New Steroidal Alkaloids from *Sarcococca saligna*

Atta-ur-Rahman,* M. Iqbal Choudhary,* M. Riaz Khan, Shazia Anjum, Afgan Farooq, and M. Zafar Iqbal

International Centre for Chemical Sciences, H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan

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Five new pregnane-type steroidal alkaloids (**1**−**5**) have been isolated from *Sarcococca saligna*. A combination of UV, IR, MS, and 1D and 2D NMR spectroscopic studies established their structures as salignarine A [(*20S*)-2*β*-hydroxy-4*β*-acetoxy-5α,6α-epoxy-20-(dimethylamino)-3*β*-(tigloylamino)pregnane] (**1**), salignarine B [(*20S*)-2*β*-hydroxy-20-(dimethylamino)-3*β*-(tigloylamino)-pregn-5-ene] (**2**), salignarine C [(*20S*)-2*β*-hydroxy-20-(dimethylamino)-3*β*-(senecioylamino)-pregn-5-ene] (**3**), salignarine D [(*20S*)-20-(dimethylamino)-3*β*-(senecioylamino)-5α-pregn-16-ene] (**4**), and salignarine E [(*20S*)-20-(dimethylamino)-3*β*-(tigloylamino)-pregn-4-ene] (**5**), respectively.

Sarcococca saligna Muell.-Arg. is an evergreen aromatic shrub belonging to the family Buxaceae, widely distributed throughout the northern areas of Pakistan and Kashmir at 5000−9000 ft altitudes. It finds extensive use in the indigenous system of medicine for the treatment of pain and rheumatic fever.[1] Crude extracts of *S. saligna* have anticholinestrase, antitumor, and antiulcer activities.[2] Our previous investigations on the plant have resulted in the isolation of several pregnane-type steroidal alkaloids.[3−8] We now report the isolation of five new pregnane-type steroidal bases whose structures were established mainly on the basis of spectroscopic studies, with ^{1}H NMR chemical shift assignments of all protons made unambiguously through 1D and 2D NMR spectroscopic studies.[9]

Results and Discussion

Alkaloid **1** was purified as a pale yellow amorphous gum, the EIMS of which showed a molecular ion at *m*/*z* 516, while the HREIMS displayed the exact molecular mass at *m*/*z* 516.3552, corresponding to the molecular formula $C_{30}H_{48}N_2O_5$ (calcd 516.3563), and indicating eight degrees of unsaturation. The characteristic fragmentation pattern indicated that the substance had a pregnane-type nucleus with two nitrogen substituents at C-3 and C-20.[10] The IR spectrum of **1** showed absorptions at 3376 (hydroxyl), 1718 (carbonyl), and 1652 and 1616 (olefinic) cm^{-1}. The UV spectrum displayed end absorption at 208 nm. The ^{1}H NMR spectrum of **1** exhibited two 3H singlets at *δ* 0.90 and 1.29 for the C-18 and C-19 methyl protons. The 3H doublet resonating at *δ* 1.20 ($J_{21,20}$ = 6.5 Hz) was ascribed to the C-21 methyl protons. Another 3H singlet at *δ* 2.07 was due to the acetyl methyl protons, while a 6H singlet at *δ* 2.36 was attributed to the $N_b(CH_3)_2$ protons. A 3H singlet and a 3H doublet at *δ* 1.83 and 1.75 ($J_{4',3'}$ = 7.0 Hz) were assigned to H-5′ and H-4′ of the tigloyl moiety, respectively. Two 1H multiplets resonating at *δ* 4.09 and 4.01 were due to H-2 and H-3, respectively. A broad singlet at *δ* 5.32 and a triplet at *δ* 3.76 ($J_{6,7}$ = 5.5 Hz) were due to H-4 and H-6 (proton geminal to oxygen linkage), respectively, and the former proton showed vicinal couplings with H-3 (*δ* 4.01) and H-2 (*δ* 4.09), while the latter proton showed the presence of an epoxide ring at C-5 and C-6 of a pregnane alkaloid **1**. A quartet centered at *δ* 6.38 ($J_{3',4'}$ = 7.0 Hz) was assigned to H-3′ (Table 1).

