Antibacterial Steroidal Alkaloids from *Sarcococca saligna*

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Two new pregnane-type steroidal alkaloids, saligcinnamide [(20*S*,2′*E*)-20-(*N*,*N*-dimethylamino)-3β-(3′-phenyl-2′-propenyl-*N*-methylamido)pregnane] (**1**) and *N*$_a$-methyl epipachysamine-D [(20*S*)-20-(*N*,*N*-dimethylamino)-3β-(*N*-methylbenzamido)pregnane] (**2**), along with a known base, epipachysamine D [(20*S*)-20-(*N*,*N*-dimethylamino)-3β-(benzamido)pregnane] (**3**), were isolated from the EtOH extracts of the roots and stems of *Sarcococca saligna*. The new bases exhibited antibacterial activity against several human pathogenic bacteria. Two derivatives of **1**, dihydrosaligcinnamide [(20*S*)-20-(*N*,*N*-dimethylamino)-3β-(3′-phenylpropionoyl-*N*-methylamido)pregnane] (**4**) and dihydrosaligcinnamine [(20*S*)-20-(*N*,*N*-dimethylamino)-3β-*N*-(3′-phenylpropyl-*N*-methylamino)pregnane] (**5**), and a derivative of **2**, *N*$_a$-methyl epipachysamine [(20*S*)-20-(*N*,*N*-dimethylamino)-3β-(*N*-benzyl, *N*-methylamino)pregnane] (**6**) were prepared and their antibacterial activity determined.

The evergreen shrub *Sarcococca saligna* (D. Don) Muell. (*Euphorbiaceae*) is widely distributed throughout the northern areas of Pakistan and Azad Kashmir (altitude 5000–9000 ft).[1] Plants of the genus *Sarcococca* exhibit anticholinesterase, antitumor, antiulcer, and ganglion-blocking properties.[2] Because the plant is frequently used for the treatment of bacterial infections by local populations, phytochemical investigations of the plant were carried out to isolate new antimicrobial compounds, and this has already resulted in the isolation and identification of some antibacterial compounds.[3] A continuation of our work on the isolation of new antibacterial constituents of the plant has resulted in the isolation and characterization of two new antibacterial principles: saligcinnamide [(20*S*,2′*E*)-20-(*N*,*N*-dimethylamino)-3β-(3′-phenyl-2′-propenyl-*N*-methylamido)pregnane] (**1**) and *N*$_a$-methyl epipachysamine-D [(20*S*)-20-(*N*,*N*-dimethylamino)-3β-(*N*-methylbenzamido)pregnane] (**2**), along with a known base epipachysamine D [(20*S*)-20-(*N*,*N*-dimethylamino)-3β-(benzamido)pregnane][4] (**3**), which is reported for the first time from this plant. LiAlH$_4$ reduction of **1** yielded dihydrosaligcinnamine [(20*S*)-20-(*N*,*N*-dimethylamino)-3β-(3′-phenylpropionoyl-*N*-methylamido)pregnane] (**4**) and dihydrosaligcininamide [(20*S*)-20-(*N*,*N*-dimethylamino)-3β-*N*-(3′-phenylpropyl-*N*-methylamino)pregnane] (**5**). LiAlH$_4$ reduction of **2** afforded *N*$_a$-methyl epipachysamine [(20*S*)-20-(*N*,*N*-dimethylamino)-3β-(*N*-benzyl-*N*-methylamino)pregnane] (**6**). Various mass spectrometric techniques were used for elucidating the structures of the new compounds isolated or prepared in this study, and the chemical shift values of all protons were assigned.[5]

Results and Discussion

Compound **1**, which showed a positive alkaloidal test with Dragendorff's reagent, was isolated as a white amorphous powder. The IR spectrum of **1** displayed absorptions at 1610 (α,β-unsaturated amide), 1590, and 1425 cm⁻¹, which are characteristic for an aromatic ring.[6] The UV spectrum showed absorption maxima at 279 and 226 nm, characteristic for a cinnamide functionality.[7]

The EIMS of **1** showed a molecular ion at *m*/*z* 490, which was further confirmed by FABMS. The HREIMS of **1** exhibited the exact molecular mass at *m*/*z* 490.3922 corresponding to a molecular formula of C$_{33}$H$_{50}$N$_2$O showing 10 degrees of unsaturation. This was suggestive of the presence of unsaturated functionalities around the carbocyclic backbone of the molecule.

