New Cholinesterase-Inhibiting Steroidal Alkaloids from *Sarcococca saligna*

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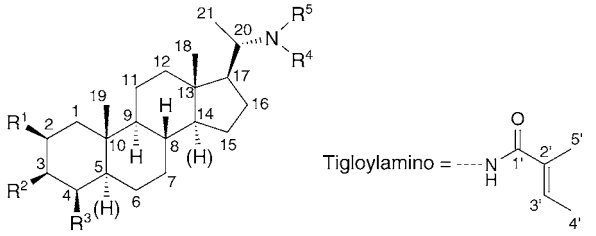
Seven new steroidal alkaloids, 2-hydroxysalignarine-E (=(2′*E*,20*S*)-20-(dimethylamino)-2*β*-hydroxy-3*β*-(tigloylamino)pregn-4-ene; **1**), 5,6-dihydrosarconidine (=(20*S*)-20-(dimethylamino)-3*β*-(methylamino)-5*α*-pregn-16-ene; **2**), salignamine (=(20*S*)-20-(methylamino)-3*β*-methoxypregna-5,16-diene; **3**), 2-hydroxysalignamine (=(20*S*)-20-(dimethylamino)-2*β*-hydroxy-3*β*-methoxypregna-5,16-diene; **4**), salignarine-F (=(2′*E*, 20*S*)-20-(dimethylamino)-4*β*-hydroxy-3*β*-(tigloylamino)pregn-5-ene; **5**), salonine-C (=(2′*E*,20*S*)-20-(dimethylamino)-3*β*-(tigloylamino)pregna-4,14-diene; **6**), and *N*-[formyl(methyl)amino]salonine-B (=(20*S*)-20-[formyl-(methyl)amino]-3*β*-methoxypregna-5,16-diene; **7**) have been isolated from the MeOH extract of *Sarcococca saligna*, along with the six known alkaloids dictyophlebine (**8**), epipachysamine-D (**9**), saracosine (**10**), iso-*N*-formylchonemorphine (**11**), sarcodinine (**12**), and alkaloid-C (**13**). The structures of **1**–**7** were deduced from spectral data. Compounds **1**–**13** demonstrated significant activity against acetyl- and butyrylcholinesterase.

Introduction. – *Alzheimer*'s disease is the fourth leading cause of death in people over 65 years of age in industrialized countries [1]. A number of studies have identified a link between the onset of the disease and the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). As part of our efforts to identify new natural cholinesterase inhibitors, the whole plant of *Sarcococca saligna* was investigated, as its crude alkaloidal fraction showed significant activity in an *in vitro* high-throughput cholinesterase-inhibition assay.

The genus *Sarcococca* belongs to the Buxaceae family, with 16–20 species. It is predominantly distributed in South Asia, extending from Afghanistan through the Himalayas [2]. Some alkaloids of *Sarcococca* exhibit anticholinesterase and ganglion-blocking activities, while others potentiate the action of naturally secreted acetylcholine in the isolated diaphragm of rats and in the serum of rabbits [3]. Previous studies on this genus have led to the isolation of a number of pregnane-type steroidal alkaloids [4–15], including fifteen new cholinesterase-inhibiting alkaloids from our laboratory [15]. Here, we report the structures of the new compounds **1**–**7** and those of the known constituents **8**–**13** isolated from *Sarcococca saligna*.

Results and Discussion. – 2-Hydroxysalignarine-E (**1**), a colorless amorphous compound, showed a molecular ion (M^+) at *m*/*z* 442.3489 ($C_{28}H_{46}N_2O_2$; calc.: 442.3559) corresponding to seven degrees of unsaturation. Comparison of its IR, ^{1}H-NMR, and ^{13}C-NMR data (*Tables 1* and *2*) with those of the known compound, salignenamide-B [11], suggested a pregnane-type steroidal skeleton, lacking one C=C bond. The presence of a tigloyl (*E*)-2-methylbut-2-enoyl) moiety in **1** (δ_H 6.51, 1.77, 1.88; δ_C 130.3,



	R^1	R^2	R^3	R^4	R^5	Unsaturation
1	OH	tigloylamino	H	Me	Me	$\Delta^{4,5}$
2	H	MeHN	H	Me	Me	$\Delta^{16,17}$
3	H	MeO	H	Me	H	$\Delta^{5,6}$, $\Delta^{16,17}$
4	OH	MeO	H	Me	Me	$\Delta^{5,6}$, $\Delta^{16,17}$
5	H	tigloylamino	OH	Me	Me	$\Delta^{5,6}$
6	H	tigloylamino	H	Me	Me	$\Delta^{4,5}$, $\Delta^{14,15}$
7	H	MeO	H	HCO	Me	$\Delta^{5,6}$, $\Delta^{16,17}$
8	H	MeHN	H	Me	Me	–
9	H	PhCONH	H	Me	Me	–
10	H	Me_2N	H	Ac	Me	$\Delta^{5,6}$
11	H	Me_2N	H	H	HCO	–
12	H	Me_2N	H	Me	Me	$\Delta^{5,6}$
13	H	MeO	H	Me	Me	$\Delta^{5,6}$