The ^{13}C NMR spectrum (broad-band decoupled) of **1** showed signals for 30 carbons. The DEPT spectra indicated the presence of eight methyl, six methylene, 10 methine, and (by difference from the broad-band spectrum) six quaternary carbons. The characteristic mass fragments at *m*/*z* 72 and 83 helped to establish the positions of the $N_b(CH_3)_2$ and tigloyl moieties at C-20 and C-3 of the pregnane skeleton, respectively.[5,11]

The HMQC spectrum of **1** was used to determine direct ^{1}H−^{13}C correlations (Table 1), while the HMBC correlations were used to establish the long-range ^{1}H−^{13}C connectivities (Table 2). The CH_3-4′ (*δ* 1.75) signal of the tigloylamide moiety displayed HMBC correlations with C-3′ (*δ* 130.9), while CH_3-5′ (*δ* 1.83) showed correlations with C-1′ (*δ* 168.8). The H-2 (*δ* 4.09), H_3-19 (*δ* 1.29), and H-6 (*δ* 3.76) protons showed correlations with C-10 (*δ* 35.0), which indicated that a hydroxyl group was present at the C-2 position of the pregnane skeleton. Similarly, H-3α (*δ* 4.01) displayed cross-peaks with C-4 (*δ* 75.0) and C-5 (*δ* 71.4), which indicated the presence of an epoxide between C-5 and C-6. The H_2-12 (*δ* 1.40, 1.70) and H_3-21 (*δ* 1.20) displayed long-range couplings with C-13 (*δ* 38.1) and C-17 (*δ* 48.7), respectively. The stereochemistry at C-2, C-4, C-5, and C-6 was assigned on the basis of the biogenetic grounds and the NOESY spectrum (Figure 1). The NOESY interactions between H-3α (*δ* 4.01), H-2α (*δ* 4.09), and H-4α (*δ* 5.32) suggested *β*-orientations of the hydroxyl, tigloylamide, and acetoxy functionalities. The α-orientation for the epoxide was assigned due to NOESY interactions for H-6*β* (*δ* 3.76) with H-19*β* (*δ* 1.29). These studies led to structure **1** for salignarine A.

Alkaloid **2** was isolated as a colorless amorphous powder. The HREIMS showed the exact M$^+$ at *m*/*z* 442.3529, which corresponds to $C_{28}H_{46}N_2O_2$ (calcd 442.3559) indicating seven degrees of unsaturation in the molecule. The IR spectrum exhibited absorptions at 3602 (NH), 3349 (OH), 1641 (C=O), and 1611 (C=C) cm^{-1}. The UV spectrum showed end absorption at 212 nm. The ^{1}H NMR spectrum exhibited two 3H singlets at *δ* 0.80 and 1.04 assigned to the C-18 and C-19 angular methyl protons. A 3H doublet at *δ* 1.07 ($J_{21,20}$ = 6.5 Hz) was due to the C-21 methyl protons, while a 6H singlet at *δ* 2.19 was ascribed to the $N_b(CH_3)_2$ protons. A 3H singlet at *δ* 1.80 and a 3H doublet at *δ* 1.70 ($J_{4',3'}$ = 6.7 Hz) were attributed to the C-5′ and C-4′ methyl protons, respectively. A 1H quartet at *δ* 6.33 ($J_{3',4'}$ = 6.7 Hz) was due to H-3′. Two multiplets integrating

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Table 1. ^{1}H and ^{13}C NMR Chemical Shift Assignments and Proton−Carbon One-Bond Connectivities (HMQC) of **1**−**3**[a]