The ^{1}H-NMR spectrum of **1** showed two 3H singlets at δ 0.71 and 0.81, which were ascribed to two angular methyl groups. A 3H doublet resonating at δ 1.36 (d, $J_{20,21}$ = 6.6 Hz) was consistent with the presence of a secondary methyl group in the compound. Three downfield singlets integrating for 3H each resonated at δ 2.65, 2.85, and 2.97 and indicated the presence of three *N*-methyl groups. Two downfield olefinic protons resonated at δ 6.80 and 7.65 as doublets (*J* = 15.0 Hz), the coupling constant showing their trans relationship. Three aromatic multiplets at δ 7.50 (2H), 7.38 (2H), and 7.30 (1H) indicated the presence of a monosubstituted benzene ring in the molecule.

The ^{13}C-NMR spectrum (broad-band decoupled) of **1** displayed resonances for 33 carbons, while the DEPT spectrum revealed the presence of six methyl, nine methylene, 14 methine, and four quaternary carbons, in accordance with the molecular formula deduced from the HREIMS. The mass spectral fragmentation pattern of the compound (Figure 1) was very informative for determining the nature and position of the substituents. The presence of a *N*,*N*-dimethylaminoethyl substituent at C-17 and of *N*-methyl, and *N*-3-phenylprop-2-en-1-one unit at C-3 was deduced from the ions at *m*/*z* 72 225, 160, and 200, respectively (Figure 2).[8]

The direct ^{1}H–^{13}C connectivities were determined from the HMQC spectrum, while vicinal ^{1}H–^{1}H couplings were observed in the COSY 45° spectrum and
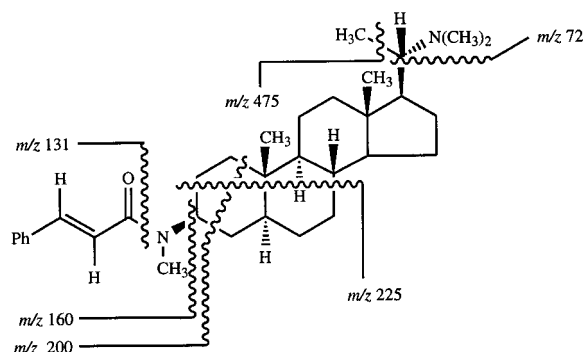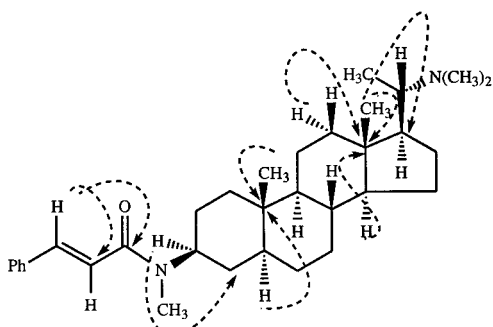
Figure 1. Mass spectral fragmentation of **1**.

Figure 2. Selected HMBC correlations for **1**.

used to assign the ¹H- and ¹³C-NMR chemical shift values to all protons (Table 1). HMBC and HOHAHA spectra were recorded to confirm the ¹H- and ¹³C-NMR chemical assignments and to elucidate the structure of the compound. The various HMBC interactions observed are shown in Figure 2. On this basis it was concluded that compound **1** bears a pregnane skeleton with *N,N*-dimethylaminoethyl and with *N*-methyl, and *N*-3-phenylprop-2-en-1-one substituents at C-17 and C-3, respectively.

Compound **2** was isolated as a colorless gum. It was found to have a structure similar to **1** with some differences in the functional groups. Characteristic absorptions for a benzamide carbonyl at 1665 cm⁻¹ and an aromatic ring at 1595 and 1450 cm⁻¹ were observed in the IR spectrum of **2**.[6] The UV spectrum showed absorption at 232 nm, which is characteristic for the aromatic amide functionalities.[7] The EIMS and FDMS of the compound showed the molecular ion peak at *m/z* 464, while the HREIMS showed the exact molecular mass at *m/z* 464.3760 for the molecular formula $C_{31}H_{48}N_2O$, indicating nine degrees of unsaturation.

