Phytochemistry, Vol. 31, No. 8, pp. 2805–2807, 1992
Printed in Great Britain.

0031-9422/92 $5.00+0.00

TRITERPENOID SAPONINS FROM LEAVES OF *CASTANOSPERMUM AUSTRALE*

VIQAR UDDIN AHMAD, WASEEMUDDIN AHMED* and KHAN USMANGHANI*

H.E.J. Research Institute of Chemistry, University of Karachi, Karachi 75270, Pakistan; *Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi 75270, Pakistan

(*Received in revised form* 3 *September* 1991)

Key Word Index—*Castanospermum australe*; Fabaceae; leaves; triterpenoid saponins.

Abstract—An investigation of the leaves of *Castanospermum australe* afforded one new and another known triterpenoid saponin as their methyl esters. The structures of these two compounds were elucidated on the basis of chemical and spectral data as 2β,23-dihydroxy-3-*O*-(4-deoxy-β-L-threo-hex-4-enopyranosiduronic acid)-olean-12-en-28-oic acid dimethyl ester and medicagenic acid 3-*O*-β-D-glucoside dimethyl ester.

INTRODUCTION

Castanospermum australe Cunn. & Fraser (known as Moreton Bay Chestnut or Black Bean) belongs to the family Fabaceae [1]. It is a large tree native of Queensland in Australia, which is, however, cultivated in the gardens of Pakistan. *C. australe* is reported to have medicinal uses [2] in treating post-pyrondial hyperglycaemia in diabetic patients, as well as being implicated as a toxic compound [3]. The sapogenin constituents already reported from the wood of this plant are castanogenin, medicagenic acid and bayogenin [4], from bark castanogenol [5] and, from the seeds, an alkaloid castanospermine [6]. Since no work has apparently been carried out on the saponin constituents of the leaves of *C. australe*, we have undertaken to examine this plant and in this communication the structure of two triterpenoid saponins is reported.

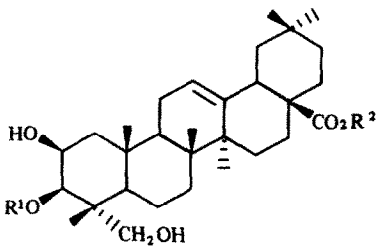
RESULTS AND DISCUSSION

The methanolic extract of the leaves of *C. australe* yielded a crude fraction containing saponin. Since the first attempt of chromatographic separation of this mixture into pure saponins was not successful, the whole fraction was methylated with diazomethane and then chromatographed on a column of silica gel. This procedure yielded the methyl esters of two saponins **1** and **2**. The genuine compounds present in the plants have, therefore, the structures **3** and **4**.

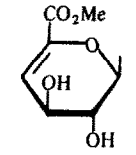
Compound **1** was obtained as colourless powder and it was established to have the composition $C_{38}H_{58}O_{10}$ by mass spectrometry. The mass spectrum of compound **1** showed the highest peak at *m/z* at 502 which was due to the sapogenin part as well as strong peaks at *m/z* 262 [a], 203 [a−COOMe] and 189. The FAB MS (negative ion mode) showed the $[M-H]^-$ ion at *m/z* 673 and the $[aglycone-H]^-$ peak at *m/z* 501. The molecular ion peak was confirmed by a peak at *m/z* 765 $[M-H+glycerol]^-$ in the negative ion FAB MS and at 697 $[M+Na]^+$ as well as *m/z* 789 $[M+Na+glycerol]^+$ peaks in the positive ion FAB MS. This indicated that compound **1** had the M_r of 674 corresponding to the molecular formula $C_{38}H_{58}O_{10}$.

In the ^1H NMR (CD_3OD, 300 MHz) compound **1** showed six singlets due to six tertiary methyl groups at δ0.75, 0.89, 0.90, 0.93, 1.17 and 1.28 and two COOMe singlets at δ3.61 and 3.78. In addition there was a double doublet at δ2.87 ($J=4.0$, 13.5 Hz) typical of H-18 of the oleanane series of triterpenes. The narrow triplet due to H-12 ($J=3.47$) was present at δ5.26. Two doublets (1H each) at δ3.39 and 3.29 ($J=11.4$ Hz) were assigned to the two H-23 protons, the H-3 signal of the aglycone moiety is partly masked by the large OMe signal. A quartet at δ4.29 ($J=3.20$ Hz) is due to H-2 of the aglycone moiety because it changes to a narrow triplet ($J=3.10$ Hz) when the partially masked doublet of H-3 at δ3.77 is irradiated. The small coupling constant $J_{2,3}$ and $J_{1\alpha,2}$ and $J_{1\beta,2}$ and the chemical shift of H-3 indicate that H-3 is axial and H-2 is equatorial. The doublet due to the anomeric proton (H-1′) was present at δ5.20 ($J=4.50$ Hz) showing finer coupling of 0.72 Hz due to long-range interaction. The smaller coupling constant of H-1′ is apparently due to the distortion of the ring as a result of the presence of the double bond in it. When the anomeric proton signal was irradiated, the triplet due to H-2′ at δ3.82 ($J=4.5$ Hz) is converted into a doublet ($J=4.5$ Hz) with fine splitting due to long range coupling. On the other hand, irradiation of signal at δ3.82 leads to the conversion of the H-1′ doublet into a singlet as well as the change of the triplet of H-3′ at δ4.03 ($J=4.0$ Hz) to a doublet ($J=4$ Hz). The double resonance experiment also clearly indicated that the H-3′ signal is coupled to H-4′ signal at δ6.10 (*d*, $J=4.0$) which is converted into a singlet when H-3′ signal is irradiated. The chemical shift of H-4′ signal indicates that it is an olefinic proton. Conversely, irradiation of H-4′ signal results into the conversion of the H-3′ triplet into a doublet ($J=4.6$ Hz). No signal corresponding to H-5′ was present (Table 1).

