New Cholinesterase Inhibiting Bisbenzylisoquinoline Alkaloids from *Cocculus pendulus*

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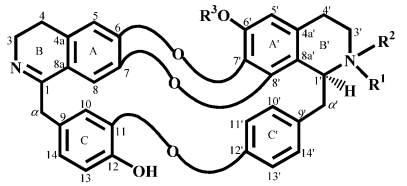
Phytochemical investigation on *Cocculus pendulus* (J. R. & G. Forst.) resulted in the isolation of two new and three known bisbenzylisoquinoline alkaloids. The structures of the new alkaloids, kurramine-2′-β-*N*-oxide (1) and kurramine-2′-α-*N*-oxide (2), were elucidated with the help of spectroscopic techniques. The cholinesterase inhibitory activities of these bisbenzylisoquinoline alkaloids are reported here for the first time.

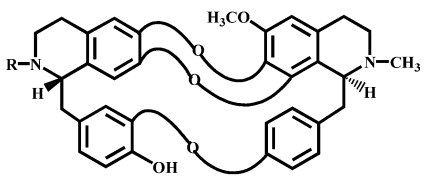
Key words *Cocculus pendulus*; Menispermaceae; bisbenzylisoquinoline alkaloid; acetylcholinesterase; butyrylcholinesterase

Bisbenzylisoquinoline alkaloids constitute one of the most well studied classes of natural products. They are reported to have a variety of biological activities, including anti-bacterial,[1] cytotoxic,[2,3] anti-malarial,[2,3] anti-cancer,[4] hypotensive[4] and anti-plasmodial[5,6] activities. In continuation of our work on medicinal plants, we have re-examined the chemistry of the plant *Cocculus pendulus*, which belongs to the family Menispermaceae.[4,7,8] A number of alkaloids, mainly bisbenzylisoquinoline alkaloids, have previously been reported from this family.[4,7,9—12] In the current investigation, an alkaloidal extract of this plant showed *in vitro* activity against acetyl- and butyryl-cholinesterase enzymes, and through a bioassay-guided fractionation, five alkaloids **1**—**5** have been isolated. The structures of the new alkaloids **1** and **2** were elucidated with the help of spectroscopic techniques and mass spectrometry. The complete ^{13}C-NMR data of known alkaloids **3**—**5** is also reported here.

All the compounds were subjected to a mechanism-based bioassay against acetyl- and butyrylcholinesterase. The enzyme acetylcholinesterase (AChE) has long been an attractive target because of its catalytic role in the hydrolysis of the neurotransmitter acetylcholine.[13] The inhibition of this enzyme is a useful approach for the treatment of Alzheimer's disease (AD), and for other therapeutic applications in Parkinson's disease, pre-mature ageing and myasthenia gravis. Recently, it has also been found that butyrylcholinesterase (BChE) inhibition may be useful in the treatment of AD and related dementias.[14] The inhibitors of AChE and BChE may act as potential leads for nervous system disorders, by potentiating the cholinergic transmission process.



1: R^1 = α-CH_3, R^2= β-O, R^3= H
2: R^1 = β-CH_3, R^2= α-O, R^3= H
3: R^1 = CH_3, R^2=, R^3= CH_3



4: R= H
5: R= CH_3

Results and Discussion

The present work on the ethanolic extracts of *Cocculus pendulus* has resulted in the isolation and characterization of two new compounds **1** and **2**, along with three known compounds **3**—**5**.[4]