The ¹H-NMR spectrum of **2** was similar to that of **1** and displayed two singlets integrating for 3H at δ 0.72 and 0.88, which were ascribed to the H-18 and H-19 angular methyls, respectively. A 3H doublet resonating at δ 1.05 (d, $J_{20,21} = 6.5$ Hz) was assigned to H-21. A 6H singlet at δ 2.86 was due to the $(CH_3)_2$-N_b protons, while a 3H singlet resonating at δ 2.36 was ascribed to the CH_3-N_a protons. Multiplets at δ 7.40−7.78 integrating for 5H indicated the presence of a monosubstituted benzene ring in the molecule.

The ¹³C-NMR spectrum (broad-band decoupled) of **2** displayed resonances for 31 carbons. The DEPT spectrum indicated that the compound contained six methyl, nine methylene, 12 methine, and four quaternary carbons, in conformity with the molecular formula. The

mass spectral fragmentation pattern of the compound was distinctively similar to that of **1** and revealed the presence of *N,N*-dimethylaminoethyl, *N*-methyl, and *N*-benzoyl substituents by the presence of ions at *m/z* 72 and at *m/z* 200, 174, 134, and 105 respectively (Figure 3). The ¹H- and ¹³C-NMR chemical shift values were assigned by the combined use of HMQC and COSY 45° spectra (Table 1) and were confirmed from the HMBC spectral data (Figure 4).

Compound **3** was identified as epipachysamine D by comparing its spectroscopic data with the literature values.[9] The assignments of ¹H- and ¹³C-NMR shifts to individual nuclei on the basis of multidimensional NMR spectra are presented here for the first time.

To study antimicrobial structure−activity relationships, LiAlH₄ reduction of **1** was carried out to afford (20*S*)-20-(*N,N*-dimethylamino)-3β-(3-phenylpropionoyl-*N*-methylamido)pregnane (**4**) and (20*S*)-20-(*N,N*-dimethylamino)-3β-(3-phenylpropyl-*N*-methylamino)pregnane (**5**). LiAlH₄ reduction of **2** yielded (20*S*)-20-(*N,N*-dimethylamino)-3β-(*N*-benzyl, *N*-methylamino)pregnane (**6**).
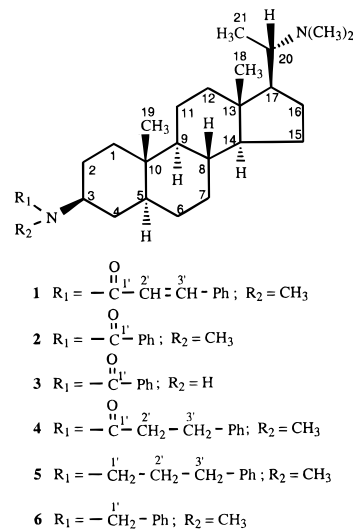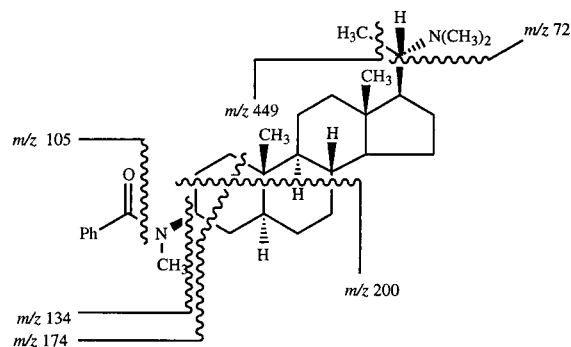

(20*S*)-20-(*N,N*-Dimethylamino)-3β-(3-phenylpropyl-*N*-methylamido)pregnane (**4**) was obtained in 39% yield from **1** and showed a molecular ion at *m/z* 492.4081 by HREIMS corresponding to the molecular formula $C_{33}H_{52}N_2O$. Olefinic absorption was missing in the IR spectrum of **4**, which was suggestive of the reduction of the olefinic bond. The ¹H-NMR spectrum of **4** displayed two 2H multiplets at δ 2.74 and 2.86 corresponding to H-2′ and H-3′, respectively, and no olefinic signals were observed. This was in agreement with the reduction of the C-2′/C-3′ double bond in **1**. (20*S*)-20-(*N,N*-Dimethylamino)-3β-*N*-(3-phenylpropyl-*N*-methylamino)-pregnane (**5**) was obtained in 33% yield in the same reduction reaction of **1**. The HREIMS of **5** displayed a molecular ion at *m/z* 478.4285, corresponding to the molecular formula $C_{33}H_{54}N_2$. The IR spectrum of **5** did not show any olefinic or ketonic absorptions. Its ¹H-NMR spectrum exhibited three 2H multiplets at δ 3.21, 3.37, and 3.84 for H-1′, H-2′, and H-3′, respectively, indicating the reduction of both keto and olefinic functionalities in **1**.

