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ISOLATION AND STRUCTURE ELUCIDATION OF THREE TRITERPENOID SAPONINS FROM *ACACIA AURICULIFORMIS*

SARASWATI GARAI and SHASHI B. MAHATO*

Indian institute of Chemical Biology, 4 Raja S.C. Mullick Road, Jadavpur, Calcutta-700032, India

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Key Word Index—*Acacia auriculiformis*; Leguminosae; proacaciaside-I; proacaciaside-II; acaciamine; triterpenoid saponins.

Abstract—Three new triterpenoid saponins, proacaciaside-I, proacaciaside-II and acaciamine isolated from the fruits of *Acacia auriculiformis*, were identified as acacic acid lactone-3-*O*-β-D-glucopyranosyl $(1\rightarrow6)$-β-D-glucopyranoside, acacic acid lactone-3-*O*-α-L-arabinopyranosyl $(1\rightarrow2)$-β-D-glucopyranoside and acacic acid lactone-3-*O*-α-L-arabinopyranosyl $(1\rightarrow6)$-2-acetamido-2-deoxy-β-D-glucopyranoside based on their spectral properties and some chemical transformations. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

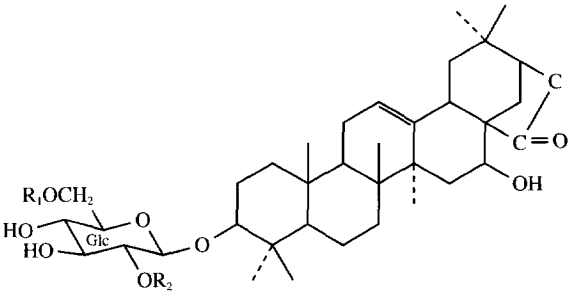
Acacia auriculiformis A. Cunn. is widely distributed throughout India and produces large amounts of fruits which give copious froth when shaken with water in powder form, thus indicating the presence of saponins. The plant is reported to have central nervous system depressant activity [1]. The water soluble saponin fraction from the fruits exhibited spermicidal [2] and filaricidal [3] activities. Our previous work on the saponins from the plant reported the isolation and structure elucidation of acaciaside, a sparingly water soluble saponin [4], and two water soluble saponins, acaciaside A and acaciaside B [5]. The wide occurrence of the plant and the biological activities of the saponin fraction prompted us to take up detailed chemical investigation of this fraction. This paper reports the structure elucidation of three new sparingly water soluble triterpenoid saponins from the plant.

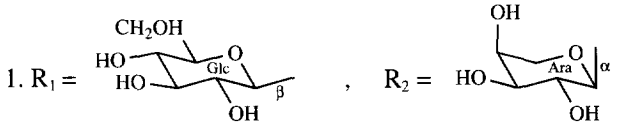
RESULTS AND DISCUSSION

The methanol extract of the defatted fruit pericarps was partitioned between water and *n*-butanol. The *n*-butanol-soluble fraction was adsorbed on silica gel and exhaustively eluted successively with chloroform, ethyl acetate, acetone and chloroform–methanol (80:20). The ethyl acetate and acetone eluted fractions on chromatographic purification afforded acaciaside (**1**) [4] and three new triterpenoid saponins proacasiaside-I (**2**), proacaciaside-II (**3**) and acaciamine (**4**). The molecular weights of **2** and **3** were determined by their positive and negative FAB-mass spectra. The positive FAB-mass spectra of **2** and **3** exhibited their $[M + Na]^+$ ions at *m/z* 817 and 787, respectively. The negative FAB-mass spectra showed their $[M - H]^-$ ions at *m/z* 793 and 763, respectively. The ^{13}C NMR spectra of both **2** and **3** displayed two anomeric carbons each. Acid hydrolysis of **2** furnished acacic acid lactone (**5**) [4] as the aglycone and D-glucose as the sugar constituent. The saponin **3** on acid hydrolysis generated **5** as the aglycone and D-glucose, and L-arabinose as sugars. The results showed that both saponins **2** and **3** are acacic acid lactone disaccharides, the former containing two glucose units and the latter containing a glucose and a terminal arabinose. Permethylation of **2** by Hakomori's method [6] and acid hydrolysis of the permethylate generated 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-glucose identified by GC of their alditol acetates and the partially methylated aglycone, 16-*O*-methylacacic acid lactone [4]. Acid hydrolysis of the permethylate of **3** liberated 3,4,6-tri-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-L-arabinose, and 16-*O*-methylacacic acid lactone [4]. The anomeric configuration of the carbohydrate moieties in **2** and **3** were ascertained as β for glucose and α for arabinose taking into consideration the ^{13}C values of the anomeric carbons observed for alkyl glycopyranosides [7]. Thus the permethylation studies, as well as the ^{13}C NMR data of **2** and **3**, suggested their structures as acacic acid lactone-3-*O*-β-D-glucopyranosyl $(1\rightarrow6)$-β-D-glucopyranoside and acacic acid lactone-3-*O*-α-L-arabinopyranosyl $(1\rightarrow2)$-β-D-glucopyranoside, respectively.