Compound **1** was obtained as a yellowish amorphous powder. The UV spectrum showed absorption maxima at 203, 235, 263, 291 and 341 nm, characteristic of bisbenzylisoquinoline alkaloids.[10] The IR spectrum of **1** exhibited absorptions at 3620 (OH), 1575 (C=N), 1507 (C=C) and 1458 (C=C) cm^{-1}. The HR-EI-MS showed the M^+ at *m*/*z* 548.2130 corresponding to the formula $C_{33}H_{28}N_2O_6$ (Calcd 548.2135) and indicating twenty-one degrees of unsaturation. The M^+ was 16 a.m.u. higher than that of a known alkaloid kurramine.[4] The EI-MS also showed a strong peak at *m*/*z* 532 formed by the loss of an oxygen atom from the M^+, suggesting an additional oxygen as an *N*-oxide.[15—18] The peak at *m*/*z* 531 represented the loss of an OH group, which also supported the presence of an *N*-oxide.[17,19] The peak at *m*/*z* 319 was due to the singly charged bisisoquinoline unit arising from the double benzylic cleavage without oxygen of *N*-oxide.[20] This mass spectral data was characteristic of a bisbenzylisoquinoline mono *N*-oxide alkaloid.

The ^{13}C-NMR spectrum of **1** (Table 2) showed the presence of thirty-three carbon resonances indicating one methyl, six methylene, eleven methine and fifteen quaternary carbons. In ^{1}H- and ^{13}C-NMR spectra of compound **1**, downfield chemical shifts of protons and carbons adjacent to 2′-*N*-oxide were observed.

The 2′-*N*-methyl singlet in the ^{1}H-NMR spectrum resonated at δ_H 3.64 correlating with the downfield carbon signal at δ_C 58.7 due to the geminal 2′-*N*-oxide.[21—23] Furthermore, chemical shifts of H-1′ (δ_H 4.55) and H-3′ (δ_H 3.90 and 3.46) correlating with carbon signals at δ_C 74.5 (C-1′) and δ_C 58.3 (C-3′) were characteristic features of *N*-oxide.[21—24]

In the ^{1}H-NMR spectrum of **1**, characteristic proton sig-

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Table 1. ^{1}H-NMR (400, 500 MHz) Spectral Data of **1**—**5**

Position	**1**[a] δ_H (J in Hz)	**2**[a] δ_H (J in Hz)	**3**[b] δ_H (J in Hz)	**4**[b] δ_H (J in Hz)	**5**[b] δ_H (J in Hz)
1	—	—	—	3.87 (br d, 3.9)	3.19 (br d, 10.2)
3	3.19 (dd, 3.0,15.7)	3.39 (m)	2.95 (m)	2.65 (m)	2.51 (m)
	3.74 (dd, 3.9,15.1)	3.83 (m)	3.65 (m)	2.93 (m)	2.76 (m)
4	2.33 (dt, 5.7, 15.9)	2.53 (dt, 5.9, 15.6), 2.70 (m)	2.82 (m)	2.42 (m)	2.55 (m)
	2.47 (br d, 13.8)		2.90 (m)	2.78 (m)	2.70 (m)
5	6.67 (s)	6.79 (s)	6.46 (s)	6.51 (s)	6.53 (s)
8	6.79 (s)	7.00 (s)	6.52 (s)	6.09 (s)	6.07 (s)
α	3.46 (m)	3.52 (m)	3.35 (m)	2.64 (m)	2.66 (m)
	3.99 (br d, 13.2)	3.56 (m)	3.55 (m)	2.78 (m)	2.85 (m)
10	6.60 (br s)	6.61 (br s)	6.38 (d, 1.3)	6.45 (d, 1.7)	6.45 (d, 1.5)
13	6.77 (d, 8.0)	6.76 (d, 8.2)	6.68 (d, 8.1)	6.76 (d, 8.1)	6.78 (d, 8.0)
14	6.75 (dd, 8.0, 1.4)	6.78 (dd, 8.2, 1.3)	6.65 (dd, 8.3, 1.3)	6.69 (dd, 8.1, 1.7)	6.72 (dd, 8.1, 1.5)
2-*N*-CH_3	—	—	—	—	2.29 (s)
1′	4.55 (br d, 10.3)	4.34 (br s)	4.02 (s)	3.49 (br d, 5.8)	3.94 (br s)
3′	3.46 (m)	3.52 (m)	2.53 (m)	2.54 (m)	2.82 (m)
	3.90 (m)	3.83 (m)	3.76 (m)	2.85 (m)	3.09 (m)
4′	3.04 (dd, 7.3, 17.3)	3.19 (m)	2.38 (m)	2.42 (m)	2.46 (m)
	3.24 (m)	3.25 (m)	3.52 (m)	2.60 (m)	2.82 (m)
5′	6.45 (s)	6.53 (s)	6.30 (s)	6.24 (s)	6.26 (s)
α′	2.85 (t, 11.7)	2.70 (m)	2.65 (m)	2.50 (m)	2.55 (m)
	3.52 (br d, 12.7)	4.28 (d, 11.9)	3.45 (m)	3.16 (br d)	3.25 (m)
10′	6.89 (br d, 7.9)	6.93 (br d, 6.5)	6.78 (dd, 8.0, 1.7)	6.98 (dd, 8.2, 2.0)	7.07 (dd, 8.2, 1.7)
11′	6.73 (br d, 7.8)	6.75 (br d, 7.8)	6.58 (dd, 8.0, 1.4)	6.71 (dd, 8.2, 2.5)	6.90 (dd, 8.2, 2.4)
13′	7.26 (br d, 7.7)	7.27 (br d, 6.5)	7.04 (dd, 8.1, 1.4)	7.09 (dd, 8.3, 2.5)	7.12 (dd, 8.2, 2.4)
14′	7.55 (br d, 8.0)	7.64 (br d, 7.7)	7.28 (dd, 8.1, 1.7)	7.39 (dd, 8.3, 1.9)	7.49 (dd, 8.2, 1.7)
2′-*N*-CH_3	3.64 (s)	3.16 (s)	2.50 (s)	2.45 (s)	2.48 (s)
6′-OCH_3	—	—	3.75 (s)	3.75 (s)	3.75 (s)