Fig. 1. *Structures of compounds* **1**–**13** *isolated from* Sarcococca saligna. Note that C=C bonds (positions) are not explicitly shown in the steroidal framework, but indicated by $\Delta^{m,n}$. The tigloyl (=2-methylbut-2-enoyl) residue is numbered arbitrarily

13.9, 12.5) was consistent with literature values. In its ^{1}H-NMR spectrum, signals for two Me groups at quaternary (δ_H 0.84, 1.27), and one Me group at a secondary C-atom(s) (δ_H 1.07), a methine H-atom (δ_H 3.91) next to an OH group, and an olefinic H-atom (δ_H 5.51) were also clearly observed. In the COSY spectrum, the signals of both H–C(2) (δ_H 3.91) and H–C(4) (δ_H 5.51) were found to be coupled with H–C(3) (δ_H 4.19). The H–C(3) methine atom also showed a COSY cross-peak with the NH *d* (δ_H 6.70). The configuration at C(2) was inferred on the basis of the coupling constant ($W_{1/2}$ = 5.6 Hz) and the ROESY interactions (*Fig. 2*) between H–C(3) and H–C(2), suggesting the β-orientation of the C(3) tigloylamino and C(2)–OH groups. Thus, the structure of **1** was assigned as (2′*E*,20*S*)-20-(dimethylamino)-2β-hydroxy-3β-(tigloylamino)pregn-4-ene.

5,6-Dihydrosarconidine (**2**) was isolated as a white powder. HR-EI-MS Analysis showed the M^+ signal at *m*/*z* 358.3193 ($C_{24}H_{42}N_2^+$; calc.: 358.3198), with five degrees of unsaturation. In its ^{1}H-NMR spectrum, two angular Me groups appeared at δ_H 0.84 (Me(18)) and 0.87 (Me(19)) (*Fig. 3*). A *d* at δ_H (1.13 (J = 6.5 Hz) was assigned to Me(21), while two downfield *s* at δ_H 2.14 and 2.23 were assigned to HN*Me* and NMe_2, respectively. A *q* at δ_H 2.68 was assigned to H–C(20), indicating the presence of a C=C bond in ring D between C(16) and C(17). The base peak at *m*/*z* 343 was due to [*M* – Me]$^+$, and the lower abundance of the ion at *m*/*z* 72 (which is normally a base peak in such alkaloids) indicated a C=C bond between C(16) and C(17). Comparison of the

Table 1. *^{1}H-NMR Data of the New Steroidal Alkaloids* **1**–**7** (δ_H in ppm, J in Hz; solvent: $CDCl_3$)