The above data indicated explicitly that the sapogenin of compound **1** was bayogenin methyl ester, whereas the sugar was methyl (4-deoxy-β-L-threo-hex-4-enopyranoside) uronate [7]. This was confirmed by the ^{13}C NMR data given in Table 2. As mentioned above

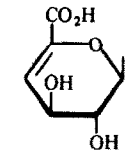


R¹ R²

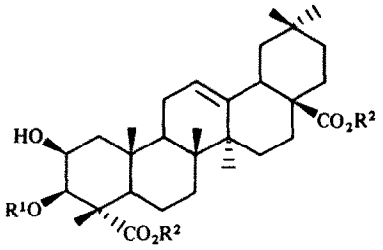


1 Me

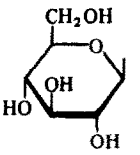
1a H Me



3 H

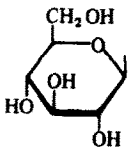


R¹ R²



2 Me

2a H Me



4 H

bayogenin has already been isolated [4] as a sapogenin after the complete hydrolysis of saponins from this plant.

Compound **2** was obtained as a colourless powder. In the mass spectrum (FAB, negative ion mode) it showed a peak at *m/z* 691 $[M-H]^-$ and the highest peak at *m/z* 783 $[M-H+\text{glycerol}]^-$, whereas the positive ion FAB spectrum in the presence of NaCl showed a strong peak at *m/z* 715 $[M+Na]^+$, and a peak at 531 $[\text{aglycone}+H]^+$. This indicates that the M_r of the compound is 692, corresponding to the formula $C_{38}H_{60}O_{11}$. In the ^{1}H NMR spectrum, (CD_3OD, 300 MHz), the compound showed six singlets due to six tertiary methyl groups at 0.75, 0.90, 0.93, 1.15, 1.27 and 1.41. Two singlets of COOMe were present at δ3.61 and 3.69. A doublet at 4.03 ($J=3.85$ Hz) is assigned to H-3 which is coupled according to the COSY spectrum to a quartet ($J=3.75$ Hz) at δ4.31. A doublet at δ4.03 ($J=7.65$ Hz) is

Table 1. Decoupling experiments on the sugar [methyl-(4-deoxy-β-L-threo-hex-4-eno-pyranosid) uronate]

Proton irradiated	Chemical shifts (δ)	Decoupling observed	Proton decoupled
H-1′	5.20	3.81 (*d*, $J=4.05$)	H-2′
H-2′	3.80	(a) 5.2 (*s*)	H-1′
		(b) 4.03 (*d*, $J=3.89$)	H-3′
H-3′	4.03	6.1 (*s*)	H-4′
H-4′	6.10	(a) 4.03 (*d*, $J=4.1$ Hz)	H-3′
H-3	3.81	4.3 (*t*, $J=4.01$ Hz)	H-2

$J_{1',2'}=4.0$ Hz, $J_{2',3'}=4.1$ Hz, $J_{3',4'}=3.9$ Hz, $J_{2,3}=4.5$ Hz.

