New Cholinesterase Inhibiting Steroidal Alkaloids from the Leaves of *Sarcococca coriacea* of Nepalese Origin

Surya K. KALAUNI,[a] Muhammad Iqbal CHOUDHARY,*,[b] Asaad KHALID,[b] Mangala Devi MANANDHAR,[a] Farzana SHAHEEN,[b] ATTA-UR-RAHMAN,[b] and Mohan Bikram GEWALI[a]

[a] *Central Department of Chemistry, Tribhuvan University; Kirtipur, Kathmandu, Nepal: and* [b] *H. E. J. Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi; Karachi-75270, Pakistan.*
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From the leaves of *Sarcococca coriacea* two new steroidal alkaloids, epoxynepapakistamine-A [(20*S*)-20-(*N*-methylamino)-3β-(tigloylamino)-5α-pregna-16α,17α-epoxy-2β,4β-di-*O*-acetate] (1), and epoxysarcovagenine-D [(20*S*)-20-(*N*-methylamino)-3β-(tigloylamino)-5α-pregna-2-en-16α,17α-epoxy-4-one] (2), and two known compounds funtumafrine C [(20*S*)-20-(*N*,*N*-dimethylamino)-5α-pregna-3-one] (3) and *N*-methylfuntumine (4) were isolated. Their structures were elucidated on the basis of their spectral properties. The compounds 1, 3 and 4 were found to have cholinesterase inhibitory activity when tested for the inhibition of electric eel acetylcholinesterase and horse serum butyrylcholinesterase. They inhibited both enzymes in a concentration dependent fashion.

Key words *Sarcococca coriacea*; Buxaceae; steroidal alkaloid; acetylcholinesterase; butyrylcholinesterase

The enzyme acetylcholinesterase (AChE) has long been an attractive target for the rational drug design and discovery of mechanism-based inhibitors because of its role in the hydrolysis of the neurotransmitter acetylcholine.[1)] The inhibition of this enzyme is considered as a promising approach for the treatment of Alzheimer's disease (AD), and for other possible therapeutic applications in the treatment of Parkinson's disease, ageing and myasthenia gravis. The role of butyrylcholinesterase (BChE) in the normal aging, and diseased brain is still unknown. It has been discovered that BChE is present in significantly higher quantities in Alzheimer's plaques than in plaques of normal age-related non-demented brains. Moreover a very potent selective BChE inhibitor, cymserine,[2)] is now in clinical trials for the treatment of AD.

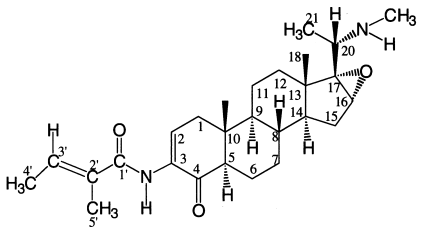
Steroidal alkaloids are the main chemical constituents of the *Sarcococca* species (Buxaceae) and some of them have interesting biological activities. The extracts and compounds isolated from *Sarcococca* are reported to have antiulcer, antitumor[3)] and antibacterial[4)] activities. The alkaloids of *Sarcococca* have been found to potentiate the action of naturally secreted acetylcholine in the isolated rat's diaphragm.[5)] There are four species of *Sarcococca* (*i.e. S. coriacea*, *S. saligna*, *S. hookeriana* and *S. wallichii*) reported from different ecological zones of Nepal.[6)] We have previously reported a number of steroidal alkaloids from various species of *Sarcococca*.[8,14,16—18)] In our continuing studies on steroidal alkamines from this genus, we recently initiated work on *S. coriacea* (HOOK. f.) SWEET which is an evergreen shrub widely distributed in central Nepal. This study has resulted in the isolation of two new steroidal alkaloids, epoxynepapakistamine-A (**1**), and epoxysarcovagenine-D (**2**), and two known alkaloids funtumafrine C (**3**) and *N*-methylfuntumine (**4**). The compounds **1**, **3** and **4** were found to have cholinesterase inhibitory activity. The structures **1**—**4** were determined by the combined use of different spectroscopic techniques.

