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TWO NEW PREGNANE-TYPE STEROIDAL ALKALOIDS FROM *SARCOCOCCA SALIGNA*

ATTA-UR-RAHMAN*, SHAZIA ANJUM, AFGAN FAROOQ, M. RIAZ KHAN and M. IQBAL CHOUDHARY

H. E. J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan

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Key Word Index—*Sarcococca saligna*; Buxaceae; steroidal alkaloids; (20*S*, 2′*Z*)-20-(*N*,*N*-dimethylamino)-3β-(2-methyl-2*Z*-butenamido)-pregn-5-en-4-one; (20*S*, 2′*Z*)-20-(*N*,*N*-dimethylamino)-3β-2-(methyl-2*Z*-butenamido)-pregna-5, 14-dien-4-one.

Abstract—Two new preganae-type steroidal alkaloids, (20*S*, 2′*Z*)-20-(*N*,*N*-dimethylamino)-3β-(2-methyl-2*Z*-butenamido)-pregn-5-en-4-one and (20*S*, 2′*Z*)-20-(*N*,*N*-dimethylamino)-3β-(2-methyl-2*Z*-butenamido)-pregna-5,14-dien-4-one, have been isolated from an ethanolic extract of the roots and stems of *Sarcococca saligna*, in addition to the two known alkaloids, *N*-formylchonemorphine and vaganine-A, which have been isolated for the first time from this species. © 1997 Elsevier Science Ltd

INTRODUCTION

Sarcococca saligna is an evergreen shrub, widely distributed throughout the northern areas of Pakistan at altitudes of 5000–9000 feet [1]. An extract of the plant has been used in the indigenous system of medicine for its anticholinesterase [2], ganglion-blocking, anti-ulcer [3] and anti-tumour activities. A new pregnane-type steroidal alkaloid has previously been reported by us from this species [4]; our recent studies have now resulted in the isolation of two new pregnane-type alkaloids, (20*S*, 2′*Z*)-20-(*N*,*N*-dimethylamino)-3β-(2-methyl-2*Z*-butenamido)-pregn-5-en-4-one (**1**) and (20*S*, 2′*Z*)-20-(*N*,*N*-dimethylamino)-3β-(2-methyl-2*Z*-butenamido)-pregna-5,14-dien-4-one (**2**), along with two known alkaloids *N*-formylchonemorphine (**3**) and vaganine-A (**4**) [5, 6], the occurrence of which in this species we are reporting for the first time. The structures of the new compounds were elucidated through the combined use of mass spectrometry (EI, HREI and FD), UV, IR and modern NMR spectroscopic techniques (DEPT, broad-band decoupled, COSY 45°, HOHAHA, HMQC and HMBC) [7]. The chemical shift values of all the protons were assigned unambiguously and the alkaloids were subjected to various pharmacological screenings where the new compounds showed some activity against human pathogenic bacteria.

* Author to whom correspondence should be addressed.

RESULTS AND DISCUSSION

Compound **1** was isolated as a colourless amorphous powder. Its IR spectrum showed absorptions at 3399 (NH), 1645 (amidic carbonyl) and 1602 (C = C) cm^{-1}, indicating the presence of an α,β-unsaturated amidic functionality in the molecule. The UV spectrum displayed absorption maxima at 257 and 237 nm, which were characteristic of enone and enamide functionalities, respectively [8]. The EI mass spectrum of **1** showed a $[M]^+$ at *m/z* 440, which was confirmed by FD mass spectrometry. The HREI mass spectrum showed the exact M_r at *m/z* 440.3361, corresponding with the molecular formula $C_{28}H_{44}H_2O_2$, having eight degrees of unsaturation.

The ^1H NMR spectrum of **1** revealed the presence of three 3H singlets at δ 0.77 (H-18), 0.90 (H-19) and 1.88 (H-5′), along with two three-proton doublets at δ 1.34 ($J_{21,20}$ = 6.6 Hz, H-21) and 1.84 ($J_{4',3'}$ = 5.5 Hz, H-4′). The *N*,*N*-dimethyl protons resonated as a 6H-singlet at δ 2.69. A multiplet at δ 2.24 was ascribed to H-3α geminal to the amidic group. Two downfield olefinic signals at σ 6.50 (*q*, $J_{3',4'}$ = 5.5 Hz, H-3′) and 7.45 (*dd*, $J_{6,7}$ = 6.7, 2.7 Hz, H-6) were also observed in this spectrum.