Table 2. ^{13}C NMR chemical shifts of compounds **1**, **1a**, **2** and **2a**

Carbon	1	1a	2	2a
1	44.89	43.57	44.66	45.48
2	71.71	70.79	71.07	71.98
3	83.42	76.16	86.60	76.41
4	43.14	41.83	54.96	52.55
5	48.10	49.77	53.33	53.07
6	18.59	18.26	21.77	22.05
7	33.41	32.43	33.55	33.58
8	40.71	39.38	40.01	40.96
9	49.36	48.13	49.59	49.56
10	37.60	36.65	37.52	37.53
11	24.15	23.10	24.64	24.60
12	123.91	123.39	123.72	123.74
13	145.14	143.74	145.04	144.98
14	43.32	41.34	43.03	42.97
15	28.76	27.64	28.73	28.68
16	24.70	23.50	24.11	24.03
17	48.10	46.76	48.16	48.10
18	42.85	41.33	42.82	42.75
19	47.13	45.92	47.14	47.07
20	31.56	30.72	31.55	31.58
21	34.86	33.90	34.83	34.77
22	33.66	32.50	33.61	33.58
23	65.46	67.00	180.03	179.96
24	14.53	13.18	13.60	12.96
25	17.54	16.74	17.10	17.09
26	17.76	16.87	17.50	17.48
27	26.42	26.03	26.53	26.50
28	180.12	178.23	180.03	180.16
29	33.54	33.12	33.49	33.52
30	24.01	23.66	23.97	23.95
OMe	52.15	51.54	52.15	52.19
	—	—	52.76	52.56
1′	103.88	1′	105.43	
2′	83.43	2′	77.98	
3′	67.72	3′	77.82	
4′	113.14	4′	71.26	
5′	141.82	5′	75.03	
6′	164.49	6′	62.42	
6′CO_2Me	52.86			

due to the anomeric H of glucose. A double doublet at 2.80 ($J = 13.5$, 3.75 Hz) is typical of H-18 in the oleanane series of triterpenes, whereas a triplet at 5.26 ($J = 3.6$ Hz) is ascribed to H-12). The mass spectrum and the ^{1}H NMR spectrum indicate that the compound is the methyl ester of medigagenic acid glucoside which is confirmed by the ^{13}C NMR (Table 1) and its hydrolysis to medicagenic acid methyl ester and glucose. It may be mentioned here that medicagenic acid has been isolated as a sapogenin from this plant [4] and medicagenic glucoside, possessing antimycotic activity against *Cryptococcus neoformans*, has been reported from *Medicago sativa* [8].

EXPERIMENTAL

The mps were determined on Büchi-535 mp apparatus and are uncorr. Optical rotations were determined on a JASCO DIP-360 polarimeter. EI and FAB mass were determined on Finnigan Mat-312 Varian Mat-112 mass spectrometer connected to a PDP-11/34 (DEC.) computer system. ^{1}H NMR (300 MHz) and ^{13}C NMR (75.4 MHz) spectra were recorded on a Bruker AM-300 spectrometer in CD_3OD, and chemical shifts are given in δ scale with tetramethyl silane as an internal standard.

Extraction and isolation. The leaves of *C. australe* were collected (6 kg) from Aaram Bagh, Karachi in November, 1987. The fresh leaves of the plant were soaked in MeOH and kept at room temp. for 1 week, this procedure was repeated twice and the combined methanolic extract was evaporated under red. pres. The dark brown material (142 g) so obtained was first fractionated with hexane, EtOAc in H_2O to remove the neutral substances and other impurities. The aq. fraction, was then extracted vigorously with H_2O-saturated *n*-BuOH. On evaporation of *n*-BuOH under high vaccum the crude saponin fraction was obtained (43 g) and 5 g of this material was methylated with diazomethane.

The methylated crude saponin mixture was chromatographed on a silica gel 60 (Merck, 230–400 mesh) column using a gradient of $CHCl_3$–MeOH as an eluent. Elution with $CHCl_3$–MeOH (95:5), afforded a fraction consisting of a new saponin **1**, which on repeated CC with $CHCl_3$–MeOH (94:6) provided a single pure compound, (19 mg), R_f value 0.20 (TLC system $CHCl_3$–MeOH, 91:9, ceric sulphate reagent in H_2SO_4). Similarly, elution with $CHCl_3$–MeOH (90:10) provided another saponin which on repeated CC with $CHCl_3$–MeOH (88:12) yielded the pure compound **2**, 25 mg, R_f value 0.42 ($CHCl_3$–MeOH, 85:15; ceric sulphate reagent in H_2SO_4).

Compound **1**. mp 149–151°, $[\alpha]_D^{25}$ +34° (MeOH; *c* 0.74); ^{1}H NMR (300 MHz, CD_3OD): δ0.75 (3H, *s*, H-26), 0.89 (3H, *s*, H-24), 0.90 (3H, *s*, H-29), 0.93 (3H, *s*, H-30), 1.17 (3H, *s*, H-27), 1.28 (3H, *s*, H-25), 2.87 (1H, *dd*, $J = 13.5$, 4.0 Hz, H-18), 3.29 (1H, *d*, $J = 11.4$ Hz, H-23A), 3.39 (1H, *d*, $J = 11.4$ Hz, H-23B), 3.62 (3H, *s*, 28-$COOCH_3$), 3.77 (1H, *d*, $J = 4.01$ Hz, H-3), 3.78 (3H, *s*, 6′-$COOCH_3$), 3.82 (1H, *t*, $J = 4.50$ Hz, H-2′), 4.03 (1H, *t*, $J = 4.0$ Hz, H-3′), 4.29 (1H, *ddd*, $J = 3.20$, 3.20, 3.20 Hz, H-2), 5.20 (1H, *d*, $J = 4.50$ Hz, H-1′), 5.26 (1H, *t*, $J = 3.47$ Hz, H-12), 6.10 (1H, *d*, $J = 4.0$ Hz, H-4′). EIMS *m/z*: 502. Negative FAB-MS *m/z*: 765 $[M - H + glycerol]^-$, 673 $[M - H]^-$. Positive FAB-MS *m/z*: 789 $[M + Na + glycerol]^+$, 697 $[M + Na]^+$.