	1		**2**		**3**	
position	δ_C	δ_H (J = Hz)	δ_C	δ_H (J = Hz)	δ_C	δ_H (J = Hz)
1	44.4	1.30, 2.20	40.3	1.15, 1.85	40.5	1.20, 1.80
2	69.8	4.09	69.6	3.92	69.6	3.95
3	51.6	4.01	50.3	4.02	50.7	4.00 dd (6.0, 3.3)
4	75.0	5.32 br s	31.2	1.20, 2.01	31.3	1.85, 2.15
5	71.4		126.8		124.0	
6	57.0	3.76 t (5.5)	118.0	5.50 t (3.6)	118.7	5.56 t (3.0)
7	32.0	1.40, 1.55	31.9	1.75, 2.02	31.8	1.65, 1.70
8	44.4	2.15	33.5	1.65	33.5	1.75
9	56.3	2.10	56.1	0.72	56.3	0.75
10	35.0		36.0		36.0	
11	20.3	1.55, 1.60	20.7	1.59, 1.65	20.7	1.35, 1.60
12	31.4	1.40, 1.70	34.7	1.70, 1.75	34.7	1.65, 1.70
13	38.1		42.0		42.0	
14	56.8	1.90	57.4	1.35	57.5	1.30
15	25.1	1.45, 1.50	28.8	1.25, 1.35	28.8	1.20, 1.30
16	26.8	1.80, 1.95	28.2	1.81, 1.90	28.2	1.95, 2.05
17	48.7	1.45	46.9	1.25	47.0	1.75
18	12.3	0.90 s	16.1	0.80 s	13.8	0.81
19	13.9	1.29 s	14.3	1.04 s	14.3	1.04
20	61.9	2.83	59.7	2.80	59.7	2.85
21	10.3	1.20 d (6.5)	14.3	1.07 d (6.5)	12.5	1.10 d (6.5)
$N_b(CH_3)_2$	43.4	2.36 s	42.4	2.19 s	42.3	2.24 s
1′	168.8		169.4		166.4	
2′	131.8		132.4		118.0	5.58 s
3′	130.9	6.38 q (7.0)	130.2	6.33 q (6.7)	151.0	
4′	13.8	1.75 d (7.0)	14.8	1.70 d (6.7)	26.1	2.10 s
5′	16.7	1.83 s	16.1	1.80 s	19.8	1.82 s
$OCOCH_3$	20.9	2.07 s				
$O\underline{C}O\overline{C}H_3$	170.0					

[a] $CDCl_3$, TMS, δ (ppm).

for one proton each at δ 3.92 and 4.02 were assigned to H-2 and H-3, respectively. A 1H triplet at δ 5.50 ($J_{6,7}$ = 3.6 Hz) was due to an olefinic H-6 proton.

The ^{13}C NMR (broad-band decoupled and DEPT) spectra of **2** exhibited signals for 28 carbons atom with seven methyl, seven methylene, nine methine, and five quaternary carbon signals. The HMQC spectrum of **2**, in conjunction with the COSY 45° spectrum, was used to assign the chemical shift values (Table 1) to all the carbons and protons, which were further deduced by HMBC (Table 2). The H-4′ (δ 1.70) and H-5′ (δ 1.80) displayed HMBC correlations with C-3′ (δ 130.2) and C-1′ (δ 169.4), respectively, and showed the presence of a tigloylamide moiety in pregnane alkaloid **2**. The H-2 (δ 3.92), H-19 (δ 1.04), and H-6 (δ 5.50) were coupled with C-10 (δ 36.0), which confirmed that a hydroxyl group was present at the C-2 position as already described for alkaloid **1**, while a double bond was present between C-5 and C-6. The H-3 (δ 4.02) methine did not show a COSY 45° coupling with the olefinic proton, which further helped to place the double bond between C-5 and C-6. The H-12 methylene protons (δ 1.70, 1.75) and H-21 (δ 1.07) methyl protons showed correlations with C-13 (δ 42.0) and C-17 (δ 46.9), respectively. The β-stereochemistry of the hydroxyl group at C-2 was deduced from the NOESY interactions of H-2α (δ 3.92) with H-3α (δ 4.02), while other important interactions are shown in Figure 2. These spectral studies led to structure **2** for salignarine B.