The ^{13}C NMR spectrum (broad-band) of **1** displayed resonances for 28 carbons, while the DEPT spectrum showed the presence of seven methyl, seven methylene, eight methine and six quaternary carbon signals. These observations revealed that the compound could be a pregnane-type steroidal alkaloid, i.e. a derivative of sarcodine, which has been isolated previously from this species [4].

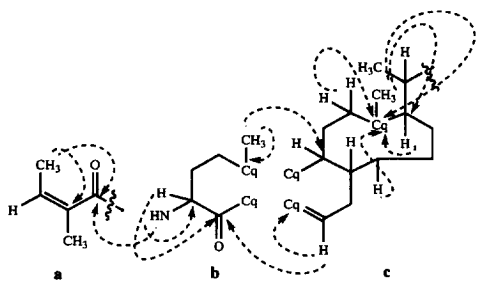
Table 1. ^{1}H NMR* and ^{13}C NMR† chemical shift assignments of compounds **1** and **2** in $CDCl_3$

Carbon	**1**		**2**	
	δ_C	δ_H	δ_C	δ_H
1	31.5	0.95 *m*, 1.81 *m*	34.2	1.49 *m*, 1.69 *m*
2	30.7	1.28 *m*, 1.32 *m*	30.7	1.32 *m*, 1.34 *m*
3	55.8	2.24 *m*	56.1	2.21 *m*
4	197.5	—	197.5	—
5	133.2	—	133.2	—
6	129.4	7.45 *dd* $J_{6,7}$ = 6.7, 2.7	133.3	7.45 *dd* $J_{6,7}$ = 6.6, 2.7
7	39.9	2.35 *m*, 2.56 *m*	39.9	2.39 *m*, 2.54 *m*
8	35.8	1.38 *m*	35.9	1.43 *m*
9	54.7	1.12 *m*	55.5	1.18 *m*
10	40.9	—	41.2	—
11	21.5	1.37 *m*, 2.01 *m*	21.5	1.41 *m*, 2.01, *m*
12	26.8	1.82 *m*, 1.92 *m*	31.4	1.28 *m*, 1.31, *m*
13	44.1	—	43.5	—
14	56.9	1.20 *m*	153.4	—
15	25.1	1.49 *m*, 1.55, *m*	153.4	—
15	25.1	1.49 *m*, 1.55, *m*	127.9	5.85 *t* $J_{15,16}$ = 1.3
16	21.7	1.59 *m*, 1.61 *m*	32.9	2.13 *m*, 2.28 *m*
17	53.2	1.72 *m*	57.8	1.42 *m*
18	12.4	0.77 *s*	16.3	0.89 *s*
19	13.4	0.90 *s*	13.6	0.92 *s*
20	66.9	3.31 *m*	62.3	3.42 *m*
21	11.9	1.34 *d* $J_{21,20}$ = 6.6	19.3	1.38 *d* $J_{21,20}$ = 6.5
$N(CH_3)_2$	40.1	2.69 *s*	42.9	2.71 *s*
1′	169.9	—	169.8	—
2′	132.8	—	132.7	—
3′	133.3	6.50 *q*, $J_{3',4'}$ = 5.5	129.1	6.58, *q*, $J_{3',4'}$ = 5.5
4′	12.3	1.84 *d*, $J_{4',3'}$ = 5.5	11.6	1.85 *q*, $J_{4',3'}$ = 5.5
5′	14.1	1.88 *s*	14.2	1.87 *s*

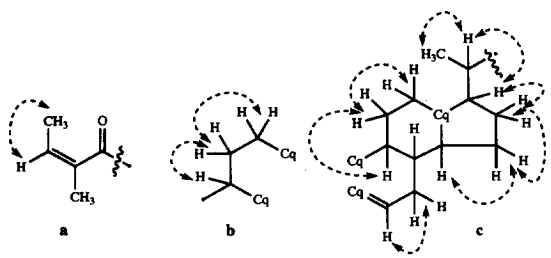
δ Values in ppm from TSM.
* Assignments made from HMQC spectrum.
† Assignments made from broad-band and DEPT spectra.

Three partial structures **a**, **b** and **c** were established by combining ^{1}H and ^{13}C NMR (Table 1) data with those obtained from COSY45° (Scheme 1) HOHAHA, HMQC and HMBC (Scheme 2) experiments. The partial structures **a**, **b** and **c** were joined together by HMBC connectivities of quaternary carbons with the protons of adjacent carbons. The angular methyls and *N*-methyls in the side-chains were assembled in order to achieve the pregnane skeleton.