Compound **2**. mp 205–206°, $[\alpha]_D^{25}$ +73° (MeOH; *c* 0.41); ^{1}H NMR (300 MHz, CD_3OD): δ0.75 (3H, *s*, H-26), 0.90 (3H, *s*, H-29), 0.93 (3H, *s*, H-30), 1.15 (3H, *s*, H-27), 1.27 (3H, *s*, H-25), 1.41 (3H, *s*, H-24), 2.80 (1H, *dd*, $J = 13.50$, 3.75, H-18), 3.15 (1H, *m*, H-5′), 3.25–3.37 (3H, *m*, H-2′, 3′ and 4′), 3.61 (3H, *s*, OCH_3), 3.68 (1H, *dd*, $J = 11.94$, 4.68 Hz, H-6′B), 3.69 (3H, *s*, OCH_3), 3.80 (1H, *dd*, $J = 11.94$, 2.40 Hz, H-6′A), 4.03 (1H, *d*, $J = 3.85$ Hz, H-3), 4.24 (1H, *d*, $J = 7.65$, H-1′), 4.31 (1H, *ddd*, $J = 3.75$, 3.75, 3.75 Hz, H-2), 5.26 (1H, *t*, $J = 3.60$ Hz, H-12). Negative FAB-MS *m/z*: 783 $[M^+ - H + glycerol]^-$, 691 $[M^+ - H]^-$. Positive FAB-MS *m/z*: 715 $[M + Na]^+$, 531 $[genin + H]^+$.

Acid hydrolysis of compounds **1** *and* **2**. The sample (10 mg) was dissolved in methanolic HCl (4 ml MeOH, 2 ml HCl, 10%) and refluxed for 6 hr. The reaction mixture was concd under red. pres. to remove MeOH. It was then diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was evaporated to dryness and chromatographed on silica gel TLC plate. Compound **1a**, TLC solvent system $CHCl_3$–MeOH (91:9), R_f value 0.32; compound **2a**, solvent system $CHCl_3$–Me (88:12), R_f value 0.84. The aglycones **1a** and **2a** were isolated and identified as methyl esters of bayogenin and medicagenic acid, respectively, through comparison of the ^{13}C NMR spectra of the aglycones of compounds **1**, **1a**, **2** and **2a** as given in the Table 1.

Acknowledgements—We are grateful to Prof. Dr S. I. Ali, Department of Botany, University of Karachi, for the identification of the plant. K.U. gratefully acknowledges the research fundings provided by the University of Karachi. Waseemuddin Ahmed is highly thankful to the University Grants Commission for a research fellowship.

REFERENCES

1. Ali, S. I. (1977) in *Flora of Pakistan* (Nasir, E. and Ali, S. I., eds), Papilionaceae, p. 30. Fakhri Printing Press, Karachi.
2. Liu, P. and Rhinehart, B. (1985) *Eur. Pat. Appl. EP* 202, 661. Through C.A. 106: 125869d (1987).
3. Nash, R. J., Evans, S. V., Fellows, L. E. and Bell, E. A. (1985) in *Plant Toxicology* (Seawright, A. A., Hegarty, M. P., James, L. F. and Keeler, R. F., eds), pp. 309–314. Dominion Press–Hedges & Bell, Melbourne.
4. Ede, R. A., Simes, J. J. and Stevenson, B. (1963) *Aust. J. Chem.* **16**, 900.
5. Rao, M. G., Row, L. R. and Rukmini, C. (1969) *Indian J. Chem.* **7**, 1203.
6. Hohenschutz, L. D., Bell, E. A., Jewess, P. J., Leworthy, D. P., Pryce, R. J., Arnold, E. and Clardy, J. (1981) *Phytochemistry* **20**, 811.
7. Kovac, P., Hirsch, J. and Kovacik, V. (1974) *Carbohydr. Res.* **32**, 360.
8. Polacheck, I., Zehavi, U., Naim, M., Levy, M. and Evron, R. (1986) *Zentralbl. Bakteriol. Mikrobiol. Hyg. Ser. A* **261**, 481.