LiAlH₄ reduction of **2** afforded (20*S*)-20-(*N,N*-dimethylamino)-3β-(*N*-benzyl-*N*-methylamino)pregnane (**6**)

Table 1. ¹H- and ¹³C-NMR Chemical Shift Assignments of 1−3

carbon	1		2		3	
	δ^{13}C	δ_H (J = Hz)	δ^{13}C	δ_H (J = Hz)	δ^{13}C	δ_H (J = Hz)
1	39.7	1.24 m, 1.85 m	39.5	1.21 m, 1.84 m	35.5	1.34 m, 1.35 m
2	29.7	1.23 m, 1.25 m	28.7	1.31 m, 1.79 m	29.7	1.21 m, 1.55 m
3	52.9	4.59 m	52.1	3.88 m	49.4	3.58 m
4	37.4	1.10 m, 1.70 m	37.3	1.10 m, 1.55 m	37.4	1.10 m, 1.75 m
5	52.6	1.42 m	47.9	1.45 m	45.4	1.22 m
6	24.3	1.25 m, 1.75 m	24.1	1.25 m, 1.74 m	24.3	1.26 m, 1.75 m
7	27.5	1.89 m, 2.10 m	27.4	1.78 m, 1.19 m	28.9	1.28 m, 1.30 m
8	35.9	1.35 m	35.0	1.35 m	35.4	1.21 m
9	56.0	1.10 m	56.1	1.10 m	54.1	0.71 m
10	35.5		39.0		39.8	
11	21.0	1.53 m, 1.60	20.7	1.52 m, 1.60 m	21.1	1.19 m, 1.55 m
12	31.7	0.93, 1.68	31.2	0.91 m, 1.65 m	31.8	0.90 m, 1.65 m
13	42.0		42.0		42.9	
14	57.0	1.32 m	57.0	1.32 m	56.3	1.10 m
15	28.5	1.23, 1.25	28.2	1.09 m, 1.21 m	28.5	1.32 m, 1.80 m
16	24.7	1.55, 1.60	24.1	1.51 m, 1.63 m	27.5	2.09 m, 2.11 m
17	53.9	1.49 m	52.4	1.49 m	53.4	1.45 m
18	12.7	0.71 s	12.5	0.72 s	12.4	0.75 s
19	12.3	0.81 s	12.2	0.88 s	12.3	0.85 s
20	65.2	3.20 m	65.1	2.76 m	63.7	3.05 m
21	12.5	1.36 d (J = 6.6)	11.9	1.05 d (J = 6.5)	11.9	1.22 d (J = 6.5)
CH₃-N_b	43.5	2.85	45.2	2.85	39.8	2.65 s
CH₃-N_b	45.5	2.97	45.2	2.85	39.8	2.65 s
1′	118.3	6.80 d (J = 15.0)				
2′	118.6	7.65 d (J = 15.0)				
3′	142.3		135.1		135.1	
4′	127.8	7.50 m	126.8	7.78 m	126.8	7.82 m
5′	128.7	7.38 m	128.5	7.51 m	128.5	7.72 m
6′	129.4	7.30 m	131.2	7.40 m	131.2	7.41 m
7′	128.7	7.38 m	128.5	7.51 m	128.5	7.72 m
8′	127.8	7.50 m	126.8	7.78 m	126.8	7.82 m
CH₃-N_a	35.9	2.65 s	35.4	2.36 s		
−CO-N	166.0		166.5		166.7	