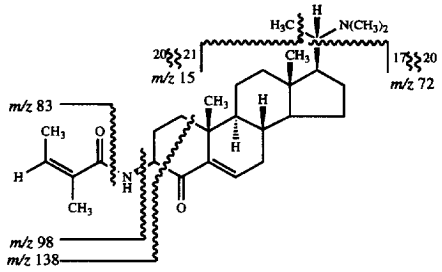
The presence of unit **b** in ring A and unit **c** in rings B, C and D was established by the HMBC connectivities of H-1, H-9 and H-19 with C-10 and the presence of keto and olefinic tertiary carbons at positions 4 and 6, respectively, but the HMBC couplings of H-3 with C-4 and of H-6 with C-5. The positions of substituted amidic functionality at C-3 and of the *N*,*N*-dimethylaminoethane side-chain at C-17 were proved by HMBC coupling of H-3 with the amidic carbonyl (C-1′) and of H-20 with C-17, respectively. The mass fragmentation pattern of **1** (Scheme 3) was also very useful in proving the presence of the 2-methyl-2-butenamido and the *N*,*N*-dimethylaminoethane side-chains at C-3 and C-17, due to the presence of ions *m*/*z* 98 and 72, respectively. The



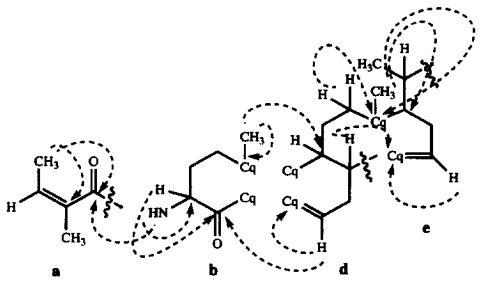
Scheme 2.



Scheme 1.



Scheme 3.



Scheme 5.

ion at m/z 138 resulted from to the well known C-3/C-4 cleavage in steroids [9].

Compound **2** was purified as a colourless amorphous powder. Its IR spectrum revealed absorptions at 3399 (NH), 1645 (amidic carbonyl) and 1602 (C = C) cm^{-1} indicating the presence of an α,β-unsaturated amidic functionality in the molecule. The absorption maxima at 257 and 237 nm in the UV spectrum of **2** were indicative of the presence of enone and enamide functionalities, respectively [8]. The EI mass spectrum exhibited a $[M]^+$ at m/z 438, which was confirmed by the FD mass spectrum. The HREI mass spectrum of **2** showed the exact M_r at m/z 438.3330 corresponding with the molecular formula $C_{28}H_{42}N_2O_2$, indicating nine degrees of unsaturation.

The ^1H NMR spectrum of **2** displayed three-proton singlets at δ 0.89 (H-18), 0.92 (H-19) and 1.87 (H-5′). Two 3H doublets resonated at δ 1.38 ($J_{21,20}$ = 6.5 Hz, H-21) and 1.85 ($J_{4',3'}$ = 5.5 Hz, H-4′). A downfield 6H singlet at δ 2.71 was due to the *N,N*-dimethyl protons. A multiplet at δ 2.21 resonated due to H-3α, which is geminal to the amidic group. Three downfield olefinic signals at δ 5.85 (1H, *t*, $J_{15,16}$ = 1.3 Hz, H-15), 6.58 (1H, *q*, $J_{3',4'}$ = 5.5 Hz, H-3′) and 7.45 (1H, *dd*, $J_{6,7}$ = 6.6, 2.7 Hz, H-6) were ascribed to H-15, H-3′ and H-6, respectively.

The ^{13}C NMR spectrum (broad-band) of **2** showed signals for all 28 carbons. The multiplicities of the signals were determined by recording DEPT spectra, which revealed the presence of seven methyl, six methylene, eight methine and seven quaternary carbons in the molecule. Compound **2** was catalytically hydrogenated with palladium on charcoal to afford **1**. These observations established that compound **2** possessed the same skeleton and substituents at **1**, with an additional double bond at C-14, 15.