The elemental analysis and FAB-mass spectra of acaciamine (**4**) revealed its molecular formula to be $C_{43}H_{67}O_{13}N$. The presence of a CONH group was indicated by the IR absorptions of 1640 and 1547 cm^{-1} and this was also supported by the ^1H NMR signal at

*Author to whom correspondence should be addressed.

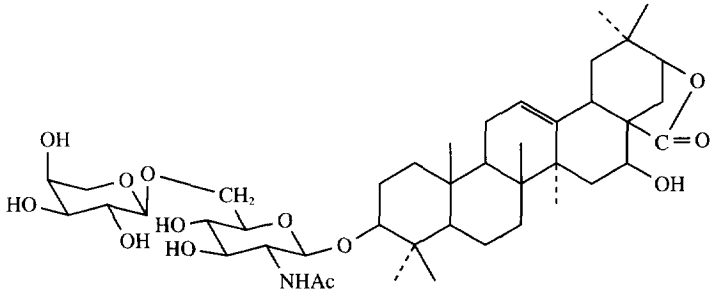




1. R_1 = , R_2 =

2. R_1 = β-D-Glc (p) R_2 = H

3. R_1 = H R_2 = α-L-Ara (p)



4

δ 8.48. Hydrolysis of **4** with 1M trifluoroacetic acid and examination of the hydrolysate in the aqueous layer indicated the presence of L-arabinose and an unidentified component giving a positive ninhydrin reaction. This component was finally indicated by TLC and GC (as peracetylated derivative) as *N*-acetyl-D-glucosamine. The organic layer of the hydrolysate was worked up in the usual way and the aglycone was identified to be acacic acid lactone **5** [4]. The ^{1}H NMR spectrum of **4** showed a singlet methyl signal at δ 2.13 assignable to an acetoxymethyl. The ^{13}C NMR spectrum displayed three characteristic signals at δ 33.6, 58.1 and 170.1 ascribable to the acetoxymethyl, C-2 carbon of *N*-acetyl-β-D-glucosamine and acetoxycarbonyl, respectively.

The positive ion FAB-mass spectrum of acaciamine (**4**) displayed ion peaks at *m/z* 828, 806, 674 and 471 ascribed to $[M + Na]^+$, $[M + H]^+$, $[M + H -$ arabinosyl$]^+$ and $[M + H -$ arabinosyl $-$ NHAc $-$ Glc$]^+$, respectively. Thus it was evident that in **4** L-arabinose is the terminal sugar which is linked to NHAc-Glc bound to the aglycone **5**. The intersugar linkage between L-arabinose and NHAc-Glc and attachment of the glycosyl moiety at the C-3 position of the aglycone were revealed by comparison of the ^{13}C NMR chemical shifts of **4**, the aglycone **5** [4], *n*-acetylglucosamine [8], and methyl-α-L-arabinopyranoside [9] taking into consideration the glycosylation shifts [9, 10]. The anomeric configuration of the glycosyl moiety in **4** was revealed as α for the arabinose and β for *N*-acetylglucosamine based on the ^{13}C NMR chemical shifts observed for alkyl glycopyranosides [7].

Thus the foregoing evidence suggested the structure of acaciamine to be acacic acid lactone-3-*O*-α-L-arabinopyranosyl (1→6)-2-acetamido-2-deoxy-β-D-glucopyranoside (**4**). This is the first report of isolation of an amino sugar containing saponin from the plant. Such saponins rarely occur in the plant kingdom.

EXPERIMENTAL

The plant material was identified at the Indian Botanic Garden, Howrah, and a voucher specimen has been deposited in the herbarium of the Indian Institute of Chemical Biology. Mps: uncorr. IR: KBr discs; ^{1}H NMR: 399.65 MHz and ^{13}C NMR: 100.40 MHz in pyridine-d_5. FAB-MS were obtained on a VG-ZAB-SE mass spectrometer using glycerol–thioglycerol as matrix for positive-ion spectra and nitrobenzyl alcohol for negative ion spectra. Cs^+ was used as a bombarding particle operating at 8 kV accelerating voltage. TLC: silica gel G (BDH) plates using the solvent system $CHCl_3$–MeOH–H_2O (81 : 18 : 1). The spots on the TLC

plates were visualized by spraying L.B. reagent. PC: Whatman No. I with solvent system *n*-BuOH–pyridine–H_2O (6:4:3), a saturated solution of aniline oxalate in water was used as staining agent. GC: ECNSS-M, 3% on Gas Chrom Q at 190° for alditol acetates and OV-225 on Gas Chrom Q at 195° for partially methylated alditol acetate.