This study was conducted to achieve two objectives, firstly to test the applicability of the cholinesterase inhibition assay to determine the cholinesterase inhibitory activities of the steroidal alkaloids and secondly to understand the structure–activity relationship in this new class of cholinesterase inhibitors.

Results and Discussion

Methanolic extract of the aerial parts of the *S. coriacea* was evaporated, the gummy extract was dissolved in distilled water and then successively partitioned with pet. ether, chloroform, ethyl acetate and butanol. The chloroform extract was subjected to column and prep. thin layer chromatography to afford compounds **1**—**4**.

Compound **1** ($C_{31}H_{48}N_2O_6$, high resolution electron impact (HR-EI)-MS *m/z* 544.3489) was isolated as a colorless crystalline solid with $[\alpha]_D^{25}$ +14° (*c*=0.07, $CHCl_3$). The UV spectrum exhibited only terminal absorptions. The IR spectrum displayed the peaks at 3451 (NH), 1724 (acetoxy C=O), 1664 (amide C=O), 1147 and 889 (C–O–C) cm^{-1}.[7)] The mass fragmentation pattern of the compound was very informative for determining the nature and position of substituents. The EI-MS showed the M^+ at *m/z* 544. The peaks



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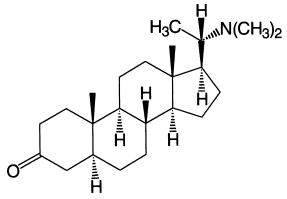

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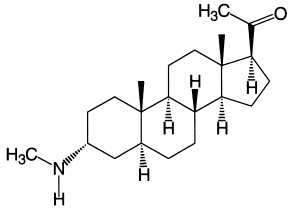

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* To whom correspondence should be addressed. e-mail: hejric@digicom.net.pk

at *m/z* 529 and 486 arose due to the loss of methyl and acetoxy groups from the M^+, respectively. The presence of an *N*-methylaminoethane substituent at C-17 was deduced from the base peak at *m/z* 58.

The ^{1}H-NMR spectrum ($CDCl_3$) of **1** showed two 3H singlets at δ 0.82 and 1.12 corresponding to the C-18 and C-19 tertiary methyls, respectively. A 3H doublet at δ 1.13 ($J_{21,20}$=6.5 Hz) was due to the C-21 secondary methyl protons. A 3H double doublet at δ 1.71 ($J_{4',3'}$=6.9 Hz, $J_{4',5'}$=1.0 Hz) and a 3H triplet at δ 1.75 ($J_{5',3'}$=1.2 Hz) were assigned to the C-4′ and C-5′ methyl protons of the tigloyl group, respectively. Two downfield 3H singlets at δ 2.05 and 2.07 were due to the two acetoxy methyl protons attached at C-2 and C-4 of ring A, respectively. Another 3H singlet at δ 2.33 was due to the N_b-methyl protons. A 1H multiplet at δ 4.29 was assigned to C-3 proton geminal to an amidic group. The amidic NH resonated as a broad doublet at δ 5.95 ($J_{NH,3}$=8.7 Hz). A split quartet, characteristic of the tigloyl C-3′ olefinic proton, resonated at δ 6.31 and showed vicinal and allylic couplings ($J_{3',4'}$=6.9 Hz, $J_{3',5'}$=1.2 Hz).