Alkaloid **3**, isolated as a white amorphous powder, had the molecular formula $C_{28}H_{46}N_2O_2$ as revealed by its HREIMS (m/z 442.3566). Close similarities in the spectral data indicated that **3** was closely related in structure to alkaloid **2**. Comparison of the ^{1}H and ^{13}C NMR spectra suggested that **3** possessed a senecioylamide substituent at C-3 instead of the tigloylamide group in **2**. The most prominent differences in the ^{13}C NMR spectra of **2** and **3** were the downfield chemical shift of C-3′ quaternary carbon (δ 151.0) and the appearance of a 6H singlet at δ 2.24 in the ^{1}H NMR spectrum compound **2**. These data suggested that alkaloid **3** possessed a senecioylamide moiety at C-3. The important stereochemical assignments of **3** were made on the basis of the NOESY interactions (see Figure 3). Thus, salignarine C was assigned structure **3** on the basis of these studies.

Alkaloid **4**, a colorless amorphous powder, showed a M^+ ion at m/z 426.3607, corresponding to the molecular formula $C_{28}H_{46}N_2O$ (calcd 426.3609) and indicating seven degrees of unsaturation. The IR spectrum showed absorptions at 3351 (NH), 1656 (C=O), and 1609 (C=C) cm^{-1}, while the UV spectrum afforded an absorption at 210 nm. The ^{1}H NMR spectrum of **4** showed a 6H singlet at δ 0.80 ascribed to the C-18 and C-19 methyl protons. A 3H doublet at δ 1.40 ($J_{21,20}$ = 6.6 Hz) was due to the H_3-21 protons. Two 3H singlets at δ 2.10 and 1.75 were ascribed to C-4′ and C-5′ of the senecioylamide functionality. A 6H singlet at δ 2.17 was assigned due to the $N_b(CH_3)_2$ protons. A multiplet at δ 4.07 was due to H-3α, while an olefinic methine singlet for H-2′ of the senecioylamide moiety resonated at δ 5.48 (br s).

The ^{13}C NMR spectrum revealed the presence of seven methyl, eight methylene, eight methine, and five quaternary carbons (Table 3). Important HMBC correlations that helped to elucidate the structure and assignments are shown in Table 2. As in salignarine C(**3**), the H-4′ (δ 2.10) and H-5′ (δ 1.75) signals displayed HMBC correlations with C-2′ (δ 119.1), which indicated the presence of a senecioylamide group in the molecule. The double bond was located at C-16/C-17 because the H-16 (δ 5.54) and H-18 (δ 0.80) protons correlated with C-13 (δ 46.7), while H-21 (δ 1.40) showed a correlation with C-17 (δ 157.0). Similarly, the H_3-19 (δ 0.80) exhibited a HMBC correlation with C-10 (δ 41.4). The important stereochemical assignments

Table 2. Long-Range ^{1}H and ^{13}C NMR Connectivities (HMBC) for **1**–**5**[a]