The air dried powdered fruits (1.5 kg) of *Acacia auriculiformis* were successively extracted in a percolator with petrol (60–80°), $CHCl_3$ and MeOH. The MeOH extract was concentrated and partitioned between H_2O and *n*-BuOH. The *n*-BuOH layer was washed with H_2O and then removed under red. pres. The residue (62 g) thus obtained was dissolved in minimum amount of MeOH, adsorbed on silica gel, dried and eluted successively with $CHCl_3$, EtOAc, Me_2CO and $CHCl_3$–MeOH (4:1). The EtAc and Me_2CO frs were chromatographed on a silica gel column $CHCl_3$–MeOH, 24:1 and 25:1) and similar frs so obtained were further purified by prep. TLC using the solvent system $CHCl_3$–MeOH–H_2O (81:18:1) followed by crystallization. Thus previously isolated acaciaside **1** (2.1 g), proacaciaside-I (**2**) (60 mg), proacaciaside-II (**3**) (50 mg) and acaciamine (**4**) (45 mg) were obtained.

Proacaciaside-I (**2**). Crystallized from MeOH as microneedles, mp 268–270°, $[\alpha]_D$ −27.5° (pyridine, *c* 1.2); IR_{max}^{KBr} cm^{-1} 3400–3500 (hydroxyl), 1760, 1640, 1550, 1460 cm^{-1}; FAB-MS (negative) *m/z*: 793 $[M-H]^-$ (100); (positive) *m/z*: 817 $[M+Na]^+$ (100), 795 $[M+H]^+$, 633 $[M+H-glucosyl]^+$ (6), 453 $[M+H-glucosyl-glucose]^+$ (33); ^{13}C NMR (Table 1) [Found: C, 63.52; H, 8.32. $C_{42}H_{66}O_{14}$ requires C, 63.45: H, 8.37%].

Proacaciaside-II (**3**). Crystallized from MeOH as microneedles, mp 263–265°, $[\alpha]_D$ −30.5° (pyridine, *c* 1.1); IR_{max}^{KBr} cm^{-1}: 3400–3600 (hydroxyl), 1764, 1639, 1547, 1464 cm^{-1}; FAB-MS (negative) *m/z*: 763 $[M-H]^-$ (100); (positive) *m/z*: 787 $[M+Na]^+$ (100), 765 $[M+H]^+$ (8), 759 $[M+Na-H_2O]^+$ (7), 633 $[M+H-arabinosyl]^+$ (6), 453 $[M+H-arabinosyl-glucose]^+$ (32), 435 $[M+H-arabinosyl-arabinose-H_2O]^+$ (40); ^{13}C NMR (Table 1) [Found: C, 64.18; H, 8.37. $C_{41}H_{64}O_{13}$ requires C, 64.24; H, 8.42%].

Hydrolysis of **2** *and* **3**. The compounds **2** and **3** (25 mg each) were hydrolysed separately with 2M HCl in aq. MeOH for 4 hr and worked up in the usual way. Both hydrolysates on chromatographic purification followed by crystallization from MeOH yielded a sapogenol which was characterized as acacic acid lactone [4] from its physical and spectral characteristics.

Table 1. ^{13}C chemical shifts δ_c (±0.1) of acacic acid lactone (**5**), proacaciaside-I (**2**), proaciaside-II (**3**) and acaciamine (**4**) (pyridine-d_5)