The ^{13}C-NMR spectra (Broad Band, distortionless enhancement by polarization transfer (DEPT)) of compound **1** showed the presence of signals with eight methyls, six methylenes, ten methines and seven quaternary carbons (Table 1). The position of the epoxide at C-16 and C-17 was determined by careful examination of correlation spectroscopy (COSY)-45°, heteronuclear multiple bond connectivity (HMBC) and ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) spectra. In the HMBC spectrum of compound **1**, the C-21 secondary methyl protons (δ 1.13) exhibited long-range interactions with the C-20 (δ 51.0) methine and the C-17 (δ 72.9) quaternary carbons. A broad singlet at δ 3.38 was due to the C-16 proton geminal to an epoxy functionality and it showed HMBC interactions with C-15 (δ 27.0) and C-14 (δ 45.7). The C-20 methine proton resonated as a quartet at δ 3.0 ($J_{20,21}$=6.5 Hz) that also indicated that C-17 has no hydrogen and is involved in epoxidation with C-16 (δ 60.0). The C-15 proton (δ 1.85) exhibited HMBC interaction with C-17 (δ 72.9), C-16 (δ 60.0) and C-13 (δ 41.6). The C-18 methyl protons (δ 0.82) also showed strong interaction with C-17 (δ 72.9). Similarly the positions of the two acetoxy groups in ring A were confirmed by COSY-45° and HMBC techniques. A double doublet at δ 5.11 ($J_{2e,1a}$=6.5 Hz, $J_{2e,1e}$=3.2 Hz) and a broad doublet at δ 5.19 ($J_{4,3}$=3.7 Hz) were due to the C-2 and C-4 protons of ring A geminal to acetoxy groups as inferred from COSY-45° spectrum. The C-2 (δ 5.11) proton showed HMBC interactions with C-3 (δ 50.0) and C-4 (δ 74.1) and it showed ^{1}H–^{1}H coupling with the C-1 protons (δ 1.23, 2.22). On the other hand, the C-4 proton which is resonated at δ 5.19 as a broad doublet, showed the HMBC interactions with C-2 (δ 71.7) and C-3 (δ 50.0) and it showed ^{1}H–^{1}H couplings with the C-5 proton (δ 1.45). The coupling constants of H-2 ($J_{2e,1a}$= 6.5 Hz, $J_{2e,1e}$=3.2 Hz) and H-4 ($J_{4,3}$=3.7 Hz) signals further indicated their equatorial orientation so that the acetoxy groups must be axially oriented which was further confirmed by comparing with reported compounds.[8,9] Important HMBC interactions are shown in the Fig. 1. These spectroscopic analysis supported a pregnane-type steroidal skeleton with a tigloyl amino group at C-3, acetoxy groups at C-2 and C-4, epoxide at C-16/C-17 and *N*-methylaminoethane substituent at C-17. The structure of the compound **1** was therefore assigned as (20*S*)-20-(*N*-methylamino)-3β-(tigloylamino)-5α-pregna-16α,17α-epoxy-2β,4β-di-*O*-acetate.

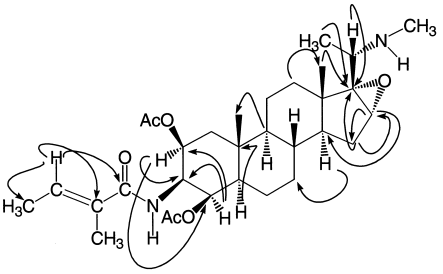

Fig. 1. Selected HMBC Interactions in Compound **1**

Compound **2** ($C_{27}H_{40}N_2O_3$ HR-EI-MS *m/z* 440.3031) was obtained as a yellowish amorphous solid having $[\alpha]_D^{25}$ +24° (*c*=0.116, $CHCl_3$). It showed UV absorptions at 223 and 203 nm. The IR spectrum displayed peaks at 3446, 3391 (NH), 1664, 1638 (C=O), 1147 and 889 (C–O–C) cm^{-1}. The EI-MS showed the M^+ at *m/z* 440 and a peak at *m/z* 425 corresponding to the loss of a methyl group from the M^+. The base peak at *m/z* 58 suggested the presence of an *N*-methylaminoethane substituent at C-17, while the peaks at *m/z* 98, 83 and 55 were indicative of the presence of 2′-methylbuten-2′-oyl amide functionality (tigloyl amino group) in the compound.

The ^{1}H-NMR spectrum ($CDCl_3$) of **2** was distinctly similar to that of compound **1**. The main differences included the absence of downfield signals for C-2 and C-4 methine protons, indicating the absence of acetoxy groups at these positions. A downfield proton resonated as a double doublet at δ 7.65 ($J_{2,1a}$=6.9 Hz, $J_{2,1e}$=2.6 Hz), and it was assigned to the C-2 olefinic proton. The downfield chemical shift and splitting pattern of the C-2 vinylic proton indicated that the carbonyl moiety is present at C-4 and not at C-1.[10] The amidic NH resonated at δ 8.15 as a singlet. The position of the oxirane ring at C-16/C-17 was again confirmed by comparing its spectral data with those of compound **1**.