1		**2**		**3**		**4**		**5**	
δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
4.09 (H-2α)	35.0 (C-10, $^3J_{CH}$)	3.92 (H-2α)	36.0 (C-10, $^3J_{CH}$)	3.95 (H-2α)	36.0 (C-10, $^3J_{CH}$)	2.10 (H-4′)	119.1 (C-2′, $^3J_{CH}$)	1.73 (H-4′)	129.9 (C-3′, $^2J_{CH}$)
4.01 (H-3α)	75.0 (C-4, $^2J_{CH}$)	1.70 (H-4′)	130.2 (C-3′, $^2J_{CH}$)	2.10 (H-4′)	118.0 (C-2′, $^3J_{CH}$)	1.75 (H-5′)	119.1 (C-2′, $^3J_{CH}$)	1.81 (H-5′)	168.0 (C-1′, $^3J_{CH}$)
	71.4 (C-5, $^3J_{CH}$)	1.80 (H-5′)	169.4 (C-1′, $^3J_{CH}$)	1.82 (H-5′)	118.0 (C-2′, $^3J_{CH}$)	5.54 (H-16)	46.7 (C-13, $^3J_{CH}$)	0.80 (H-18)	41.8 (C-13, $^2J_{CH}$)
1.75 (H-4′)	130.9 (C-3′, $^2J_{CH}$)	5.50 (H-6)	36.0 (C-10, $^2J_{CH}$)	5.56 (H-6)	36.0 (C-10, $^3J_{CH}$)	0.80 (H-18)	46.7 (C-13, $^2J_{CH}$)	0.81 (H-19)	36.3 (C-10, $^3J_{CH}$)
1.83 (H-5′)	168.8 (C-1′, $^3J_{CH}$)	1.04 (H-19)	36.0 (C-10, $^3J_{CH}$)	0.81 (H-18)	42.0 (C-13, $^2J_{CH}$)	0.80 (H-19)	41.4 (C-10, $^2J_{CH}$	1.13 (H-21)	47.3 (C-17, $^3J_{CH}$)
3.76 (H-6)	35.0 (C-10, $^3J_{CH}$)	1.07 (H-21)	46.9 (C-17, $^3J_{CH}$)	1.04 (H-19)	36.0 (C-10, $^2J_{CH}$)	1.40 (H-21)	157.0 (C-17, $^3J_{CH}$)	5.93 (H-4)	128.0 (C-5, $^2J_{CH}$)
1.70 (H-12)	38.1 (C-13, $^2J_{CH}$)	1.70 (H-12β)	42.0 (C-13, $^2J_{CH}$)	1.10 (H-21)	47.0 (C-17, $^3J_{CH}$)				36.3 (C-10, $^3J_{CH}$)
1.29 (H-19)	35.0 (C-10, $^2J_{CH}$)	1.75 (H-12α)							
1.20 (H-21)	48.7 (C-17, $^3J_{CH}$)								

[a] $CDCl_3$, TMS, δ (ppm).

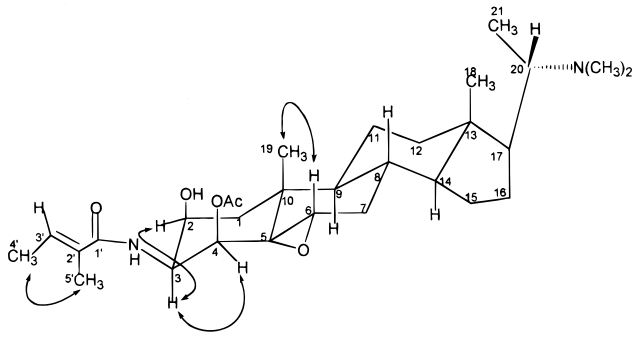
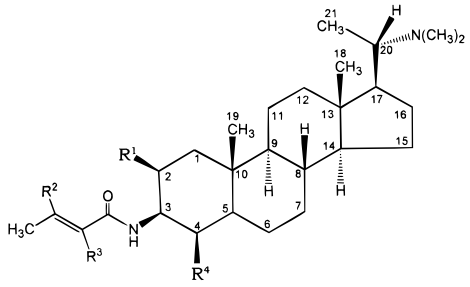


Figure 1. Selected NOESY interactions of salignarine A (**1**).

of **4** were determined on the basis of the NOESY spectrum (Figure 4). Hence, structure **4** was assigned to salignarineD.

Alkaloid **5** was obtained as a pale yellow gum. It displayed the molecular ion at *m*/*z* 426.3590, which corresponds to the molecular formula $C_{28}H_{46}N_2O$ (calcd 426.3609). Its IR spectrum showed absorptions at 3580, 1658, and 1618 cm^{-1} indicating the presence of NH, C═O, and C═C functional groups. The UV spectrum of **5** displayed end absorption only. The ^{1}H NMR spectrum showed two 3H singlets at δ 0.80 and 0.81, which were ascribed to the C-18 and C-19 angular methyl protons, respectively. A 3H doublet at δ 1.13 ($J_{21,20}$ = 6.5 Hz) was assigned to the H_3-21. A 6H singlet at δ 2.29 was due to the $N_b(CH_3)_2$ protons. Two downfield signals resonating at δ 1.73 (d, $J_{4',3'}$ = 6.7 Hz) and a singlet at δ 1.81 along with a 1H quartet at δ 6.38 ($J_{3',4'}$ = 6.7 Hz) indicated the presence of a tigloyl moiety. A 1H multiplet at δ 4.12 and a 1H doublet at δ 5.93 ($J_{4,3}$ = 6.5 Hz) were ascribed to the mutually coupled H-3 and H-4, respectively, since they showed strong cross-peaks in the COSY 45° spectrum.