C	5	2	3	4	C		2	3	4
1	38.9[a]	38.8[a]	38.9[a]	38.6[a]	Glc	1	105.1[a]	104.6	
2	27.2	27.2	26.7	26.5		2	75.2	83.5	
3	78.0	89.1	89.1	89.2		3	78.2	76.4	
4	39.3	39.4	39.3	39.3		4	71.5	72.0	
5	56.0	55.8	55.7	55.9		5	78.1	78.3	
6	18.7	18.5	18.5	18.3		6	69.8	62.6	
7	32.6	32.5	32.5	32.6	Glc'	1	105.0[a]		
8	40.4	40.3	40.5	40.4		2	75.0		
9	47.4	47.3	47.3	47.3		3	78.3		
10	37.3	37.0	37.0	37.0		4	71.7		
11	23.8	23.7	23.8	23.7		5	78.0		
12	124.6	124.5	124.4	124.6		6	62.7		
13	140.2	140.2	140.3	140.2	Ara-1			106.0	105.2
14	43.4	43.1	43.4	43.3		2		72.3	72.5
15	38.0[a]	38.1[a]	38.0[a]	38.1[a]		3		74.2	74.3
16	66.6	66.7	66.8	66.7		4		69.0	68.9
17	49.9	50.1	50.3	50.0		5		66.0	66.2
18	41.7	41.7	41.7	41.7	GlcNAc	1			104.8
19	42.8	42.9	42.9	42.9		2			58.1
20	34.1	34.2	34.2	34.2		3			75.9
21	83.4	83.4	83.3	83.4		4			73.7
22	28.0	28.0	27.3	27.2		5			76.7
23	28.6	28.6	28.6	28.1		6			69.9
24	15.7	15.7	15.6	15.6	Me(CO-NH-)				23.6
25	116.3[b]	16.2[b]	16.4[b]	16.3[b]	Me(CO-NH-)				170.1
26	16.4[b]	16.8[b]	16.8[b]	16.9[b]					
27	28.6[c]	28.6[c]	28.6[c]	28.6[c]					
28	181.0	181.2	181.0	181.1					
29	28.7[c]	28.8[c]	28.7[c]	28.7[c]					
30	24.3	24.3	24.4	24.3					

[a–c]Assignments within a column may be interchanged.

The filtrate from each hydrolysate was neutralized with Ag_2CO_3 and filtered. A portion of the filtrate was concd under red. pres. and tested for sugars by PC with the solvent system as mentioned earlier. Only D-glucose was identified in the filtrate from saponin **2**, and D-glucose and L-arabinose were identified in the filtrate from **3**. The identification of the sugars was also supported by GC after preparation of their alditol acetates by reaction with $NaBH_4$ followed by acetylation in the usual way.

Permethylation and hydrolysis of **2** *and* **3**. Both saponins **2** and **3** (25 mg each) were completely methylated by the Hakomori method [6]. Usual work-up followed by purification by silica gel CC and elution with EtOAc–hexane (2:2) yielded the corresponding permethylates. The permethylate of **2** on hydrolysis by refluxing with 2M HCl in MeOH for 3 hr and usual work-up afforded 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-glucose identified by GC of their alditol acetates by comparison with authentic samples. Similarly the partially methylated sugars obtained from saponin **3** were identified as 2,3,4-tri-*O*-methyl-L-arabinose and 3,4,6-tri-*O*-methyl-D-glucose.

Acaciamine (**4**). Crystallized from MeOH as micro needles, mp 242–243°, $[\alpha]_D$ +48° (MeOH, *c* 1.0); IR_{max}^{KBr} cm^{-1}: 3400–3600 (hydroxyl) 1760, 1737, 1640, 1547 cm^{-1}; FAB-MS (positive) *m/z*: 828 $[M+Na]^+$ (25), 806 $[M+H]^+$ (100), 674 $[M+H-arabinosyl]^+$ (5), 471 $[M+H-arabinosyl-NHAc-Glc]^+$ (2), 453 $[M+H-arabinosyl-NHAc-Glc-H_2O]^+$ (5), 435 $[M+H-arabinosyl-NHAc-Glc-2H_2O]^+$ (10); ^1H NMR (pyridine-d_5): δ 0.79 (3H, *s*), 0.80 (3H, *s*), 0.96 (3H, *s*), 0.99(3H, *s*), 1.07 (3H, *s*), 1.14 (3H, *s*), 1.33 (3H, *s*), 2.13 ($NHCOC\underline{H}_3$), 4.87 (1H, *d*, *J*=5 Hz, H-1 of arabinose unit), 5.01 (1H, *d*, *J*=7 Hz, H-1 of NHAc-glucosamine), 5.46 (1H, *t*-like, H-12) and 8.48 (1H, *brs*, $CONH$); ^{13}C NMR (Table 1) [Found: C, 64.14; H, 8.32; N, 1.70. $C_{43}H_{67}O_{13}N$ requires C, 64.07; H, 8.38; N, 1.74%].

Hydrolysis of **4**. Compound **4** (25 mg) was hydrolysed with 1M CF_3COOH (5 ml) under reflux for 3 hr. and worked up in the usual way. The residue on chromatographic purification over silica gel followed by crystallization from MeOH yielded an aglycone characterized as acacic acid lactone [4].

The aq. phase from acid hydrolysis was worked up as usual and acetylated with Ac_2O–pyridine (1:1) and the acetylated sugars were identified by GC as acetyl derivatives of L-arabinose and *N*-acetyl-D-glucosamine by comparison with authentic samples.

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