Pergamon

PII: S0031-9422(96)00211-7

Phytochemistry, Vol. 43, No. 4, pp. 907–911, 1996

0031-9422/96 $15.00 + 0.00

STEROIDAL ALKALOIDS FROM *VERATRUM ALBUM*

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(*Received in revised form* 30 *January* 1996)

Key Word Index—*Veratrum album*; Liliaceae; rhizomes; steroidal alkaloids; veramanine; neojerminalanine.

Abstract—Two new steroidal alkaloids, veramanine and neojerminalanine, have been isolated from the rhizomes of *Veratrum album*, along with the known glycoalkaloid, pseudojervine, whose NMR spectral data are reported for the first time. The structures of **1** and **2** were elucidated on the basis of extensive spectroscopic investigations.

INTRODUCTION

Veratrum, a genus of perennial herbs, is distributed in the temperate regions of the northern hemisphere. The alkaloidal extracts of *Veratrum* species are well known for their pharmacological properties [1–9]. Our phytochemical investigations on *V. album* L. have resulted in the isolation of many new steroidal alkaloids [10–12]. We describe herein the isolation of two more new steroidal bases, veramanine (**1**) and neojerminalanine (**2**), as well as the known alkaloid, pseudojervine (**3**). The structures of **1** and **2** have been established on the basis of detailed spectroscopic studies.

RESULTS AND DISCUSSION

Veramanine (**1**) $C_{27}H_{43}NO_5$, a jerveratrum-type alkaloid, was obtained as a yellow-coloured solid. Its spectral data showed a close resemblance to jervine (**4**) [13], a major jerveratrum alkaloid isolated from various *Veratrum* species. The UV spectrum showed absorption maximum at 247 nm, characteristic of α,β-unsaturated cyclopentenone-type jerveratrum alkaloids [10, 11]. The IR spectrum displayed intense bands at 3660 (N–H), 3500–3300 (OH), 1708 (C=O), 1622 (C=C), 1100 and 985 (C–O).

The ^{1}H NMR spectrum ($CDCl_3$–CD_3OD, 400 MHz) of **1** was also similar to that of jervine. However, the notable difference was the appearance of a downfield methine signal at δ 3.73, assigned to the hydroxyl-bearing C-6 methine proton, and the lack of any vinylic signal. The two three-proton doublets at δ 0.78 and 0.77 were assigned to H_3-27 and H_3-21, respectively, while a three-proton close doublet at δ 1.91 was attributed to the allylic H_3-18. Another three-proton singlet at δ 0.76 was assigned to H_3-19.

Two-dimensional NMR experiments, such as COSY–45°, *J*-resolved, HOHAHA, HMQC, HMBC, etc., were carried out in order to verify the chemical shift assignments and connectivities. The COSY–45° spectrum showed a strong cross-peak between H-20 (δ 2.34) and H-22 (δ 2.53). H-20 also showed vicinal coupling interactions with H_3-21 (δ 0.77). The coupling interaction between H-22 and H-23, resonating at δ 2.53 and 3.17, respectively, was also observed in the spectrum. H_{ax}-3 (δ 3.80) exhibited strong interactions with H_{ax}-4 (δ 1.85) and H_{ax}-2 (δ 1.40).

The HOHAHA spectrum of **1** recorded using a longer mixing interval (100 ms) revealed all the long-range proton–proton interactions within the individual spin-systems. The downfield H-6 resonated at δ 3.73 and showed cross-peaks with protons resonating at δ 2.00 (H-9), 1.95 (H-7), 1.50 (H-5), 1.85 (H_{ax}-4), and 1.48 (H_{eq}-4). Similarly H_{ax}-3 at δ 3.80 showed HOHAHA interactions with the protons resonating at δ 1.98 (H_{ax}-1), 1.80 (H_{eq}-2), 1.85 (H_{ax}-4) and 1.50 (H-5). H-23 at δ 3.17 showed HOHAHA interactions with the protons at δ 2.87 (H_{ax}-26), 2.53 (H-22), 2.34 (H-20), 2.10 (H-24), 1.55 (H-25) and 0.77 (H-21).

The broad-band decoupled ^{13}C NMR spectrum showed 27 carbon resonances which were in agreement with the molecular formula. Multiplicity assignments were made from a DEPT experiment, which indicated the presence of four methyl, eight methylene and ten methine carbons. The remaining five quaternary carbon signals were located in the broad-band decoupled ^{13}C NMR spectrum. The chemical shift assignments of the various carbon atoms are presented in Table 1 [13].