	1	**2**	**3**	**4**	**5**	**6**	**7**
H–C(1)	1.15, 1.85 (*m*)	1.90, 1.35 (*m*)	1.35, 2.20 (*m*)	1.15,2.20 (*m*)	1.85, 1.23 (*m*)	1.69, 1.15 (*m*)	1.35, 1.90 (*m*)
H–C(2)	3.91 (*m*, $W_{1/2}=5.6$)	1.80, 1.75 (*m*)	1.80, 1.75 (*m*)	4.14 (br. *s*)	1.6, 1.21 (*m*)	2.6, 1.94 (*m*)	1.75, 1.80 (*m*)
H–C(3)	4.19 (*m*)	2.68 (*m*)	3.04 (*m*)	3.11 (br. *s*)	3.95 (br. *s*)	4.15 (*m*)	3.03 (*m*, $W_{1/2}=17.7$)
H–C(4)	5.51 (br. *s*)	2.30, 2.60 (*m*)	2.11, 2.55 (*m*)	2.15, 2.55 (*m*)	3.99 (br. *s*)	5.78 (br. *s*)	2.14, 2.39 (*m*)
H–C(5)	–	1.65	–	–	–	–	–
H–C(6)	1.65, 2.11 (*m*)	1.32, 1.75 (*m*)	5.36 (br. *s*)	5.39 (br. *s*)	5.78 (br. *s*)	1.49, 2.15 (*m*)	5.34 (br. *s*)
H–C(7)	1.32, 1.84 (*m*)	2.00, 2.61 (*m*)	2.00, 2.60 (*m*)	2.00, 2.60 (*m*)	2.05, 2.5 (*m*)	1.88, 1.34 (*m*)	2.30, 1.5 (*m*)
H–C(8)	1.82	2.40	2.38	1.65	1.55	1.55	1.45
H–C(9)	0.75	1.38	1.27	1.00	1.23	0.88	1.35
H–C(10)	–	–	–	–	–	–	–
H–C(11)	1.61, 1.42 (*m*)	1.79, 1.99 (*m*)	1.20, 1.60 (*m*)	1.20, 1.60 (*m*)	2.13, 1.41 (*m*)	2.12, 1.39 (*m*)	1.7, 1.99 (*m*)
H–C(12)	1.36, 1.84 (*m*)	1.58, 1.50 (*m*)	1.40, 1.70 (*m*)	1.40, 1.70 (*m*)	1.52, 1.20 (*m*)	1.35, 1.23 (*m*)	1.53, 1.22 (*m*)
H–C(13)	–	–	–	–	–	–	–
H–C(14)	1.36	2.45	2.14	1.35	1.32	–	1.75
H–C(15)	2.01, 1.88 (*m*)	1.85, 2.06 (*m*)	1.65, 2.10 (*m*)	1.70, 2.10 (*m*)	1.7, 1.92 (*m*)	5.41 (br. *s*)	1.6, 1.20 (*m*)
H–C(16)	1.59, 1.61 (*m*)	5.55 (br. *s*)	5.55 (br. *s*)	5.64 (br. *s*)	1.63, 1.73 (*m*)	2.06, 1.65 (*m*)	5.78 (br. *s*)
H–C(17)	1.25	–	–	–	1.2	1.1	–
H–C(18)	0.84 (*s*)	0.84 (*s*)	0.84 (*s*)	0.86 (*s*)	0.82 (*s*)	0.86 (*s*)	0.75 (*s*)
H–C(19)	1.27 (*s*)	0.87 (*s*)	1.03 (*s*)	1.21 (*s*)	1.04 (*s*)	1.06 (*s*)	1.01 (*s*)
H–C(20)	2.80 (*m*)	2.68 (*q*, $J=6.6$)	3.08 (*d*, $J=6.6$)	2.90 (*q*, $J=6.6$)	2.80 (*s*)	2.82 (*m*)	4.19 (*m*)
H–C(21)	1.07 (*d*, $J=6.6$)	1.13 (*d*, $J=6.5$)	1.17 (*d*, $J=6.5$)	1.14 (*d*, $J=6.5$)	1.38 (*d*, $J=6.5$)	1.73 (*d*, $J=6.5$)	1.3 (*d*, $J=6.5$)
C(3)–NMe	–	2.14 (*s*)	–	–	–	–	–
C(20)–NMe	2.20 (*s*)	2.23 (*s*)	2.33 (*s*)	2.28 (*s*)	2.23 (*s*)	2.91 (*s*)	2.64/2.72[a])
H–C(1)	–	–	–	–	–	–	–
H–C(2′)	–	–	–	–	–	–	–
H–C(3′)	6.51 (*q*, $J=6.9$)	–	–	–	6.34 (*q*, $J=6.9$)	6.34 (*q*, $J=6.9$)	–
H–C(4′)	1.77 (*d*, $J=6.9$)	–	–	–	1.73 (*d*, $J=6.9$)	1.78 (*d*, $J=6.9$)	–
H–C(5′)	1.88 (*s*)	–	–	–	1.83 (*s*)	1.82 (*s*)	–
HCO	–	–	–	–	–	–	8.02/8.19[a])
MeO	–	–	3.34 (*s*)	3.38 (*s*)	–	–	3.33 (*s*)

[a]) Signal splitting due to rotational isomerism of the amido group.

Table 2. *^{13}C-NMR Data of the New Steroidal Alkaloids* **1**–**7** (δ_C in ppm; solvent: $CDCl_3$)

	1	**2**	**3**	**4**	**5**	**6**	**7**
C(1)	33.3	34.3	42.4	42.4	34.5	34.5	34.3
C(2)	69.6	30.5	29.6	67.0	36.5	37.4	29.6
C(3)	50.7	53.4	79.2	81.2	45.2	45.5	81.8
C(4)	115.3	35.0	32.3	32.4	68.8	126.4	32.5
C(5)	151.8	42.5	139.0	141.0	140.5	149.4	141.1
C(6)	41.8	27.6	121.2	121.2	129.1	30.3	121.3
C(7)	34.4	32.7	31.8	31.8	30.2	31.6	33.3
C(8)	32.8	34.1	33.1	30.0	35.5	33.5	32.3
C(9)	54.5	55.7	53.7	51.4	54.5	57.3	49.2
C(10)	38.1	35.4	35.5	37.0	39.5	39.5	35.5
C(11)	21.3	20.4	20.6	20.6	20.8	20.4	20.9
C(12)	34.4	31.9	34.6	34.6	31.8	34.3	31.9
C(13)	46.8	46.5	45.3	47.3	45.6	46.8	47.5
C(14)	56.9	57.2	54.7	56.7	55.5	154.6	56.4
C(15)	31.2	31.2	30.2	31.3	34.4	123.3	31.2
C(16)	118.7	123.3	130.3	130.3	32.7	31.3	125.8
C(17)	151.8	156.0	142.3	149.3	51.5	51.5	153.4
C(18)	15.9	12.7	19.7	19.7	15.8	15.9	18.3
C(19)	19.4	16.9	20.2	20.2	18.7	18.8	19.2
C(20)	59.2	59.3	61.7	61.7	57.9	59.5	57.3
C(21)	16.0	15.8	14.0	16.0	19.3	19.7	15.3
C(3)–N*Me*	–	30.3	–	–	–	–	–
C(20)–N*Me*	42.3	42.3	42.3	42.3	42.5	42.5	22.3/21.9[a])
C(1′)	169.0	–	–	–	168.3	168.3	–
C(2′)	132.1	–	–	–	131.3	131.5	–
C(3′)	130.3	–	–	–	130.8	130.3	–
C(4′)	13.9	–	–	–	11.5	12.3	–
C(5′)	12.5	–	–	–	13.1	13.9	–
MeO	–	–	55.2	55.9	–	–	55.5
HCO	–	–	–	–	–	–	168.6