The ^{13}C-NMR spectra (Broad Band and DEPT) of **2** (Table 1) showed signals for twenty-seven carbons with six methyls, six methylenes, eight methines, and seven quaternary carbons. On the basis of spectroscopic analysis, the structure for epoxysarcovagenine-D (**2**) was elucidated as [(20*S*)-20-(*N*-methylamino)-3β-(tigloylamino)-5α-pregna-2-en-16α,17α-epoxy-4-one].

Compound **3** ($C_{23}H_{39}NO$, HR-EI-MS *m/z* 345.3027) was isolated for the first time from this genus as a white crystalline solid. This compound was earlier isolated from *Funtumia africana.*[11] The ^{1}H-NMR ($CDCl_3$) spectrum of **3** displayed two 3H singlets at δ 0.66 and 0.99 for the C-18 and C-19 tertiary methyl protons, respectively. A 3H doublet resonating at δ 0.85 ($J_{21,20}$=6.5 Hz) was due to the C-21 secondary methyl protons. The *N*,*N*-dimethyl protons resonated as a 6H singlet at δ 2.15. The C-17 proton resonated at δ 2.29 as a multiplet. The C-20 methine proton appeared as a multiplet at δ 2.82.

The ^{13}C-NMR spectra (Broad Band, DEPT) of compound **3** exhibited twenty-three carbon resonances with five methyls, nine methylenes, six methines and three quaternary carbons (Table 1). The spectral data of compound **3** has been

Table 1. The ^{13}C-NMR ($CDCl_3$, 100 MHz) Chemical Shift Data (δ ppm) of Compounds **1**—**4**

Carbon	Compound			
	1	**2**	**3**	**4**
1	40.2	38.9	38.2	37.1
2	71.7	125.9	29.0	28.6
3	50.0	132.1	212.0	54.3
4	74.1	196.2	44.7	34.9
5	48.7	54.2	56.4	56.1
6	20.4	20.4	24.1	22.7
7	25.0	20.5	21.7	24.4
8	33.2	33.1	35.3	35.5
9	56.0	54.9	53.9	58.7
10	35.0	40.0	35.7	36.0
11	31.4	30.2	31.7	21.4
12	32.9	32.8	39.6	32.0
13	41.6	41.6	41.7	44.3
14	45.7	45.2	46.7	45.3
15	27.0	27.0	27.6	28.1
16	60.0	60.1	38.6	35.1
17	72.9	72.9	54.9	63.1
18	16.0	15.9	12.3	13.4
19	15.3	14.1	11.5	12.3
20	51.0	51.0	61.1	209.7
21	17.9	17.8	10.0	31.5
$N_b(CH_3)_n$	34.6 (n=1)	34.6 (n=1)	40.0 (n=2)	—
$N_a(CH_3)$	—	—	—	33.3
1′ C=O	168.0	167.6	—	—
2′	131.5	131.4	—	—
3′	131.2	131.8	—	—
4′	12.2	12.2	—	—
5′	13.9	13.2	—	—
O$\underline{C}$O$\underline{C}H_3$ (C-2)	170.1, 20.9	—	—	—
O$\underline{C}$O$\underline{C}H_3$ (C-4)	170.4, 21.3	—	—	—

Table 2. *In Vitro* Quantitative Inhibition of AChE and BChE by Compounds **1**, **3** and **4**

Compound	IC_{50} (μM)		Selectivity	
	AChE	BChE	AChE	BChE
Epoxynepapakistamine-A (**1**)	>200	77.4±0.024		>2.58
Funtumafrine C (**3**)	45.75±1.122	6.56±0.123		6.97
N-Methylfuntumine (**4**)	97.61±1.731	12.69±0.126		7.69
Eserine (control)	0.041±0.001	0.857±0.008	20.9	

presented for the first time in this paper.