Figure 3. Mass spectral fragmentation of **2**.
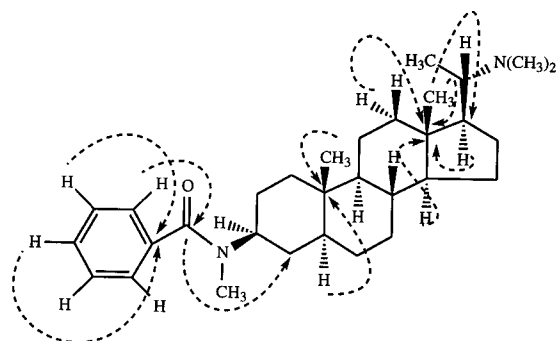
Figure 4. Selected HMBC correlations **2**.

in 85% yield. It showed a molecular ion at m/z 450.3974 corresponding to the molecular formula $C_{31}H_{50}N_2$. Its ¹H-NMR spectrum displayed a 2H diagnostic singlet at δ 2.18, which was assigned to H-1′.

Compounds **1** and **2** showed good antibacterial activity against seven human pathogenic bacteria, while **3−6** showed weak activity. It was therefore suspected that the 3β-substituent was an important functionality responsible for imparting antibacterial properties to bases **1** and **2**. A methyl group at the 3β-position in pregnane-type steroidal alkaloids appears to enhance the antibacterial activity (Table 2).

Experimental Section

General Experimental Procedures. The melting points were determined on a Buchi 510 melting point apparatus and are uncorrected. A Polatronic D polarimeter was used for measuring the optical rotations. The UV spectra were recorded on a Shimadzu 240 spectrophotometer, and IR spectra were recorded on a JASCO IRA-1 IR spectrophotometer. The ¹H-NMR spectra were recorded on Bruker spectrometers at 300, 400, or 500 MHz, while the ¹³C-NMR spectra were recorded on an AMX 500 NMR spectrometer at 125 MHz. The mass spectra were recorded on Finnigan MAT 112 and Finnigan MAT 312 double-focusing mass spectrometers. The HRMS measurements were carried out by peak matching, using perfluorokerosine as an internal standard. Flash chromatography on Si gel was used for preliminary column chromatography. The purity of the samples was checked by TLC (Si gel G₂₅₄ precoated plates). TLC spots were detected under UV light at 250 and 336 nm. Iodine vapors were used to detect the alkaloids, and they also gave an orange coloration with Dragendorff's spray reagent.

Table 2. Antibacterial Activity of Compounds 1–6 Against Some Pathogenic Bacteria

	zones of inhibition (mm)							
organism	ampicillin	amoxacillin	**1**	**2**	**3**	**4**	**5**	**6**
Klebsiella pneumoniae	17	17	9	8		10.5		6.0
Proteus mirabilis	17	16	7	8				
Pseudomonas aeruginosa	15	15	6	6		7.5	5.5	5.5
Staphylococcus aureus	18	16	12	12	7	11		9.0
Streptococcus pyogenes	17	16	8	6				
Salmonella typhii	12	12	6	6				
Shigella boydii	17	17	6	6				

Plant Material. The plant material was collected from the District Bagh of Azad Kashmir in July 1995, and was identified by Dr. Tahir Ali, taxonomist, 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 roots and stems of *S. saligna* (8 kg) were air-dried and ground followed by soaking in EtOH–H$_2$O (8:2) (20 L) for 15 days. The gum (780 g), which was obtained after evaporating the solvent on a rotary evaporator, was dissolved in H$_2$O (2 L) and extracted with petroleum ether (6 L) and CHCl$_3$ (8 L), respectively. The crude alkaloidal fraction (55 g) obtained on removal of CHCl$_3$ *in vacuo* was adsorbed on an equal quantity of Si gel and purified on a column prepacked with Si gel (230–240 mesh, 1.5 kg). Elution was carried out with increasing polarties of petroleum ether–CHCl$_3$ and CHCl$_3$–MeOH. The fraction eluted with CHCl$_3$–MeOH (8:2) (2.0 g) was rechromatographed under vacuum using Si gel (type 60, PF$_{254}$ art. 7749 Merck), with elution by EtOAc–petroleum ether–diethylamine (1:8.8:0.2) to afford an alkaloidal subfraction (500 mg). This fraction was subjected to preparative TLC using Me$_2$CO–petroleum ether–diethylamine (0.5:9.3:0.2) as eluent to yield (20*S*,2′*E*)-20-(*N*,*N*-dimethylamino)-3β-(3′-phenyl-2′-propenyl-*N*-methylamido)pregnane (**1**), (20*S*)-20-(*N*,*N*-dimethylamino)-3β-(*N*-methylbenzamido)pregnane (**2**), and (20*S*)-20-(*N*,*N*-dimethylamino)-3β-(benzamido)pregnane (**3**).

