **1** were made by comparison with those of the sapogenin **2** and the appropriate methyl glycosides [14] by the application of the known chemical shift rules [15, 16] and glycosylation shifts [14, 17].

On the basis of the foregoing evidence, the structure of acaciaside (**1**) is proposed to be acacic acid lactone-3-*O*-β-D-glucopyranosyl(1→6)-[α-L-arabinopyranosyl (1→2)] β-D-glucopyranoside (**1**). In steroid- and triterpenoid oligosaccharides arabinosee, if present, occurs as the inner sugar and acaciaside is one of the rare saponins containing arabinose at the terminal position.

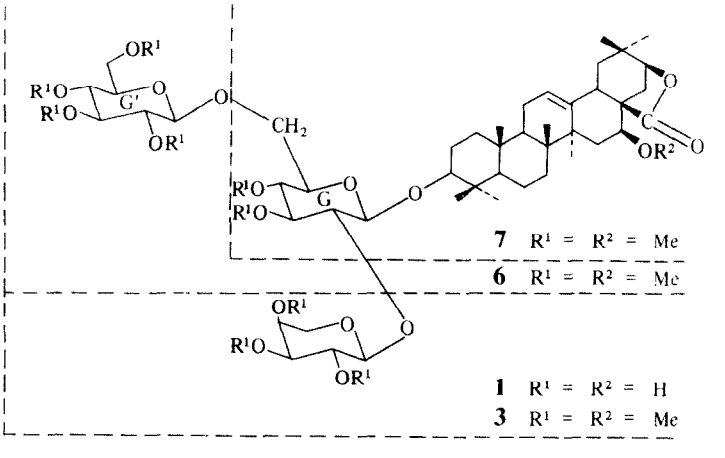
EXPERIMENTAL

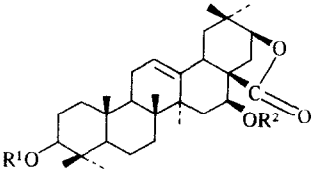
The plant material was identified by Mr U. Bhattacharya (Indian Botanic Garden, Howrah) and a voucher specimen has been deposited at the herbarium of IICB. Mps: uncorr. TLC was performed on silica gel G (BDH) using solvent system (a) $CHCl_3$–MeOH–H_2O (14:6:1); PC was carried out on Whatman paper No. 1 using the solvent system (b) *n*-BuOH–C_5H_5N–H_2O (6:4:3); a saturated soln of aniline oxalate

Table 1. ^{13}C chemical shifts δ_C ($\pm$0.1) of acacic acid lactone (**2**) and acaciaside (**1**) (pyridine-d_5)

C	2	1	C	2	1
1	38.9[a]	38.7[a]	27	28.6[a]	28.6[a]
2	27.2	27.2	28	181.0	181.2
3	78.0	89.0	29	28.7[a]	28.8[a]
4	39.3	39.5	30	24.3	24.3
5	56.0	55.9	G-1		105.3[b]
6	18.7	18.4	G-2		83.3
7	32.6	32.5	G-3		77.1
8	40.4	40.4	G-4		71.7
9	47.4	47.2	G-5		78.2[c]
10	37.3	36.9	G-6		69.7
11	23.8	23.8	G′-1		105.0[b]
12	124.6	124.4	G′-2		76.4
13	140.2	140.2	G′-3		78.3
14	43.4	43.0	G′-4		71.7
15	38.0[a]	38.2[a]	G′-5		78.0[c]
16	66.6	66.7	G′-6		62.8
17	49.9	50.0	A-1		106.1
18	41.7	41.7	A-2		72.3
19	42.8	42.9	A-3		74.3
20	34.1	34.2	A-4		69.0
21	83.4	83.4	A-5		66.3
22	28.0	28.0			
23	28.6	28.6			
24	15.7	15.7			
25	16.3[b]	16.2[b]			
26	16.4[b]	16.8[b]			

G, G′ = glucose, A = arabinse.
[a–c]Assignments within a column may be interchanged.





2 R¹ = R² = H
4 R¹ = H, R² = Me
5 R¹ = Ac, R² = Me

in water [18] was used for staining. GLC was performed using the column (i) 3% ECNSS-M on Gas chrom Q at 190° for alditol acetates and (ii) 3% OV-225 on Gas Chrom Q at 195° for partially methylated alditon acetates. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or d_5-pyridine using a JEOL GX-400 or JEOL FX-100 spectrometer operating at 400 MHz and 100 MHz with TMS as internal standard. FABMS were obtained on a Kratos MS 9/50 TC spectrometer. The samples, loaded on to the copper probe tip with glycerol, were bombarded with a fast atom beam of xenon produced by an Jon-Tech 11 NP atom gun operating at a potential of 9 kV. The spectra were recorded using a UV galvanometer recorder. EIMS were recorded at an ionising potential of 70 eV.

Isolation of acaciaside (**1**). The air-dried and powdered seeds (1 kg) of *A. auriculiformis* were successively extracted with petrol (bp 60–80°) and 90% EtOH. The ethanolic extract, on removal of the solvent under red. pres., yielded a viscous dark brown mass (25 g). This extract was chromatographed on silica gel (320 g) with petrol, petrol–$CHCl_3$ (1:1), $CHCl_3$, $CHCl_3$–MeOH (9:1, 4:1, 7:3 and 3:2) as successive eluents. The $CHCl_3$–MeOH (7:3 and 3:2) eluates (2.5 g) were combined and a portion (0.5 g) was subjected to rechromatography through a reversed - phase silica gel (25 g) column (octadecylsilane bonded to silica gel) using MeOH–H_2O (3:2) mixture as the eluent. Column fractions were monitored on TLC to give a chromatographically pure fraction (80 mg) and two impure fractions. The pure fraction was crystallized from MeOH to give colourless needles of **1**, mp 245–248° (dec), $[\alpha]_D$-21.73° (pyridine ; *c* 0.23). (Found: C, 60.82; H, 8.0 Calc. for $C_{47}H_{74}O_{18}$: C, 60.88; H, 8.04%.)