1	R^1 = OH,	R^2 = H,	R^3 = CH_3,	R^4 = OAc , 5, 6-α-epoxide
2	R^1 = OH,	R^2 = H,	R^3 = CH_3,	R^4 = H, $\Delta^{5,6}$
3	R^1 = OH,	R^2 = CH_3,	R^3 = H,	R^4 = H, $\Delta^{5,6}$
4	R^1 = H,	R^2 = CH_3,	R^3 = H,	R^4 = H, $\Delta^{16,17}$
5	R^1 = H,	R^2 = H,	R^3 = CH_3,	R^4 = H, $\Delta^{4,5}$

The ^{13}C NMR spectra of **5** showed seven methyl, eight methylene, eight methine, and five quaternary carbon signals. The HMQC assignments are summarized in Table 3. The important HMBC correlations are shown in Table 2. The H-4′ (δ 1.73) and H-5′ (δ 1.81) protons exhibited HMBC correlations with C-3′ (δ 129.8) and C-1′ (δ 168.0), respectively, as encountered in salignarines A (**1**) and B (**2**). The presence of a C-4/C-5 double bond was confirmed by the HMBC correlation of H-4 (δ 5.93) with C-5 (δ 128.0) and C-10 (δ 36.3). The two angular methyl protons resonating at δ 0.80 (CH_3-18) and 0.81 (CH_3-19) exhibited correlations with quaternary carbons resonating at δ 36.3 (C-10) and 41.8 (C-13), respectively. The secondary methyl protons resonating at δ 1.13 (CH_3-21) showed a HMBC correlation with C-17 (δ 47.3). All important stereochemical

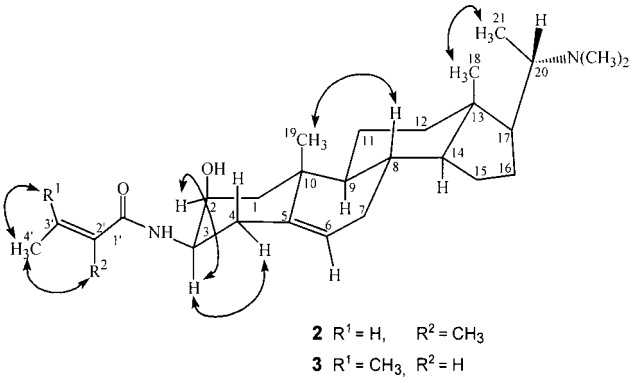


Figure 2. Selected NOESY interactions of salignarines B (**2**) and C (**3**).

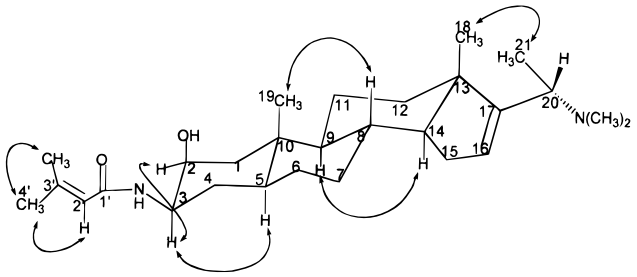


Figure 3. Selected NOESY interactions of salignarine D (**4**).