(20*S*,2′*E*)-20-(*N*,*N*-Dimethylamino)-3β-(3′-phenyl-2′-propenyl *N*-methylamido)pregnane (1): (60 mg 7.5 × 10^{-4} %; R_f = 0.41) white powder; mp 156–160 °C; [α]$^{25}_D$ 96° (*c* 0.02, CHCl$_3$); UV (MeOH) λ$_{max}$ (log ε) 279 (3.78) nm; IR (KBr) ν$_{max}$ 2910, 1610 (C=O), 1538, 1425, 1405, 1315 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; EIMS *m*/*z* 490 (20) [M]$^+$, 475 (21) [M$^+$ − CH$_3$], 445 (11), 419 (42), 404 (29), 160 (20), 131 (98), 72 (100); FABMS *m*/*z* 491 [M + H]$^+$; HREIMS *m*/*z* 490.3922 (calcd for C$_{33}$H$_{50}$N$_2$O, 490.3923).

(20*S*)-20-(*N*,*N*-Dimethylamino)-3β-(*N*-methylbenzamido)pregnane (2): (4.5 mg, 5.6 × 10^{-5} %); R_f = 0.43; [α]$^{25}_D$ 66° (*c* 0.04, CHCl$_3$); UV (MeOH) λ$_{max}$ (log ε): 232 (1.62) nm; IR (CHCl$_3$) ν$_{max}$ 2900, 1665, 1595, 1450 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; EIMS *m*/*z* 464 (7) [M$^+$], 449 (12) [M − 15]$^+$, 419 (3), 360 (5), 200 (2), 174 (3), 134 (3), 105 (90), 72 (100), 58 (19); FABMS *m*/*z* 465 [M + H]$^+$; FABMS (positive) *m*/*z* 465; HREIMS *m*/*z* 464.3760 (calcd for C$_{31}$H$_{48}$N$_2$O, 464.3766).

(20*S*)-20-(*N*,*N*-Dimethylamino)-3β-(benzamido)pregnane (epipachysamine D) (3): (25 mg, 3.1 × 10^{-4} %); R_f = 0.7; mp 245–248 °C; [α]$^{25}_D$ 24.4° (*c* 0.02, CHCl$_3$); UV (MeOH) λ$_{max}$ (log ε) 223 (5.08) nm; IR

(CHCl$_3$) ν$_{max}$ 3490, 2903, 1652, 1546, 1510 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; EIMS *m*/*z* 450 (10) [M$^+$], 435 (17) [M − 15]$^+$, 363 (2), 133 (7), 122 (46), 105 (100), 84 (27), 72 (100); FDMS *m*/*z* 450; HREIMS *m*/*z* 450.3572 (calcd for C$_{30}$H$_{46}$N$_2$O, 450.3573).

LiAlH$_4$ Reduction of Saligcinnamide (1). LiAlH$_4$ (25 mg) was added in a three-necked round-bottomed flask containing Et$_2$O (5 mL) and stirred, followed by addition of a solution of 10 mg (2.04 × 10^{-2} mmol) of **1** in Et$_2$O (5 mL) over a period of 10 min with continuous stirring. The reaction mixture was refluxed for 1 h. Excess LiAlH$_4$ was destroyed by adding a saturated aqueous solution of sodium sulfate (30 mL), and the solution was filtered. The crude products were recovered by extracting with Et$_2$O (30 mL). The organic extract was dried over anhydrous Na$_2$SO$_4$ (3 g) and the solvent removed under vacuum to afford the crude product mixture (9 mg), which was purified on precoated Si gel TLC plates using Me$_2$CO–petroleum ether (0.4:9.6) to yield compounds **4** (R_f = 0.77) and **5** (R_f = 0.95).