[a]) Signal splitting due to rotational isomerism of the amido group.

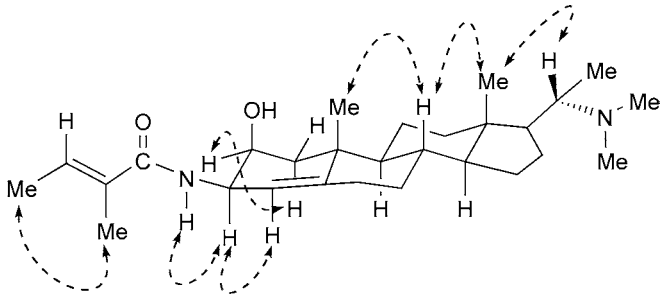


Fig. 2. *Selected ROESY interactions in 2-hydroxysalignarine-E* (**1**)

UV, IR, and ^{1}H-NMR data of **2** with those of the known alkaloid sarconidine [4] indicated close structural similarity, except for the lack of the C(4)=C(5) bond in **2**. Thus, the structure of compound **2** was assigned as (20*S*)-20-(dimethylamino)-3β-(methylamino)-5α-pregn-16-ene.

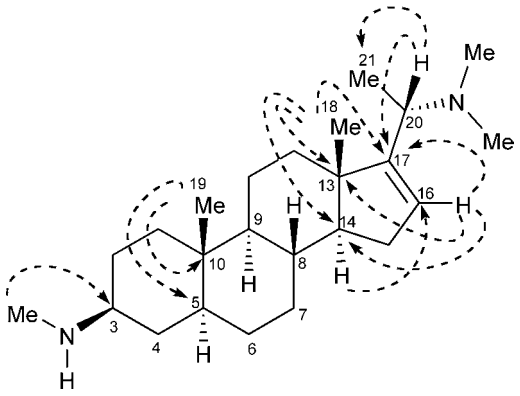


Fig. 3. *Selected HMBC correlations in 5,6-dihydrosarconidine* (**2**)

Salignamine (**3**) has a molecular formula of $C_{23}H_{37}NO$, as deduced by HR-EI-MS (*m*/*z* 343.2880 for M^+). The ^{1}H- and ^{13}C-NMR spectra of **3** (*Tables 1* and *2*) were comparable to those of the known alkaloid-C (**13**), which was also isolated during this study, with an additional olefinic H-atom at δ_H 5.55, and a downfield δ-shifted Me signal at δ_H 2.33 in **3**. The EI mass spectrum of **3** indicated that the extra double bond was between C(16) and C(17) (lower abundance of $H_3C-HC=N^+HMe$ at *m*/*z* 58, generally a base peak in HNMe-containing steroidal alkaloids with a saturated ring D [17]). The MeO group in **3** was placed at C(3) based on comparison of its ^{1}H- and ^{13}C-NMR data with those of alkaloid-C (**13**) [5]. This was further supported by a HMBC experiment (*Fig. 4*), in which a correlation was observed between H–C(3) (δ_H 3.04) and C(4) (δ_C 32.3), which, in turn, were coupled with C(5) (δ_C 139.0). Consequently compound **3** was deduced as (20*S*)-20-(methylamino)-3β-methoxypregna-5,16-diene.

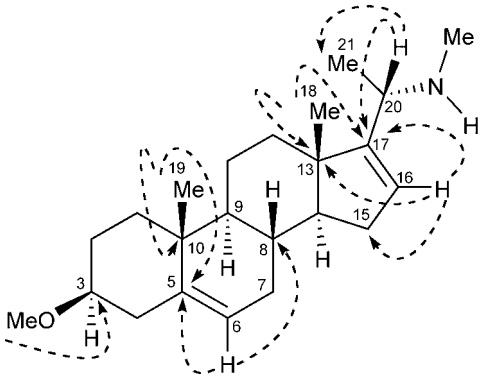


Fig. 4. *Selected HMBC correlations in salignamine* (**3**)