The EI-, FD-, FAB- (+ve) and HREI-mass spectra of **1** showed the $[M]^+$ at *m/z* 461.3062 (18 mu more than jervine) matching with the molecular formula

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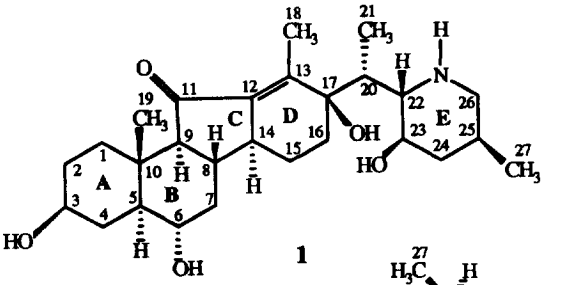

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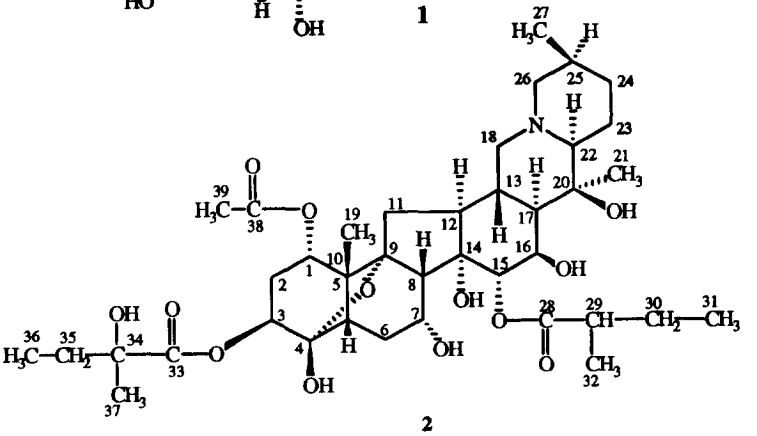

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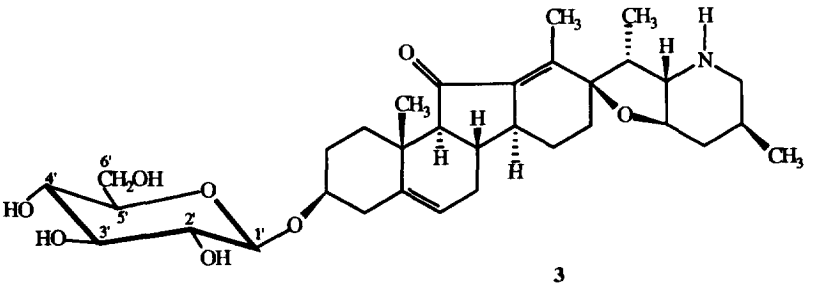

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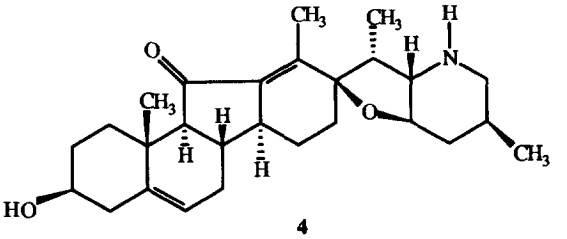

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$C_{27}H_{43}NO_5$ (Calcd 461.3141) and indicating seven degrees of unsaturation in the molecule. The HREI-mass spectrum also showed several characteristic fragments. For instance, the peak at *m/z* 443 ($C_{27}H_{41}NO_4$) is due to the loss of a water molecule from the $[M]^+$. The peak at *m/z* 114 ($C_6H_{12}NO$) could arise by cleavage of the C-20/C-22 bond, while the base peak at *m/z* 110 ($C_7H_{12}N$) was due to the cleavage of the C-20/C-21 and C-17/C-20 bonds, and loss of a water molecule. Another ion at *m/z* 126 ($C_8H_{16}N$) is again due to the cleavage of the C-17/C-20 bond and the loss of a water molecule. This fragmentation pattern is characteristic of jerveratrum-type steroidal alkaloids [14]. The presence of two additional oxygen atoms and two less degrees of unsaturation were the key differences between compound **1** and jervine. One oxygen was placed as a hydroxyl group at C-6, while ring B lacked any double bond as inferred from the ^{13}C and ^{1}H NMR spectra. This left one additional oxygen to be incorporated in the structure. Since the ^{13}C NMR spectra of **1** did not have any other additional signal for an oxygen-bearing carbon, it was not difficult to infer, that unlike jervine, compound **1** lacked any ethereal ring; the additional oxygen can be therefore placed on C-17. This also satisfied one of the observed degrees of unsaturation. The FAB-mass spectrum of the acetylated product showed a $[M + 1]^+$ at *m/z* 588 indicating the presence of three secondary hydroxyl groups.