(20*S*)-20-(*N*,*N*-Dimethylamino)-3β-(3′-phenylpropan-*N*-methylamido)pregnane (4): amorphous powder (3.5 mg, 39%); [α]$^{25}_D$ 100° (*c* 0.012, CHCl$_3$); IR (CHCl$_3$) ν$_{max}$ 2900, 1595, 1453 cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz) δ 0.71 (3H, s, CH$_3$-18), 0.81 (3H, s, CH$_3$-19), 1.32 (3H, d, $J_{21,20}$ = 6.6 Hz, CH$_3$-21), 2.66 (6H, s, CH$_3$-N$_b$), 2.74 (2H, m, CH$_2$-2′), 2.86 (2H, m, CH$_2$-3′), 2.95 (3H, s, CH$_3$-N$_a$), 3.20 (1H, m, H-20), 7.17–7.33 (5H, m, ArH); EIMS *m*/*z* 492 (5) [M$^+$], 477 (9) [M − 15]$^+$, 419 (2), 360 (6), 198 (2), 174 (3), 134 (3), 72 (100); FABMS *m*/*z* 493 [M + H]$^+$; HREIMS *m*/*z* 492.4081 (calcd for C$_{33}$H$_{52}$N$_2$O, 492.4079).

(20*S*)-20-(*N*,*N*-Dimethylamino)-3β-*N*-(3′-phenylpropan, *N*-methylamino)pregnane (5): amorphous powder (3 mg, 33%); [α]$^{25}_D$ 91° (*c* 0.003, CHCl$_3$); IR (CHCl$_3$) ν$_{max}$ 2910, 1704, 1645, 1592, 1520 cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz) 0.69 (3H, s, CH$_3$-18), 0.77 (3H, s, CH$_3$-19), 1.49 (3H, d, $J_{21,20}$ = 6.5 Hz, CH$_3$-21), 2.86 (3H, s, CH$_3$-N$_a$), 3.02 (6H, s, CH$_3$-N$_b$), 3.04 (2H, m, CH$_2$-1′), 3.37 (2H, m, H-2′), 3.48 (2H, m, CH$_3$-3′), 3.15 (1H, m, H-20), 7.38–7.47 (5H, m, Ph); EIMS *m*/*z* 478 (79) [M$^+$], 459 (100) [M − 15]$^+$, 431 (20), 361 (6); FABMS *m*/*z* 479 [M + H]$^+$; HREIMS *m*/*z* 478.4286 (calcd for C$_{33}$H$_{54}$N$_2$, 478.4287).

LiAlH$_4$ Reduction of *N*$_a$-Methylsaligbenzamide (2). A similar procedure was applied as used for the reduction of **1**. The crude reduction product obtained was purified by preparative TLC using Me$_2$CO–petroleum ether (0.4:9.6) to afford (20*S*)-20-(*N*,*N*-dimethylamino)-3β-(*N*-benzyl, *N*-methylamino)pregnane **6** (2 mg, 85%); R_f 0.75 [α]$^{25}_D$ 166° (*c* 0.012, CHCl$_3$); IR (CHCl$_3$) ν$_{max}$ 2902, 1597, 1448 cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz) δ 0.68 (3H, s, CH$_3$-18), 0.81 (3H, s, CH$_3$-19),

1.25 (3H, d, $J_{21,20}$ = 6.5 Hz, CH_3-21), 2.18 (2H, s, CH_2-1'), 2.47 (3H, s, CH_3-N_a), 2.66 (6H, s, CH_3-N_b), 3.15 (1H, m, H-20), 7.28−7.58 (5H, m, Ar−H); EIMS m/z 450 (5) [M^+], 435 (4) [M − 15]$^+$, 379 (14), 186 (11), 160 (36), 91 (30), 72 (100); FDMS m/z 450; HREIMS m/z 450.3975 (calcd for $C_{31}H_{50}N_2$, 450.3974).

Antibacterial Assay. The antibacterial activity of compounds **1**−**6** was evaluated by the agar well diffusion method.[10] Loopfuls of 24-h-old cultures containing approximately 10^4−10^6 CFU were spread over the surface of Mueller−Hinten agar plates, and wells were made in the media with the help of a sterile metallic borer. Test samples and standard antibiotics at dilutions of 200 μg/100 μL were added in the wells, and the plates were incubated at 37 °C for a period of 24 h after which zones of inhibition were measured (Table **2**).

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