2-Hydroxysalignamine (**4**) had a molecular formula of $C_{24}H_{39}NO_2$ (*m*/*z* 373.2982; calc.: 373.2980), and its mass-fragmentation pattern, IR, and ^{1}H- and ^{13}C-NMR spectra (*Tables 1* and *2*) were distinctly similar to those of compound **13**, with an additional OH group (IR: 3425 cm^{-1}). The location of the C(2)–OH group in **4** was assigned by a

HMBC experiment, in which correlations were observed between H–C(2) (δ_H 4.14) and C(3) (δ_C 81.2), and between H–C(3) (δ_H 3.11) and C(5) (δ_C 141.0). The configuration at C(2) was assigned as β on the basis of the coupling constant ($W_{1/2}$ = 5.5 Hz) and ROESY interaction (*Fig. 5*) between H–C(1a) and H–C(2), indicating that both were α-oriented. Thus, compound **4** was assigned as (2′*E*,20*S*)-20-(dimethylamino)-2β-hydroxy-3β-methoxypregna-5,16-diene.

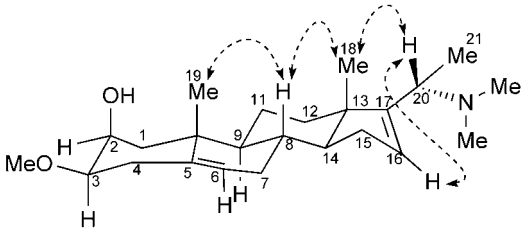


Fig. 5. *Selected ROESY interactions in 2-hydroxysalignamine* (**4**)

Salignarine-F (**5**) was obtained as a yellow gum. Its HR-EI mass spectrum displayed M^+ at 442.3577 ($C_{28}H_{46}N_2O_2^+$; calc.: 442.3559), with seven degrees of unsaturation. The ^{1}H- and ^{13}C-NMR spectra were distinctly similar to those of compound **1**, except for a double bond between C(5) and C(6). Two broad *s* at δ_H 3.95 and 3.99 were ascribed to H–C(3) and H–C(4), respectively. The ^{13}C-NMR DEPT spectrum displayed resonances for 28 C-atoms, with seven Me, seven CH_2, nine CH and five quaternary C-atoms. The presence of a C(5)=C(6) bond was also inferred from the HMBC interactions of H–C(6) (δ_H 5.78) with C(10) (δ_C 39.5), and of C(5) (δ_C 140.5) with H–C(19) (δ_H 1.04) (*Fig. 6*). The β-orientation of C(4)–OH was inferred from the $W_{1/2}$ value of H–C(4) (δ_H 3.99, $W_{1/2}$ = 5.5 Hz). Finally, the structure of **5** was deduced as (2′*E*,20*S*)-20-(dimethylamino)-4β-hydroxy-3β-(tigloylamino)pregn-5-ene.

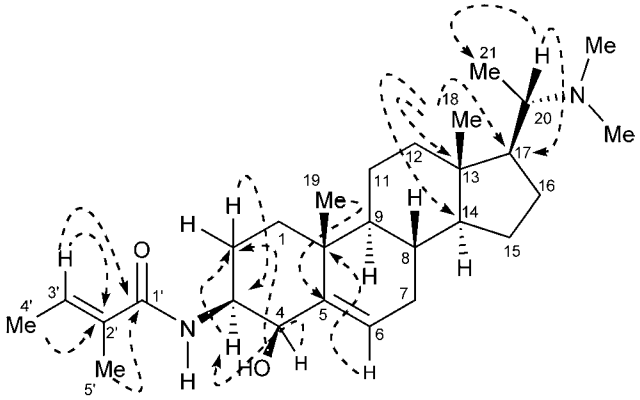


Fig. 6. *Selected HMBC interactions in salignarine-F* (**5**)

Salonine-C (**6**), $C_{28}H_{44}N_2O$, was obtained as a white amorphous solid. Its ^{1}H-NMR spectrum revealed the presence of 3 *s* at δ_H 0.86, 1.06, and 1.82, assigned to Me(18), Me(19), and Me(5′), respectively. Me(21) and Me(4′) resonated at δ_H 1.73 (*d*, *J* =

6.5 Hz) and 1.78 ($d, J = 6.9$ Hz), respectively, while the Me_2N group resonated at δ_H 2.91 (s). The ^{13}C-NMR spectra displayed resonances for 28 C-atoms, with seven Me, six CH_2, eight CH, and seven quaternary C-atoms. In the HMBC spectrum, the olefinic H-atom (δ_H 5.78) showed an interaction with C(3) (δ_H 45.5), which indicated a C(4)=C(5) bond. Another downfield ^{1}H-NMR signal at δ_H 5.41 showed HMBC interactions with C(16) (δ_C 31.3) and C(17) (δ_C 51.5), and the quaternary C(14)-atom at δ_C 154.6 showed interaction with H–C(18) (δ_H 0.86), indicating the presence of the second C=C bond between C(14) and C(15). Compound **6** was, thus, assigned as (2′*E*,20*S*)-20-(dimethylamino)-3β-(tigloylamino)pregna-4,14-diene.