Table 1. ^{13}C NMR data of veramanine (**1**), neojerminalanine (**2**) and pseudojervine (**3**)

	1		**3**			**2**	
Carbon	Chemical shift (δ)	Multiplicity†	Chemical shift (δ)	Multiplicity†	Carbon	Chemical shift (δ)	Multiplicity†
1	37.2	CH_2	37.6	CH_2	1	75.6	CH
2	29.5	CH_2	30.3	CH_2	2	26.8	CH_2
3	65.6	CH	73.2	CH	3	66.7	CH
4	42.5	CH_2	36.5	CH_2	4	105.2	C*
5	48.0	CH	141.3	C*	5	46.2	CH
6	71.3	CH	121.1	CH	6	28.5	CH_2
7	39.7	CH_2	38.3	CH_2	7	73.9	CH
8	37.0	CH	37.7	CH	8	48.0	CH
9	63.2	CH	62.3	CH	9	93.2	C*
10	38.3	C*	37.5	C*	10	47.0	C*
11	206.0	C*	206.9	C*	11	33.2	CH_2
12	125.0	C*	137.1	C*	12	47.2	CH
13	137.0	C*	145.4	C*	13	33.3	CH
14	43.8	CH	44.4	CH	14	80.9	C*
15	23.8	CH_2	23.6	CH_2	15	69.8	CH
16	32.5	CH_2	30.5	CH_2	16	67.0	CH
17	85.6	C*	85.4	C*	17	46.2	CH
18	12.0	CH_3	11.7	CH_3	18	60.5	CH_2
19	17.5	CH_3	18.3	CH_3	19	19.0	CH_3
20	40.8	CH	39.8	CH	20	72.7	C*
21	10.4	CH_3	10.8	CH_3	21	21.1	CH_3
22	65.6	CH	65.9	CH	22	69.5	CH
23	75.4	CH	75.8	CH	23	18.9	CH_2
24	30.5	CH_2	28.7	CH_2	24	29.7	CH_2
25	30.6	CH	30.9	CH	25	25.8	CH
26	53.5	CH_2	53.8	CH_2	26	61.2	CH_2
27	18.4	CH_3	17.9	CH_3	27	16.6	CH_3
1′	—	—	100.9	CH	28	175.5	C*
2′	—	—	75.6	CH	29	41.2	CH
3′	—	—	76.0	CH	30	26.4	CH_2
4′	—	—	70.0	CH	31	11.6	CH_3
5′	—	—	78.6	CH	32	14.1	CH_3
6′	—	—	61.7	CH_2	33	176.5	C*
					34	76.0	C*
					35	32.4	CH_2
					36	7.8	CH_3
					37	13.1	CH_3
					38	174.5	C*
					39	22.1	CH_3

*Quaternary carbon signal appeared in broad-band decoupled spectrum.
†Multiplicity confirmed by DEPT.

According to the above mentioned spectroscopic data, veramanine was shown to possess the structure (22*S*,23*R*,25*S*)-3β,6α,17,23-tetrahydroxy-22,26-epimono-14(13→12) *abeo*-5α,17β(H)-cholest-12-en-11-one (**1**).