Compound **4** ($C_{22}H_{37}NO$, HR-EI-MS *m/z* 331.2806)[16] was also isolated for the first time from this genus as a yellowish crystalline solid. This compound was earlier isolated from the leaves of *Holarrhena febrifuga*.[16]

The stereochemical assignments of stereogenic centers in the compounds **1**—**4** were made on the basis of nuclear Overhauser effect spectroscopy (NOESY) interactions, chemical shifts comparisions with reported compounds[9—18] and biogenetic considerations keeping in view of the fact that all pregnane-type steroidal alkaloids are biosynthesized from cholesterol *via* pregnenolone.[19] The β-orientation of the C-20 methine proton in compounds **1**—**3**, and equatorial position of the C-3 tigloyl group in compounds **1**—**2** were confirmed by comparing the spectral data with reported compounds.[9—18,25] In compounds **1** and **2**, the *cis* configuration of tigloyl methyl groups at C-2′ and C-3′ were inferred from NOESY interactions between the C-3′ olefinic proton with C-4′ methyl protons.

Compounds **1**, **3** and **4** were screened for their cholinesterase inhibitory activity. Electric eel acetylcholinesterase was used for two reasons; firstly, the oligometeric forms of AChE in the electrical organ of the electric fish is structurally similar to those in vertebrates nerve and muscle AChE.[22,23] Secondly the results obtained with this enzyme allow molecular modeling studies to be conducted using the already published eel AChE X-ray structure.[22] On the other hand, serum butyrylcholinesterase has similarities with synaptic acetylcholinesterase in primary amino acid sequence, deduced secondary structure and active site chemistry. The two enzymes also have overlapping specificity for substrate and inhibitors.[24]

The concentrations of compounds **1**, **3** and **4** that inhibited the enzymes activity by 50% (IC_{50}) are presented in Table 2. Eserine [(−)-physostigmine] was used as standard inhibitors. Compounds **1**, **3** and **4** were found to be more selective inhibitors of BChE than the AChE.

Experimental

The melting points were recorded on a Buchi 535 melting point apparatus and are uncorrected. Optical rotations were measured on Polartronic D polarimeter. The ^{1}H-NMR spectra were recorded on Bruker AM-400 and AMX-500 instruments, while the ^{13}C-NMR spectra were recorded on Bruker AM-400 operating at 100 MHz. The chemical shift (δ) values are reported in ppm and coupling constants (J) are measured in Hz. The IR spectra were recorded on a Jasco 302A spectrophotometer. The UV spectra were recorded on a Hitachi UV 3200 spectrophotometer. The EI mass spectra were recorded on a Varian MAT 311A mass spectrometer, while HR-EI measurements were carried on a Jeol HX 110 mass spectrometer. Precoated TLC plates (silica gel, Merck GF 254) were used to check the purity of compounds.

Plant Material The fresh plant material (leaves, stems) was collected from the Hattiban areas of Kathmandu valley (Nepal) during August–September, 1999. The plant was identified by Prof. R. P. Choudhary, Taxonomist, Central Department of Botany, Kirtipur, Kathmandu, Nepal. A voucher specimen # SK 2057 has been deposited in the Central Department of Botany of Tribhuvan University.

Extraction and Isolation Air-dried aerial parts (14 kg) of *S. coriacea* were extracted with methanol (35 l). The concentrated methanolic extract (968 g) was dissolved in cold distilled water (3 l) and defatted with pet. ether (10 l). The aqueous layer was then extracted with chloroform to obtain a reddish gummy material (32 g), which was adsorbed on equal amount of silica gel and loaded onto a Si gel (500 g, E. Merck, type 60, 70—230 mesh) packed column and eluted with gradients of pet. ether, chloroform and methanol to afford a number of fractions (F_1—F_4). These fractions (F_1—F_4) were further subjected to CC and TLC to afford pure compounds. Fraction F_4 (15.0 g) eluted with 10% MeOH in $CHCl_3$ was subjected to repeated column chromatography to obtain many subfractions. A subfraction F_4S_3 (0.73 g) was loaded on a column and eluted with pet. ether, acetone and diethylamine (87 : 12 : 1) to afford the crystalline compound **1** (18.2 mg, % yield 1.3×10^{-4}). Another subfraction (F_4S_4, 1.03 g) of this column was reloaded on another column and eluted with pet. ether, acetone and diethylamine (84 : 15 : 1) to afford the yellowish crystalline compound **4** (14.3 mg, % yield 1.02×10^{-4}).