N-(Formyl(methyl)amino)salonine-B (**7**), $C_{24}H_{37}NO_2$, was obtained as a yellow gum. The base peak at *m*/*z* 356.3233 in the HR-EI mass spectrum was due to the loss of a Me group, which indicated the presence of a C=C bond. The peak at *m*/*z* 58 indicated the presence of a formyl(methyl)amino group. The ^{1}H- and ^{13}C-NMR spectra of **7** were distinctly similar to those of compound **3**. A *m* at δ_H 4.19 was ascribed to H–C(20), a split *s* at δ_H 2.64/2.72 for the MeN group, and a splitted *s* at δ_H 8.02/8.19 for the formyl H-atom, peak doubling being due to the rotational isomerism of the formamido group. The ^{13}C-NMR spectra displayed resonances for 24 C-atoms, with five Me, seven CH_2, eight CH, and four quaternary C-atoms. The β-orientation of the MeO–C(3) group was inferred from a *m* resonating at δ_H 3.03 ($W_{1/2} = 17.7$ Hz). The presence of a C(16)=C(17) bond was supported by the HMBC spectrum (*Fig. 7*). H–C(16) (δ_H 5.78) showed an interaction with C(15) (δ_C 31.2), and C(17) (δ_C 153.4) with H–C(18) (δ_H 0.75). The presence of a second double bond between C(5) and C(6) was inferred from the HMBC interactions of H–C(6) (δ_H 5.34) with C(10) (δ_C 35.5), and of C(5) (δ_C 141.1) with H–C(19) (δ_H 1.01). Finally, the structure of compound **7** was deduced as (20*S*)-20-(formyl(methyl)amino)-3β-methoxypregna-5,16-diene.

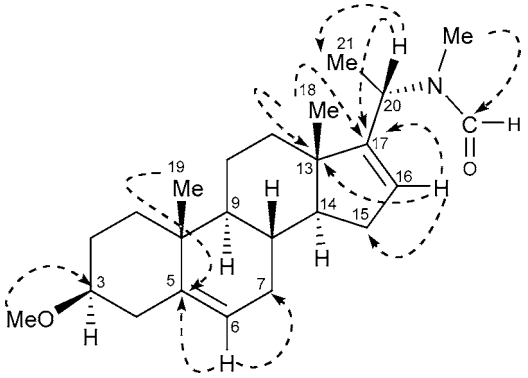


Fig. 7. *Selected HMBC interactions in* N-*(formyl(methyl)amino)salonine-B* (**7**)

The steroidal alkaloid dictyophlebine (**8**) was isolated for the first time from *S. saligna*. This compound has previously been isolated from *Dictyophleba lucida* [18]. The UV, IR, NMR, and mass spectra of this compound were identical to those reported in the literature. Five more known compounds, epipachysamine-D (**9**), saracosine (**10**), iso-*N*-formylchonemorphine (**11**), sarcodinine (**12**), and alkaloid-C (**13**) were also

isolated from *S. saligna* and structurally identified by comparison with the literature data.

The anticholinesterase activities of **1**–**13** are reported here for the first time. All compounds were found to be nontoxic in brine-shrimp lethality assay (LD_{50} > 1,000 mg/ml). *Table 3* shows their inhibitory activities against the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). All these compounds were found to be more selective towards BChE, whereas the standard drug eserine was 21-times more selective towards AChE. Compounds **1**–**13** were found to be non-competitive enzyme inhibitors.

Table 3. In vitro *Activities* (in terms of IC_{50} [μM] ± SEM) *of Compounds* **1**–**13** *against Acetylcholinesterase* (AChE) *and Butyrylcholinesterase* (BChE). For comparison, the respective IC_{50} values for eserine as a reference compound are also shown

Compound	AChE	BChE
1	15.99 ± 0.13	6.91 ± 0.06
2	20.29 ± 1.82	1.89 ± 0.06
3	249 ± 10.23	25.7 ± 0.63
4	82.5 ± 2.22	20.95 ± 3.2
5	30.2 ± 2.0	1.9 ± 0.2
6	7.8 ± 0.5	32.2 ± 0.5
7	48.6 ± 2.7	10.5 ± 0.3
8	6.21 ± 0.23	3.65 ± 0.02
9	28.93 ± 0.54	2.82 ± 0.02
10	20.0 ± 1.30	3.86 ± 0.01
11	6.36 ± 0.22	4.07 ± 0.11
12	40.04 ± 0.13	12.51 ± 0.06
13	42.2 ± 0.26	22.13 ± 0.14
Eserine	0.041 ± 0.00	0.857 ± 0.01