Neojerminalanine (**2**), $C_{39}H_{61}NO_{13}$, was isolated as an amorphous powder. Its IR spectrum showed strong absorptions indicative of the presence of hydroxyl (3405 cm^{-1}) and carbonyl groups (1710–1715 cm^{-1}). The ^1H NMR spectrum ($CDCl_3$, 400 MHz) exhibited a six-proton triplet at δ 0.89 (J = 7.5 Hz), which was assigned to H_3-31 and H_3-36. Two three-proton doublets at δ 1.12 (J = 7.0 Hz) and 1.14 (J = 7.0 Hz) were assigned to H_3-27 and H_3-32, respectively. Three three-proton singlets, appearing at δ 1.34, 1.29 and 1.27, were assigned to H_3-21, H_3-37 and H_3-19 respectively. A doublet of doublets at δ 5.06 (J_1 = 4.4 Hz, J_2 = 1.8 Hz) was ascribed to H-3 coupled with H_2-2. A multiplet at δ 5.04 ($W_{1/2}$ = 10.0 Hz) and a doublet at δ 5.34 (J = 3.8 Hz) were assigned to the protons at H-1 and H-15, respectively. Two broad singlets at δ 4.56 and 4.29 were due to H-7 and H-16, respectively. A three-proton singlet at δ 1.98 was due to the acetate methyl group (H_3-39).

The ^1H–^1H COSY–45° spectrum of **2** displayed interactions between H-15 and H-16 resonating at δ 5.34 and 4.29, respectively. The H-3 proton resonated at δ 5.06, while H-1 appeared at δ 5.04 and showed COSY interactions with H_2-2 at δ 2.15 and 1.30. These interactions indicated a substructure CH(O)–CH_2–CH(O) as a part of ring A. The H-7 proton at δ 4.56 showed interactions with H-8 (δ 2.76) and H-6

(δ 2.25), which were not mutually coupled to each other. H-6 showed strong COSY interactions with its geminal partner appearing at δ 2.0. These interactions led to an arrangement CH_2-CH(O)–CH in ring B of the steroidal skeleton. H_3-31 showed vicinal couplings with H-30α and with the β protons, which resonated as multiplets at δ 1.45 and 1.70. The multiplet at δ 1.70 was, in turn, coupled with H-29 (δ 2.20).

The ^{13}C NMR spectra (100 MHz, $CDCl_3$) of **2** further confirmed its structure. The broad-band decoupled spectrum showed the presence of 39 carbon resonances, which were in agreement with the molecular formula $C_{39}H_{61}NO_{13}$. Multiplicities were determined from DEPT experiments, indicating the presence of eight methyls, nine methylenes and thirteen methines. The remaining nine quaternary carbons were deduced from the broad-band decoupled spectrum. The presence of a ketal carbon (C-4) was inferred from a signal at δ 105.2 in the broad-band spectrum. The signals at δ 176.5, 175.5 and 174.5 were due to three ester carbonyl functions. The carbon chemical shift assignments showed close resemblance to those of verabenzoamine and neojermerine alkaloids [11, 15]. The ^{13}C NMR data are presented in Table 1.

The EI-mass spectrum of **2** displayed a $[M]^+$ at *m/z* 751.4130 ($C_{39}H_{61}NO_{13}$, Calcd 751.4143) which was further confirmed by FAB (+ve)- and FD mass spectrometry. The base peak at *m/z* 112 resulted from cleavage of the C-13/C-18 and C-20/C-22 bonds and is characteristic of cerveratrum alkaloids. The ion at *m/z* 733 corresponded to the loss of a water molecule from the $[M^+]$. The ion at *m/z* 692 could arise due to the loss of an acetate moiety. The ion at *m/z* 456 resulted from the loss of 2-methylbutyryl-*O*, 2-hydroxy-2-methylbutyryl-O, AcO and a water molecule from the $[M]^+$.

The stereochemical assignments at the various asymmetric centres in **2** were based on chemical shift comparisons with those of the known *Veratrum* alkaloid, neojermerine [15]. According of these studies, neojerminalanine was shown to possess the structure 1α-acetoxy-3-*O*-(2-hydroxy-2-methylbutyryl)-15-*O*-(2 - methylbutyryl) - jermine (**2**). The stereochemistry of the two substituted chiral centres in the butyryl moieties were not investigated due to the small quantity of sample recovered after spectroscopic studies.

Compound **3**, $C_{33}H_{49}NO_8$, was isolated as a crystalline solid. Spectroscopic observations indicated that this compound is the reported base pseudojervine (jervine 3-*O*-β-D-glucopyranoside) (**3**). The structure of **3** was earlier identified through UV, IR and mass spectroscopy, as well as from identification of hydrolytic products, i.e. isojervine and D-glucose [16–20]. The specific rotation of **3** reported earlier was $-139°$ in $CHCl_3$–EtOH [21], while we measured it as $-97°$ ($CHCl_3$–EtOH)). Since the spectroscopic data are virtually identical, the observed differences in the magnitude of the specific rotation could be the result of a measurement error. The present report further confirms structure **3** based on detailed NMR studies.