Fraction F_3 (3.95 g) eluted with 5% MeOH in $CHCl_3$ was further chromatographed on the silica gel (flash silica, 240—300 mesh, 50 g) column and eluted with pet. ether, acetone and diethylamine to afford many subfractions. A subfraction F_3S_3 (0.52 g) was again chromatographed on column and eluted with pet. ether, acetone and diethylamine (90 : 9 : 1) to afford the pure compound **2** (11.4 mg, % yield 8.1×10^{-5}).

Fraction F_2 (1.2 g) eluted with 3% MeOH in $CHCl_3$ was also loaded on flash silica gel packed column and eluted with pet. ether, acetone and diethylamine (97 : 2 : 1) to afford pure compound **3** (13.2 mg, % yield 9.4×10^{-5}).

Epoxynepapakistamine-A (**1**): Colorless crystalline solid ($CHCl_3$) mp 119—120 °C. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 0.82 (3H, s, CH_3-18), 1.12 (3H, s, CH_3-19), 1.13 (3H, d, $J_{21,20}$=6.5 Hz, CH_3-21), 1.71 (3H, dd, $J_{4',3'}$=6.9 Hz, $J_{4',5'}$=1.0 Hz, CH_3-4′), 1.75 (3H, t, $J_{5',3'}$=1.2 Hz, CH_3-5′),

2.05 (3H, s, $OCOCH_3$), 2.07 (3H, s, $OCOCH_3$), 2.23 [3H, s, $N_b(CH_3)$], 3.0 (1H, q, $J_{20,21}$=6.5 Hz, H-20), 3.38 (1H, bs, H-16), 4.29 (1H, m, H-3), 5.11 (1H, dd, $J_{2e,1a}$=6.5 Hz, $J_{2e,1e}$=3.2 Hz, H-2), 5.19 (1H, bd, $J_{4,3}$=3.7 Hz, H-4), 5.95 (1H, bd, $J_{NH,3}$=8.7 Hz, NH), 6.31 (1H, dd, $J_{3',4'}$=6.9 Hz, $J_{3',5'}$=1.2 Hz, H-3′). ^{13}C-NMR ($CDCl_3$, 100 MHz): Table 1. IR ν_{max} ($CHCl_3$) cm^{-1}: 3451, 1724, 1664, 1147, 889. UV λ_{max} (MeOH) nm (log ε): 209 (5.3). EI-MS *m/z* (rel. int., %): $[M]^+$ 544 (1.7), 529 (5), 486 (27), 98 (3), 58 (100), 55 (43). HR-EI-MS *m/z* 544.3489 (Calcd for $C_{31}H_{48}N_2O_6$, 544.3496). $[\alpha]_D^{25}$ +14° (*c*=0.07, $CHCl_3$).