Experimental Part

General. Column chromatography (CC) was carried out on silica gel *60* (70–230 mesh). Pre-coated silica-gel *60* F_{254} (*Merck*; 0.2 mm) glass plates for TLC were inspected at 254 and 366 nm, and developed with *Dragendorff*'s spraying reagent. Melting points (m.p.) were determined in glass capillary tubes using a *Büchi-535* melting-point apparatus. Optical rotations were measured on a *Schmidt–Haensch Polartronic-D* polarimeter. UV Spectra were measured on a *Hitachi U-3200* spectrophotometer. The IR spectra were recorded on a *Jasco A-302* spectrophotometer. ^{1}H- and ^{13}C-NMR Spectra were recorded on *Bruker AM-300* and *AMX-500* spectrometers at 300 or 500, and at 75 or 125 MHz, respectively, FAB- and HR-EI-MS: on *Jeol JMS-600* and *HX-110* mass spectrometers, resp.

Collection and Identification of Plant Material. The whole plants of *Sarcococca saligna* (D.DON.) Muel. (50 kg) were collected in October 1999 from the District Bagh of Azad Kashmir (Pakistan) and were identified by Mr. *Tahir Ali* (taxonomist at the Department of Botany, University of Karachi, Pakistan). A voucher specimen (KU#19290) was deposited in the Herbarium of the University of Karachi.

Extraction. The air-dried plant material (14 kg) was crushed and soaked in MeOH (50 l) for 15 d. The MeOH extract was filtered and evaporated to a gum (1.25 kg), triturated in distilled H_2O (5 l), and subsequently extracted with petroleum ether (251 g, *Fraction A*); $CHCl_3$ at pH 3 (6.0 g; *Fraction B*), pH 7 (220 g; *Fraction C*) and pH 9 (25.0 g; *Fraction D*); AcOEt (95 g; *Fraction E*); and, finally, with BuOH (145 g; *Fraction F*). *Fractions B* and *D* showed 100% inhibition against acetylcholinesterase and butyrylcholinesterase (25 mg/ml). These two fractions were subjected to repeated CC (1.5 kg SiO_2), eluting with petroleum ether/acetone/Et_2NH mixtures of increasing polarity. The resulting subfractions were individually subjected to further CC on SiO_2

(petroleum ether/acetone/Et_2NH mixtures) to obtain semipure sub-subfractions, which were finally purified by prep. TLC (SiO_2; solvent mixtures as above) to obtain pure **1**–**13**.

2-Hydroxysalignarine-E (=*(2′*E*,20*S*)-20-(dimethylamino)-2β-hydroxy-3β-(tigloylamino)pregn-14-ene*; **1**). Yellow gum. Yield: 19.7 mg (141 ppm). $[\alpha]_D^{20} = +38.9$ ($c = 0.3$, MeOH). UV (MeOH): 211 (1.32), 229 (2.76). IR ($CHCl_3$): 3329 (NH, OH), 2911 (CH), 1663 (C=O), 1624 (C=C). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 442 (34) (M^+), 427 (100, $[M-15]^+$), 328 (2), 296 (5), 100 (9), 83 (17), 72 (39), 55 (7). FD-MS: 442 ($C_{28}H_{46}N_2O_2$). HR-EI-MS: 442.3489 (M^+, $C_{28}H_{46}N_2O_2^+$; calc.: 442.3559), 83.0504 ($C_5H_7O^+$; calc.: 83.0496), 72.0803 ($C_4H_{10}N^+$; calc.:72.0813).

5,6-Dihydrosarconidine (=*(20*S*)-20-(dimethylamino)-3β-(methylamino)-5α-pregn-16-ene*; **2**). Colorless powder. Yield: 21.03 mg (150 ppm). $[\alpha]_D^{20} = -60$ ($c = 0.2$, $CHCl_3$). UV (MeOH): 212 (2.66). IR ($CHCl_3$): 3650 (NH), 2810 (CH), 1601 (C=C). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 358 (3) (M^+), 343 (100, $[M-15]^+$), 72 (47), 58 (14). FD-MS: 358 ($C_{24}H_{42}N_2^+$). HR-EI-MS: 358.3193 (M^+, $C_{24}H_{42}N_2$; calc.: 358.3198), 72.0811 ($C_4H_{10}N^+$; calc.: 72.0813).

Salignamine (=*(20*S*)-20-(methylamino)-3β-methoxypregn-5,16-diene*; **3**). Yellow gum. Yield: Yellow gum. Yield: 9.9 mg, (70.7 ppm) $[\alpha]_D^{20} = -23$ ($c = 0.12$, $CHCl_3$). UV (MeOH): 202 (2.9). IR ($CHCl_3$): 3303 (NH), 2901 (CH), 1386 (C–O). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 343 (16, M^+), 328 (100, $[M-15]^+$; 312 (14), 58 (37). FD-MS: 343 ($C_{23}H_{37}NO^+$). HR-EI-MS: 343.2880 (M^+, $C_{23}H_{37}NO$; calc.: 343.2875).