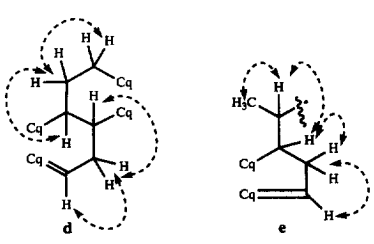
The COSY 45° (Scheme 4), HOHAHA, HMQC and HMBC (Scheme 5) spectra helped in the formation of four spin-systems **a**, **b**, **d** and **e**, which were joined together by HMBC connectivities of quaternary carbons and their vicinal protons. The angular methyls and *N*-methyls in the side-chains were assembled in order to achieve the pregnane skeleton. The presence of unit **b** in ring A, unit **d** in rings B and C, and unit **e** in ring D was established by the HMBC connectivities of H-1, H-9 and H-19 with C-10. The presence of keto and olefinic tertiary carbons at positions 4, 6 and 15, respectively, was established by the HMBC couplings of H-3 and C-4, of H-6 with C-5 and of H-15 with C-14. The positions of the substituted amidic functionality at C-3 and the *N,N*-dimethylaminoethane side-chain at C-17 were established by HMBC coupling of H-3 with the amidic carbonyl (C-1′) and of H-20 with C-17, respectively. The mass fragmentation pattern of **2** (Scheme 6) supported the presence of 2-methyl-2-butenamido and *N,N*-dimethylaminoethane side-chains at C-3 and C-17, respectively, by the presence of fragments at m/z 98 and 72. The ion at m/z 138 resulted from C-3/C-4 cleavage. The ^1H and ^{13}C NMR chemical shift assignments of **1** and **2** are presented in Table 1.

The known compounds **3** and **4** were identified by comparing their physical and spectroscopic data with literature values. The ^1H and ^{13}C NMR chemical shift assignments of **3** and **4** are presented here for the first time (Table 2).

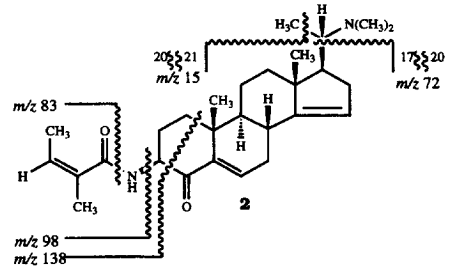
The antibacterial activities against some human pathogenic bacteria were studied (Table 3) where **1** and **2** showed some antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*, respectively.

EXPERIMENTAL

General. Mps are uncorr. Purity of samples was checked by TLC (silica gel G-254 precoated plates)



Scheme 4.



Scheme 6.

Table 2. ^{1}H NMR* and ^{13}C NMR† chemical shift assignments of compounds **3** and **4** in $CDCl_3$

Carbon	**3**		**4**	
	δ_C	δ_H	δ_C	δ_H
1	39.4	1.10 *m*, 1.60 *m*	39.7	1.28 *m*, 1.80 *m*
2	30.2	1.25 *m*, 1.50 *m*	24.3	1.61 *m*, 1.69, *m*
3	47.0	4.10 *m*	49.7	4.00 *m*
4	37.5	0.95 *m*, 1.75 *m*	75.3	5.13 *dd* J = 8.1, 9.0
5	46.0	1.08 *m*	48.9	1.35 *m*, 1.45 *m*
6	23.7	1.20 *m*, 1.77 *m*	20.4	1.25 *m*, 1.45 *m*
	27.8	1.05 *m*, 1.50, *m*	25.2	1.32 *m*, 1.5 *m*
8	35.0	1.33 *m*	35.1	1.38 *m*
9	54.0	0.60 *m*	53.9	0.65 *m*
10	36.2	—	39.5	—
11	20.9	1.18 *m*, 1.46 *m*	20.3	1.19 *m*, 1.47 *m*
12	31.9	0.85 *m*, 1.63 *m*	31.9	1.21 *m*, 1.71 *m*
13	42.3	—	42.1	—
14	56.5	1.00 *m*	55.9	1.01 *m*
15	28.8	1.13 *m*, 1.24 *m*	28.4	1.25 *m*, 1.27 *m*
16	23.9	1.04 *m*, 1.50 *m*	24.2	1.60 *m*, 1.62 *m*
17	57.1	1.30 *m*	52.5	1.45 *m*
18	12.2	0.80 *s*	12.7	0.85 *s*
19	12.4	0.90 *s*	14.1	0.95 *s*
20	64.5	2.42 *m*	65.1	3.21 *m*
21	21.7	1.18 *d* $J_{21,20}$ = 6.5	11.2	1.24 *d* $J_{21,20}$ = 6.6
$N(CH_3)_2$	40.9	2.40 *s*	42.0	2.61 *s*
1′	161.4	—	166.0	—
2′	—	—	118.4	5.45 *s*
3′	—	—	132.5	—
4′	—	—	27.5	1.84 *s*
5′	—	—	27.5	2.09 *s*
$\underline{OCOCH_3}$	—	—	171.0	—
$OCO\underline{CH_3}$	—	—	21.1	1.89

δ Values in ppm from TMS.
* Assignments made from HMQC spectrum.
† Assignments made from broad-band and DEPT spectra.