Epoxysarcovagenine-D (**2**): Yellowish amorphous solid ($CHCl_3$): mp 114—116 °C. ^{1}H-NMR ($CHCl_3$, 400 MHz) δ: 0.86 (3H, s, CH_3-18), 0.87 (3H, s, CH_3-19), 1.18 (3H, d, $J_{21,20}$=6.5 Hz, CH_3-21), 1.76 (3H, dd, $J_{4',3'}$=6.9 Hz, $J_{4',5'}$=1.0 Hz, CH_3-4′), 1.87 (3H, t, $J_{5',3'}$=1.2 Hz, CH_3-5′), 2.37 [3H, s, $N_b(CH_3)$], 3.08 (1H, q, $J_{20,21}$=6.5 Hz, H-20), 3.45 (1H, bs, H-16), 6.49 (1H, dd, $J_{3',4'}$=6.9 Hz, $J_{3',5'}$=1.2 Hz, H-3′), 7.65 (1H, dd, $J_{2,1a}$=6.9 Hz, $J_{2,1e}$=2.6 Hz, H-2), 8.15 (1H, s, NH). ^{13}C-NMR ($CDCl_3$), 100 MHz): Table 1. IR ν_{max} ($CHCl_3$) cm^{-1}: 3446, 3391, 1664, 1638, 1147, 889. UV λ_{max} (MeOH) nm (log ε): 223 (5.2). EI-MS *m/z* (rel. int., %): $[M]^+$ 440 (0.9), 425 (4), 98 (11), 83 (24), 58 (100), 55 (19). HR-EI-MS *m/z* 440.3031 (Calcd for $C_{27}H_{40}N_2O_3$, 440.3038). $[\alpha]_D^{25}$ +24° (*c*=0.116, $CHCl_3$).

Funtumafrine C (**3**): White crystalline solid ($CHCl_3$): mp 169—170 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.66 (3H, s, CH_3-18), 0.99 (3H, s, CH_3-19), 0.85 (3H, d, $J_{21,20}$=6.5 Hz, CH_3-21), 2.15 [6H, s, $N_b(CH_3)_2$], 2.29 (1H, m, H-17), 2.82 (1H, m, H-20). ^{13}C-NMR ($CDCl_3$, 100 MHz): Table 1. IR ν_{max} ($CHCl_3$) cm^{-1}: 1708, 1692, 1353. UV λ_{max} (MeOH) nm (log ε): 202 (4.7). EI-MS *m/z* (rel. int., %): $[M]^+$ 345 (0.6), 330 (2.3), 72 (100). HR-EI-MS *m/z* 345.3027 (Calcd for $C_{23}H_{39}NO$, 345.3031). $[\alpha]_D^{25}$ +50° (*c*=0.06, $CHCl_3$).

N-Methylfuntumine (**4**): Yellowish crystalline solid ($CHCl_3$): mp 123—124 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.58 (3H, s, CH_3-18), 0.76 (3H, s, CH_3-19), 2.09 (3H, s, CH_3-21), 2.34 (1H, m, H-3), 2.40 (3H, s, N_a-CH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz): Table 1. IR ν_{max} ($CHCl_3$) cm^{-1}: 3391, 2848, 1692, 1353. UV λ_{max} (MeOH) nm (log ε): 202 (4.8). EI-MS *m/z* (rel. int., %): $[M]^+$ 331 (14), 316 (2), 288 (5), 70 (100). HR-EI-MS *m/z* 331.2806 (Calcd for $C_{22}H_{37}NO$, 331.2875). $[\alpha]_D^{25}$ +81° (*c*=0.074, $CHCl_3$).

***In Vitro* Cholinesterase Inhibition Assay** Acetylcholinesterase and butyrylcholinesterase inhibiting activities were measured by the spectrophotometric method developed by Ellman *et al.*[20] Electric eel AChE (type VI-S, Sigma), and horse serum BChE (Sigma) were used as sources of both the cholinesterases, acetylthiocholine iodide and butyrylthiocholine chloride (Sigma) were used as substrates of the reaction, and 5,5-dithiobis (2-nitrobenzoic) acid (DTNB Sigma) were used for the measurement of the cholinesterase activity. All the other reagents and conditions were same as described previously.[21] In this procedure 140 μl of 0.1 mM sodium phosphate buffer (pH 8.0), 10 μl of DTNB, 20 μl of test compound solution, and 20 μl of acetylcholinesterase/butyrylcholinesterase solution were mixed and incubated for 15 min at 25 °C. The reaction was then started by the addition of 10 μl of acetylthiocholine/butyrylthiocholine. The hydrolysis of acetylthiocholine and butyrylthiocholine was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine or butyrylthiocholine at a wavelength of 412 nm. Microtiter plate spectrophotometer (Molecular Devices, U.S.A.). Test compounds were dissolved in 5% ethanol while controls also received the same volume of the solvent. All the reactions were in triplicate.

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