2-Hydroxysalignamine (=*(20*S*)-20-(dimethylamino)-2β-hydroxy-3β-methoxypregn-5,16-diene*; **4**). Yellow gum. Yield: 6.9 mg, (44.7 ppm). $[\alpha]_D^{20} = -26$ ($c = 0.02$, $CHCl_3$). UV (MeOH): 206 (3.8). IR ($CHCl_3$): 2905 (CH), 1663 (C=O). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 373 (34, M^+), 358 (100, $[M-15]^+$, 342 (2), 72 (39). FD-MS: 373 ($C_{24}H_{39}NO_2^+$). HR-EI-MS: 373.2982 ($C_{24}H_{39}NO_2^+$; calc.: 373.2980), 72.0803 ($C_4H_{10}N^+$; calc.: 72.0813).

Salignarine-F (=*(2′*E*,20*S*)-20-(dimethylamino)-4β-hydroxy-3β-(tigloylamino)pregn-5-ene*; **5**). Yellow gum. Yield: 6.79 mg, (40.3 ppm). $[\alpha]_D^{20} = -71$ ($c = 0.014$, MeOH). UV (MeOH): 203 (2.6). IR (KBr): 3650 (NH), 2810 (=CH), 1601 (C=C), 1511 (NH). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 442 (3, M^+), 427 (70, $[M-15]^+$), 84 (37), 72 (100), 58 (14). FD-MS: 442 ($C_{28}H_{46}N_2O_2^+$). HR-EI-MS: 442.3577 (M^+, $C_{28}H_{46}N_2O_2^+$; calc.: 442.3559), 427.3233 ($C_{27}H_{43}N_2O_2^+$; calc.: 427.3273), 83.0503 ($C_5H_7O^+$; calc.: 83.0496), 72.0811 ($C_4H_{10}N^+$; calc.: 72.0813).

Salonine-C (=*(2′*E*,20*S*)-20-(dimethylamino)-3β-(tigloylamino)pregna-4,14-diene*; **6**). White amorphous solid. Yield: 20.03 mg, (140 ppm). $[\alpha]_D^{20} = -120$ ($c = 0.048$, MeOH). UV (MeOH): 206 (2.23). IR ($CHCl_3$): 3650 (NH), 2810 (=CH), 1601 (C=C), 1511 (NH). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 424 (3, M^+), 409 (25, $[M-15]^+$, 84 (37), 72 (100), 58 (14). FD-MS: 424 ($C_{28}H_{44}N_2O^+$). HR-EI-MS: 424.3489 (M^+, $C_{28}H_{44}N_2O^+$; calc.: 424.3508), 409.3233 ($C_{27}H_{41}N_2O^+$; calc.: 409.3273), 83.0503 ($C_5H_7O^+$; calc.: 83.0496), 72.0811 ($C_4H_{10}N^+$; calc.: 72.0813).

N-*[Formyl(methyl)amino]salonine-B* (=*(20*S*)-20-[formyl(methyl)amino]-3β-methoxypregna-5,16-diene* (**7**). Yellow gum. Yield: 5.03 mg, (15.0 ppm). $[\alpha]_D^{20} = -21$ ($c = 0.075$, MeOH). UV (MeOH): 237 (2.86). IR ($CHCl_3$): 2810 (=CH), 1601 (C=C). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. EI-MS: 371 (3, M^+), 356 (100, $[M-15]^+$), 58 (14). FD-MS: 371 ($C_{24}H_{37}NO_2^+$). HR-EI-MS: 371.2982 (M^+, $C_{24}H_{37}NO_2$; calc.: 371.2980), 356.3233 ($C_{23}H_{34}NO_2^+$; calc.: 356.3273).

Inhibition Assays. Electric eel acetylcholinesterase (AChE), horse butyrylcholinesterase (BChE), acetylthiocholine iodide, butyrylcholine chloride, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB; =5-[(3-carboxy-4-nitrophenyl)disulfanyl]-2-nitrobenzoic acid), and eserine were purchased from *Sigma* (St. Louis, MO). Buffer and other chemicals were of analytical grade. AChE Inhibition was determined spectrophotometrically, with acetylthiocholine as substrate, by modifying the method of *Ellman* [16]. The reaction was carried out in 100 μM sodium phosphate buffer (pH 8.0) at 25°. In a typical assay, 140 μl of buffer, 20 μl of enzyme preparation, and 20 μl of test-compound soln. were mixed and incubated for 30 min. DTNB (10 μl) was added, and the reaction was initiated by adding 10 μl of acetylthiocholine. Butyrylthiocholine chloride was used as a substrate to assay BChE under similar conditions as above. The rate of hydrolyses of acetylthiocholine and butyrylthiocholine were determined by monitoring the formation of the yellow 2-nitro-5-sulfanylbenzoate anion (as a result of the reaction of DTNB with the thiocholine released by the enzymatic hydrolysis) at a wavelength of 412 nm.

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