Table 3. Antibacterial activity of compounds **1** and **2** against some human pathogens

Bacterium	Zones of inhibition (mm)				
	Standards			Compounds	
	Amp	Amox	TOB	**1**	**2**
Staphylococcus aureus	18	16	—	11	7
Streptococcus pyogenes	17	16	20	6	9
Pseudomonas aeruginosa	17	16	—	6	—
Escherichia coli	19	—	20	—	7
Salmonella typhi	12	12	—	6	—
Shigella boydii	17	17	20	7	6
Klebsiella pneumoniae	14	14	20	10	9
Proteus mirabilis	17	16	—	7	—
Shigella flexnariae	18	—	20	—	8
Corynebacterium diphtheriae	17	—	18	—	6

AMP = ampicillin; AMOX = amoxacillin; TOB = tobramycine.

and flash CC on silica gel was used for CC. ^{1}H NMR spectra were recorded at 500 MHz, ^{13}C NMR at 125 MHz.

Plant material. Collected from the Bagh District of Azad Kashmir in July, 1995 and identified by Mr Tahir Ali, Taxonomist, Department of Botany, University of Karachi, Pakistan. A voucher specimen is deposited in the herbarium of Department of Botany, University of Karachi (KU 19290).

Extraction and isolation. Air-dried and ground roots and stems of *S. saligna* (D.Don) Muell. (8 kg) were soaked in EtOH–H_2O (4:1) (20 l) for 15 days.

The solvent was then evapd *in vacuo* and the remaining gum (780 g) dissolved in H_2O (2 l) and extracted with petrol (6 l) and $CHCl_3$ (8 l), respectively. Evapn of the $CHCl_3$ yielded the crude alkaloidal fr. (55 g), which was adsorbed on an equal quantity of silica gel and chromatographed to afford various frs. The fr. eluted with $CHCl_3$–MeOH (9:1) (6.7 g) was subjected to further CC. Elution with EtOAc–petrol (3:2) yielded a subfr. (600 mg), which was subjected to TLC on precoated silica gel plates using Me_2Co–petrol–Et_2N (0.5:9.3:0.2), to afford (20*S*, 2′*Z*)-20-(*N*,*N*-dimethylamino)-3β-(2*Z*-methyl-2-butenamido)-pregn-5-en-4-one (**1**) (2×10^{-4}%) (R_f 0.64) and (20*S*, 2′*Z*)-20-(*N*,*N*-dimethylamino)-3β-(2-methyl-2*Z*-butenamido)-pregna-5, 14-dien-4-one **2** (2.5×10^{-4}%) (R_f 0.66). The fr. eluted with $CHCl_3$–MeOH (4:1) (3 g) was subjected to CC. Elution with $CHCl_3$–petrol (9:1) yielded subfr. which was chromatographed over precoated silica gel plates using Me_2CO–petrol–Et_2N (1.0:8.8:0.2) to afford (**3**) 1×10^{-4}%) (R_f 0.35) and (**4**) 1.4×10^{-4}%) (R_f 0.52).

Compound **1**. Mp. 278° $[\alpha]_D^{25} -106.4°$ (*c* 0.1, MeOH). UV λ_{max}^{MeOH} nm (log ε): 257 (2.9), 237 (2.9. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 339 (NH), 1645 (amidic carbonyl), 1602 (C = C). EIMS *m/z* (rel. int.): 440 (20) $[M]^+$, 425 (28) $[M-15]^+$, 411 (3) 353 (4), 138 (16), 98 (8), 83 (100), 72 (98), 55 (38). FDMS: *m/z* 440 ($C_{28}H_{44}N_2O_2$). HREIMS: *m/z* 440.3361 ($C_{28}H_{44}H_2O_2$ requires 440.3403). ^1H and ^{13}C NMR: Table 1.