Table 3. ^{1}H and ^{13}C NMR Chemical Shift Assignments and Proton−Carbon One-Bond Connectivities (HMQC) of **4** and **5**[a]

	4		**5**	
position	δ_C	δ_H (J = Hz)	δ_C	δ_H (J = Hz)
1	33.0	1.30, 1.70	31.5	1.40, 1.90
2	33.3	1.55, 2.01	32.8	1.35, 1.60
3	44.3	4.07	44.6	4.12
4	31.9	1.40, 1.80	120.1	5.93 d (6.5)
5	44.2	2.09	128.0	
6	26.1	1.60, 1.75	33.3	1.45, 1.52
7	31.0	1.30, 1.65	31.7	1.70, 1.83
8	34.0	2.20	34.0	1.66
9	55.3	1.79	55.1	0.80
10	41.4		36.3	
11	20.6	1.35, 1.55	20.6	1.45, 1.50
12	34.7	1.10, 1.50	34.6	1.40, 1.43
13	46.7		41.8	
14	57.8	1.30	57.6	1.35
15	28.5	1.85, 2.00	25.9	1.59, 1.75
16	123.6	5.54 br s	28.3	1.90, 1.25
17	157.0		47.3	1.25
18	11.5	0.80	12.5	0.80 s
19	15.9	0.80	13.8	0.81 s
20	59.2	2.79	61.0	3.09
21	16.1	1.40 d (6.6)	11.5	1.13 d (6.5)
$N_b(CH_3)_2$	42.4	2.17 s	42.0	2.29 s
1′	166.2		168.0	
2′	119.1	5.48 br s	132.6	
3′	149.3		129.8	6.38 q (6.7)
4′	27.5	2.10 s	14.2	1.73 d (6.7)
5′	19.6	1.75 s	16.0	1.81 s

[a] $CDCl_3$, TMS, δ (ppm).

assignments of **5** were made on the basis of the NOESY spectrum (Figure 4) and were similar to those of compound **4**. These spectral studies supported structure **5** for salignarine E.

Experimental Section

General Experimental Procedures. The purity of the alkaloids was checked by TLC (Si gel G_{254} precoated plates), and the spots were detected under UV light at 250 and 336 nm and using Dragendorff's reagent. The melting points, which are uncorrected, were determined on a Büchi 510 melting point apparatus. The optical rotations were measured on a Polatronic D polarimeter. UV spectra were recorded on a Shimadzu 240 spectrophotometer, while a JASCO A-302 infrared spectrophotometer was used for recording the IR spectra. Bruker AM 400 or AMX 500 spectrometers were used for recording the ^{1}H NMR spectra at 400 or 500 MHz, while the AMX 500 NMR spectrometer was used for recording the ^{13}C NMR spectra at 125 MHz. The mass spectra were recorded on a Finnigan MAT 311A and Finnigan MAT 312 double-focusing mass spectrometers. HREIMS measurements were carried out by peak matching, using perfluorokerosine as an internal standard.

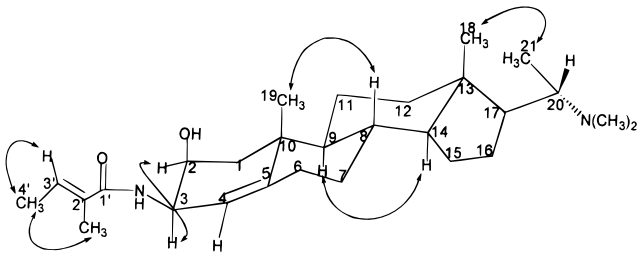


Figure 4. Selected NOESY interactions of salignarine E (**5**).

Plant Material. The plant material was collected in July 1995, from District Bagh of Azad Kashmir (Pakistan), and identified by Dr. Tahir Ali, Department of Botany, University of Karachi, Pakistan. A voucher specimen (KU # 19290) has been deposited in the Herbarium of the Department of Botany, University of Karachi.