EXPERIMENTAL

General. Optical rotations were measured in $CHCl_3$ and MeOH, UV spectra in MeOH and IR spectra in $CHCl_3$. ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ and CD_3OD using TMS as an int. standard.

Plant material. Rhizomes of *V. album* L. (50 kg dry wt) were collected from Trabzan, northern Anatolia, Turkey, in June, 1989. Plant material was identified by B.S. Air-dried material was extracted with EtOH (200 l) and evapn of the EtOH extract afforded a crude gum (200 g).

Extraction and isolation. Crude ethanolic gum was dissolved in 10% HOAc (pH 3.5). This acidic aq. extract was partitioned with petrol (40–60°) and then with $CHCl_3$. The pH of the aq. soln was re-adjusted with NH_4OH to 8.2 and extracted with $CHCl_3$. Chromatography of the $CHCl_3$ extract (pH 8.2) was carried out on a silica gel column using $CHCl_3$–MeOH (9:1) as eluent. This yielded a fr. containing a white crystalline solid (80 mg, 1.6×10^{-4}% yield, $R_f = 0.03$), named as pseudojervine (**3**). The other frs were combined and again chromatographed by med. pres LC and eluted with $CHCl_3$–MeOH (9:1). The partially purified frs thus obtained was further purified by prep. TLC (silica gel) using acetone–$CHCl_3$–$(Et)_2NH$ (7:2:1) to afford veramanine (**1**) as a yellow-coloured solid (40 mg, 8.0×10^{-5}% yield, $R_f = 0.4$). CC of the $CHCl_3$ extract (27 mg) obtained at pH 3.5 on a silica gel column (140 × 6 cm) resulted in several frs. The fr. (2.4 g) obtained on elution with $CHCl_3$–MeOH (9:1) was subjected to med. pres. LC. The fr. obtained on elution with $CHCl_3$–MeOH (9.5:0.5) afforded a mixt. of alkaloids which was separated by prep. TLC (silica gel) using acetone–CH_2Cl_2–petrol–$(Et)_2NH$ (1.5:1.0:6.5:1.0) as eluent. This afforded neojerminalanine (**2**) (35 mg, 7.0×10^{-5}% yield, $R_f = 0.48$).

Veramanine (**1**). $[\alpha]_D^{25} = -33.5°$ ($c = 0.08$, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3660 (N–H), 3500–3300 (OH), 1708 (C=O), 1622 (C=C), 1100, 985, 920 (C–O–C). UV λ_{max} (MeOH) nm: 247. EIMS *m/z* (rel. int. %): 461.3062 [$C_{27}H_{43}NO_5$, M^+] (30), 443 [$C_{27}H_{41}NO_4$], $[M - H_2O]^+$ (5), 346 (5), 328 (5), 129 (5), 126 [$C_8H_{16}N]^+$ (100), 114 [$C_6H_{12}NO]^+$ (78), 110 [$C_7H_{12}N]^+$ (92). ^1H NMR ($CDCl_3$–CD_8OD, 400 MHz): δ 0.76 (3H, *s*, H_3-19), 0.77 (3H, *d*, $J_{20,21}$ = 6.6 Hz, H_3-21), 0.78 (3H, *d*, $J_{25,27}$ = 7.3 Hz, H_3-27), 1.91 (3H, *d*, $J_{14,18}$ = 2.3 Hz, H_3-18), 2.13 (1H, *dd*, $J_{26\alpha,\beta}$ = 12.4 Hz, $J_{25,26\alpha}$ = 12.0 Hz, H_{ax}-26), 2.34 (1H, *m*, H-20), 2.53 (1H, *dd*, $J_{20,22}$ = 9.1 Hz, $J_{22,23}$ = 10.2 Hz, H_{ax}-22), 2.87 (1H, *dd*, $J_{26\alpha,\beta}$ = 12.7 Hz, $J_{25,26\beta}$ = 4.0 Hz, H_{eq}-26), 3.17 (1H, *ddd*, J_1 = 11.0 Hz, J_2 = 10.2 Hz, J_3 = 3.9 Hz, H_{ax}-23), 3.73 (1H, *bd*, J = 5.0 Hz, H-6), 3.80 (1H, *bs*, H-3). ^{13}C NMR ($CDCl_3$, 100 MHz): Table 1.