Compound **2**. Mp 20°. $[\alpha]_D^{25} -113.6°$ (*c* 0.1, MeOH). UV λ_{max}^{MeOH} nm (log ε): 257 (2.9), 237 (2.9). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3399 (NH), 1645 (amidic carbonyl), 1602 (C = C). EIMS *m/z* (rel. int.): 438 (13) $[M]^+$, 423 (100) $[M-15]^+$, 393 (3), 311 (2), 258 (3), 150 (2), 138 (9), 98 (11), 83 (28), 72 (18), 55 (6). FDMS: *m/z* 438 ($C_{28}H_{42}N_2O_2$). HREIMS: *m/z* 438.3330 ($C_{28}H_{42}N_2O_2$ requires 438.3246). ^1H and ^{13}C NMR: Table 1.

Compound **3**. Mp 288–290°. $[\alpha]_D^{25} +25°C$ (*c* 0.05, MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (NH), 1685, 1595 (amidic carbonyl). EIMS *m/z* (rel. int.): 374 (36) $[M]^+$, 359 (2) $[M-15]^+$, 315 (11), 110 (83), 84 (100), 72 (23). FDMS: *m/z* 374 ($C_{24}H_{42}N_2O$). HREIMS: *m/z* 374.3317 ($C_{24}H_{42}N_2O$ requires 374.3296). ^1H and ^{13}C NMR: Table 2.

Compound **4**. $[\alpha]_D^{25} +$ 119° (*c* 0.17, MeOH). UV λ_{max}^{MeOH} nm (log ε): 204 (3.8). IR ν cm^{-1}: 3410 (NH) 1710 ($OCOCH_3$). EIMS: *m/z* (rel. int.): 486 (81) $[M]^+$, 471 (60) $[M-15]^+$, 449 (17), 415 (23), 258 (17), 150 (2), 138 (8), 100 (65), 83 (100), 72 (100). FDMS: *m/z* 486 ($C_{30}H_{50}N_2O_3$). HREIMS: *m/z* 486.3788 ($C_{30}H_{50}N_2O_3$ requires 486.3821). ^1H and ^{13}C NMR: Table 2.

Hydrogenation of **2**. Compound **2** (3.5 mg) was dissolved in dry CH_2Cl_2 (2.5 ml), Pd-C (10%) (15 mg) added and air was removed by evacuation. H_2 was bubbled for 20 min. The catalyst was then removed by filtration over silica gel and the solvent evapd to yield compound **1** (85%), which was identified by comparing its spectroscopic data with an authentic sample.

Antibacterial activities. Antibacterial activities of the compounds were determined by the agar well diffusion method. A loop-full of 24 h-old cultures containing *ca* 10^4–10^6 CFU were spread on the surface of MHA plates and wells dug in the media with the help of a sterile metallic borer. Test samples and standard antibiotics were added to the wells, the plates were incubated at 37° for 24 h and the zones of inhibition measured.

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REFERENCES

1. Nasir, E. and Ali, S. I., *The Flora of W. Pakistan*, 1972. Fakhari Printing Press, Karachi, p. 457.
2. Kiamuddin, M. and Hye, H., *Pakistani Journal of Scientific and Industrial Research*, 1970, **13**, 59.
3. Qiu, M.-H., Nie, R.-L. and Li, Z.R., *Yunnan Zhiwu Yanjiu*, 1994, **16**, 286.
4. Naeem, I., Khan, N., Atta-ur-Rahman and Choudhary, M. I., *Phytochemistry*, 1996, **43**, 905.
5. Banerji, J., Chatterjee, A., Itoh, Y. and Kikuchi, T. (1978) *Indian Journal of Chemistry*, 1978, **16B**, 346.
6. Qiu, M.-H., Li, Z. R., Cao, D. Y. and Nie, R.-L., *Acta Botanica Sinica*, 1993, **35**, 885.
7. Atta-ur-Rahman and Choudhary, M. I., *Solving Problems with NMR Spectroscopy*, Academic Press, New York, 1996, p. 398.
8 Scott, A. I., *Interpretation of the Ultraviolet Spectra of Natural Products*, Pergamon Press, London, 1964, p. 79.
9. Budzkiewicz, H., Djerassi, C. and Williams, D. H., *Structure of Elucidation of Natural Products by Mass Spectrometry*, Vol. 2. Holden-Day, Amsterdam, 1964, p. 5.