a) Measured in CD_3OD. *b*) Measured in $CD_3OD+CDCl_3$.

Table 2. ^{13}C-NMR (125 MHz) Spectral Data of **1**—**5**

Position	**1**[a]	**2**[a]	**3**[b]	**4**[b]	**5**[b]	Position	**1**[a]	**2**[a]	**3**[b]	**4**[b]	**5**[b]
1	169.3	172.0	167.7	59.0	66.9	1′	74.5	74.4	58.8	59.2	60.0
3	46.7	45.3	44.7	43.8	50.3	3′	58.3	60.0	40.2	42.0	44.4
4	26.0	25.9	22.8	23.1	26.5	4′	24.8	26.6	24.8	27.5	23.1
4a	136.8	137.6	134.6	130.7	131.4	4a′	127.3	127.3	127.1	129.1	129.4
5	116.4	117.6	115.4	115.5	115.4	5′	113.0	113.4	107.7	106.4	106.6
6	140.0	140.2	138.5	138.6	139.3	6′	146.0	147.1	146.8	145.8	146.0
7	144.5	146.0	143.4	139.4	139.5	7′	145.4	145.6	138.3	132.3	134.1
8	116.3	117.8	114.9	112.4	113.8	8′	139.9	139.5	135.2	138.9	139.0
8a	124.0	123.2	122.3	135.0	133.2	8a′	114.9	114.9	128.4	119.6	119.6
α	41.7	41.1	43.7	41.6	41.3	α′	42.4	39.3	40.8	40.7	40.0
9	128.8	126.5	126.6	126.5	126.7	9′	134.2	135.3	134.1	138.8	138.9
10	118.7	118.6	117.2	117.8	117.0	10′	132.8	132.9	131.1	131.0	130.9
11	146.2	146.7	144.5	144.5	144.0	11′	122.5	122.4	121.1	120.5	121.2
12	148.6	149.0	147.1	147.9	148.3	12′	156.3	156.0	154.2	155.1	154.3
13	117.6	116.8	116.3	116.4	116.0	13′	124.4	124.5	122.8	121.7	122.2
14	123.7	123.8	122.7	122.4	122.1	14′	131.7	132.1	130.3	128.4	128.3
2-*N*-CH_3	—	—	—	—	41.3	2′-*N*-CH_3	58.7	56.5	41.1	40.9	41.1
						6′-OCH_3	—	—	56.0	55.7	56.0

a) Measured in CD_3OD. *b*) Measured in $CD_3OD+CDCl_3$.