Extraction and Isolation. The air-dried plant material (155 kg) was ground and soaked in EtOH−H_2O (8:2) (35 L) for 15 days. The extract was evaporated on a rotary evaporator to yield the crude gum (1.5 kg), which was dissolved in H_2O (2 L) and defatted with petroleum ether (10 L). The aqueous extract was acidified with 10% acetic acid to pH 3.5 and extracted with $CHCl_3$ (10 L) to yield the alkaloidal fraction (220 g). This was adsorbed on an equal quantity of Si gel and eluted with different gradients of petroleum ether, acetone, and methanol. Elution with pure acetone yielded a subfraction (5.5 g) that, on chromatography on Si gel and elution with petroleum ether−acetone (7:3), afforded a subfraction (73.7 mg) that was purified by preparative TLC on precoated Si gel (GF_{254}) plates using petroleum ether−acetone−diethylamine (10:9:1) as a mobile phase to afford salignarine E (**5**) (23 mg) as a pale yellowish gum. Elution with MeOH−acetone (1.1:9.1) afforded a fraction (7.8 g), which was dissolved in acetone and precipitated. The insoluble material was filtered, and the filtrate was subjected to column chromatography using petroleum ether−acetone−diethylamine (8.5:1.0:0.5) to yield salignarine D (**4**) (16.7 mg). Elution with MeOH−acetone (1:3) afforded a subfraction (5.2 g) that was chromatographed on a Si gel column and eluted with petroleum ether−$CHCl_3$−diethylamine (5.0:4.9:1) to obtain a subfraction (362 mg). This fraction was subjected to TLC on precoated Si gel (GF_{254}) plates using petroleum ether−acetone−diethylamine (14:5:1) to afford salignarine A (**1**) (10 mg), salignarine B (**2**) (31 mg), and salignarine C (**3**) (18 mg).

Salignarine A (1): 10 mg, 6.5×10^{-5} %; R_f 0.23; brown gum; $[\alpha]^{20}_D$−60 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 208 (2.4) nm; IR ($CHCl_3$) ν_{max} 3376 (OH), 1718 (C=O), 1652 (C=O), 1616 (C=C) cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; EIMS *m*/*z* 516 (14) [M$^+$], 501 (9), 427 (26), 100 (32), 83 (39), 72 (100), 55 (22); HREIMS *m*/*z* 516.3552 (calcd for $C_{30}H_{48}N_2O_5$).

Salignarin B (2): 31 mg, 2.02×10^{-4} %; R_f 0.39; colorless amorphous powder; $[\alpha]^{20}_D$ = +94 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 212 (1.8) nm; IR ($CHCl_3$) ν_{max} 3602 (NH), 3349 (OH), 1641 (C=O), 1611 (C=C) cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; EIMS *m*/*z* 442 (10) [M$^+$], 427 (86), 328 (12), 100 (73), 83 (99), 72 (100), 55 (44), HREIMS *m*/*z* 442.3529 (calcd for $C_{28}H_{46}N_2O_2$).

Salignarine C (3): 18 mg, 1.16×10^{-4} %; R_f 0.36; white powder; $[\alpha]^{20}_D$ −12 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 211 (1.9) nm; IR ($CHCl_3$) ν_{max} 3413 (NH), 1658 (C═O), 1627 (C═C) cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 1; EIMS *m/z* 442 (10), [M^+], 427 (100), 83 (33), 72 (29); HREIMS *m/z* 442.3566 (calcd for $C_{28}H_{46}N_2O_2$).

Salignarine D (4): 16 mg; 1.07×10^{-4}; R_f 0.61 colorless powder; $[\alpha]^{23}_D$ +66 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 210 (1.7) nm; IR ($CHCl_3$) ν_{max} 3351 (NH), 1656 (C═O), 1609 (C═C); ^1H NMR, see Table 3; ^{13}C NMR, see Table 3; EIMS *m/z* 426 (17) [M^+], 411 (92), 381 (9), 100 (24), 83 (30), 72 (52); HREIMS *m/z* 426.3607 (calcd for $C_{28}H_{46}N_2O$).

Salignarine E (5): 23 mg, 1.48×10^{-4}; R_f 0.60; brown gum; $[\alpha]^{25}_D$ +53 (*c* 6.13, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 204 (1.6) nm; IR ($CHCl_3$) ν_{max} 3580 (NH), 1658 (C═O), 1618 (C═C); ^1H NMR, see Table 3, ^{13}C NMR, see Table 3; EIMS *m/z* 426 (21) [M^+], 411 (90), 381 (13), 100 (100), 83 (32), 72 (87), 55 (43); HREIMS *m/z* 426.3590 (calcd for $C_{28}H_{46}N_2O$).

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