Hydrolysis of acaciaside (**1**). Compound **1** (100 mg) was hydrolysed with 2 M HCl in aq. MeOH for 4 hr and worked-up in the usual way. The residue, on chromatographic purification over silica gel followed by crystallization from MeOH, yielded a sapogenol (25 mg), mp 250–252°, $[\alpha]_D$ + 53.7°, which was characterized as acacic acid lactone (**2**) from its physical and spectral characteristics.

The filtrate from the hydrolysate was neutralized with Ag_2CO_3 and filtered. A portion of the filtrate was concd under red. pres and tested for carbohydrates by paper chromatography with solvent system (b). D-Glucose and L-arabinose 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.

Periodate oxidation of acaciaside (**1**) *and hydrolysis of the product.* To a soln of compound (**1**) (25 mg) in 95% EtOH (3 ml) was added dropwise a solution of sodium metaperiodate (25 mg) in H_2O (2 ml) and the mixture was stirred at 15° for 3 hr, kept at room temp. overnight and worked-up in the usual manner. The residue was hydrolysed with 2 M HCl. The aq. phase was tested for carbohydrates by PC, which revealed the absence of any sugar.

Permethylation of acaciaside (**1**) *and hydrolysis.* Compound (**1**) (50 mg) was completely methylated by the Hakomori method. Usual work-up followed by purification by a silica gel chromatography and elution with EtOAc–petrol (2:3) yielded compound **3** (35 mg) as a white powder, mp 112–114° (no hydroxy absorption in the IR spectrum); ^{1}H NMR ($CDCl_3$): δ0.80 (3H, *s*), 0.84 (3H, *s*), 0.88 (3H, *s*), 1.04 (6H, *s*), 1.20 (3H, *s*), 1.24 (3H, *s*), 4.20 (1H, *d*, J = 5 Hz, H-1 of arabinose unit), 4.28 (1H, *d*, J = 7 Hz, H-1 of glucose unit), 4.64 (1H, *d*, J = 7 Hz H-1 of glucose unit) and 5.4 (1H, *br s*, H-12).

The permethylate **3** (15 mg) was hydrolysed by refluxing with 2 M HCl in MeOH (6 ml) for 3 hr. The reaction mixture was cooled, evapd to dryness *in vacuo*, diluted with H_2O and filtered. The filtrate was neutralized with Ag_2CO_3 and filtered. The filtrate was reduced with $NaBH_4$ and worked-up in the usual manner. The residue was acetylated with Ac_2O–pyridine (1:1) at steam bath temp for 1 hr, purified by chromatography over silica gel and subjected to GLC analysis using column (ii). The peaks corresponding to alditol acetates of 2,3,4,6-tetra-*O*-methyl-D-glucose (R_t 1.0), 3,4-di-*O*-methyl-D-glucose (R_t 5.27) and 2,3,4-tri-*O*-methyl-L-arabinose (R_t 0.73) by comparison of their R_t values with those reported in the literature [19]. The residue on chromatographic purification yielded the partially methylated aglycone (**4**), mp 242–244°, $[\alpha]_D$ +48° ($CHCl_3$; *c* 0.75). The compound **4** (12 mg) on acetylation with Ac_2O (0.5 ml) and pyridine (0.5 ml) furnished the acetate **5** which crystallized from MeOH as needles, mp 237–239°, $[\alpha]_D$ + 44°($CHCl_3$; *c* 0.5), ^{1}H NMR ($CDCl_3$): δ0.84, 0.87, 0.95, 1.01, 1.03, 1.20 (all *s*, together 7 × Me), 1.95 (3H, *s*, OAc), 3.32 (3H, *s*, OMe), 4.82 (1H, *dd*, J = 10.5 and 6.5 Hz, H-3), 5.34 (1H, *t*-like H-12). (Found: C, 75.30; H, 9.41. Calc. for $C_{33}H_{50}O_5$; C, 75.24, H, 9.57%)

Partial hydrolysis of acaciaside (**1**) *and methylation of the prosapogenin mixture followed by their separation.* Compound **1** (40 mg) was heated in 2 MHCl (3 ml) and *n*-BuOH (3 ml) on a steam-bath for 1 hr. To the reaction mixture were added *n*-BuOH (50 ml) and H_2O (20 ml) and the mixture was shaken. The *n*-BuOH layer was separated, washed with H_2O and evapd under red. pres. to give a prosapogenin mixture (30 mg). This was methylated in the same way as described for **1** to give a resinous substance (25 mg), which was subjected to PLC [silica gel; developing solvent petrol–EtOAc (3:1)] to give two fractions. Each fraction was further purified by PLC to give the pure permethylates **6** (7 mg) and **7** (5 mg).

Permethylate **6**. ^{1}H NMR ($CDCl_3$): δ4.30 (1H, *d*, J = 7 Hz, H-1 of glucose unit) and 4.62 (1H, *d*, J = 7 Hz, H-1 of glucose unit). On acid hydrolysis it yielded 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-glucose, identified by GLC analysis of the alditol acetates.

Permethylate **7**. ^{1}H NMR ($CDCl_3$): δ4.25 (1H, *d*, J = 7 Hz, H-1 of glucose unit). The compound **7**, on acid hydrolysis liberated 2,3,4,6-tetra-*O*-methyl-D-glucose only.

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