Veramanine-3,6,23-O-*triacetate.* Veramanine (2 mg) was dissolved in pyridine (1 ml) and then Ac_2O (2 ml) was added. The soln was kept overnight. Evapn of the remaining solvent yielded a triacetylated product which was confirmed by EI and FAB (+ve) MS.

Neojerminalanine (**2**). $[\alpha]_D^{25} = -44°$ ($c = 0.05$, C_5D_5N). IR ν_{max} ($CHCl_3$) cm^{-1}; 3405 (OH), 1710–1715 (C=O). UV: terminal absorption. EIMS *m/z* (rel. int. %): 751.4130 $[C_{39}H_{61}NO_{13}M]^+$ (1), 733 $[M-18]^+$ (1), 708 (1), 692 (1), 650 (1), 632 (2), 574 (4), 474 (3), 456 (4), 154 (1), 112 (100), 111 (20), 98 (18). ^1H NMR ($CDCl_3$, 400 MHz): δ 0.89 (3H, *t*, $J_{30,31}$ = 7.5 Hz, H_3-31), 0.90 (3H, *t*, $J_{37,38}$ = 7.4 Hz, H_3-36), 1.12 (3H, *d*, $J_{25,27}$ = 7.0 Hz, H_3-27), 1.14 (3H, *d*, $J_{29,32}$ = 7.0 Hz, H_3-32), 1.27 (3H, *s*, H_3-19) 1.29 (3H, *s*, H_3-37), 1.34 (3H, *s*, H_3-21), 1.98 (3H, *s*, H_3-39), 4.29 (1H, *bs*, H-16), 4.56 (1H, *bs*, H-7), 5.04 (1H, *m*, $W_{1/2}$ = 10 Hz, H-1), 5.06 (1H, *dd*, $J_{2\alpha,3}$ = 4.4 Hz, $J_{2\beta,3}$ = 1.8 Hz, H-3), 5.34 (1H, *d*, $J_{15,16}$ = 3.8 Hz, H-15). ^{13}C NMR ($CDCl_3$, 100 MHz): Table 1.

Pseudojervine (**3**). $[\alpha]_D^{25} = -97°$ ($c = 0.12$, $CHCl_3$–EtOH). IR ν_{max} ($CHCl_3$) cm^{-1}; 3402 (O–H), 1720 (C=O), 1598 (C=C). UV λ_{max} (MeOH) nm (log ε): 248 (4.02). EIMS *m/z* (rel. int. %): 587.3450 [$C_{33}H_{49}NO_8$, Calcd. *m/z* 587.3458 M]$^+$ (10), 559 (8), 425 (6), 408 (8), 167 (23), 149 (62), 125 (70), 110 (100), 83 (40). ^1H NMR ($CDCl_3$–CD_3OD, 300 MHz): δ 0.80 (3H, *d*, $J_{25,27}$ = 7.5 Hz, H_3-27), 0.81 (3H, *d*, $J_{20,21}$ = 6.6 Hz, H_3-21), 1.01 (3H, *s*, H_3-19), 2.00 (3H, *d*, $J_{14\alpha,18}$ = 2.3 Hz, H_3-18), 2.53 (1H, *dd*, $J_{20,22}$ = 9.7 Hz, $J_{22,23}$ = 9.0 Hz, H-22), 2.88 (1H, *dd*, $J_{26\alpha,\beta}$ = 12.9 Hz, $J_{25,26\beta}$ = 3.7 Hz, H-26), 3.05 (1H, *m*, H-23), 3.60 (1H, *dd*, J_1 = 12.9 Hz, J_2 = 4.8 Hz, H_a-6′), 3.65 (1H, *dd*, J_1 = 12.9 Hz, J_2 = 3.2 Hz, H_b-6′), 4.22 (1H, *d*, J = 7.8 Hz, H-1′). ^{13}C NMR ($CDCl_3$, 100 MHz): Table 1.

Acknowledgements—This work was partially supported by a grant [BC-91-016] from TWAS (Trieste, Italy) to M.I.C. The authors are grateful to Glaxo Laboratories (Pakistan), Zulfeqar Industries Ltd and the University Grants Commission for providing financial support to R.A.A.

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