nals of bisbenzylisoquinoline skeleton (sub group K)[25] were resonated at δ_H 6.45, 6.67, 6.79 (each 1H, s), δ_H 6.60 (br s) and an ABX spin system for the ring C and four broad doublets for the protons of ring C′ were also observed. The C-8 proton of ring A usually resonates as a singlet in the region at δ_H 5.90—6.20[25] when the ring B is at tetrahydropyridine oxidation level, but in compound **1** it appeared at δ_H 6.79 as a singlet. This downfield shift of C-8 proton suggested the presence of an imine function in ring B of the molecule, which was further inferred from the absence of H-1 in the ^{1}H-NMR spectrum. The HMBC and HMQC spectra of **1** clearly indicated the presence of an imine function and three diaryl ether linkages. Imine function at C-1 position was also supported by ^2J correlations of C-α-methylene protons and ^3J correlation of H-8 with C-1 (δ_C 169.3) in the HMBC spectrum (Fig. 1). The COSY 45° spectrum of **1** showed interactions of H-10′ (δ_H 6.89, br d) with H-11′ (δ_H 6.73, br d) and H-13′ (δ_H 7.26, br d) with H-14′ (δ_H 7.55, br d). The HMBC correlations of H-10′, H-11′, H-13′ and H-14′ with C-12′ further supported the presence of an ether linkage at C-12′.

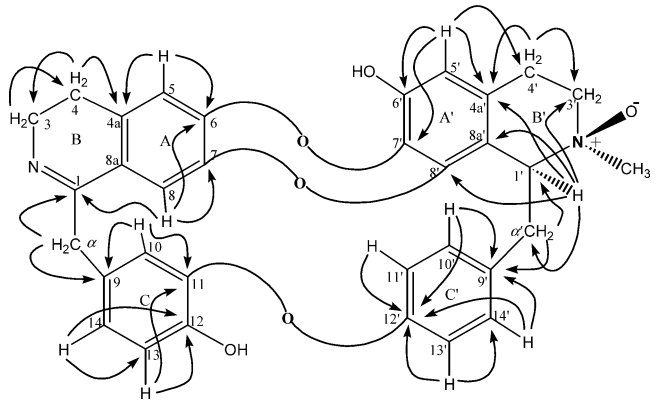

Fig. 1. Key HMBC Interactions in Compound **1**

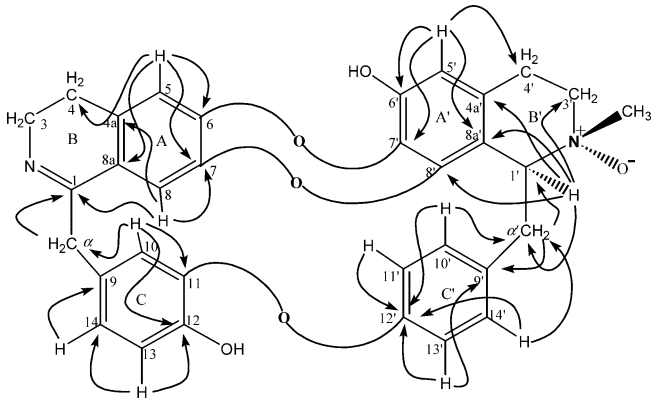

Fig. 2. Key HMBC Interactions in Compound **2**

H-13 and H-14 also showed correlations in the COSY 45° spectrum. The presence of the ether linkage at C-11 and an OH group at C-12 was supported by ^3J correlations of H-13 with C-11 (δ_C 146.2) and H-14 with C-12 (δ_C 148.6) in the HMBC spectrum. Similarly, the ether linkages between C-6 and C-7′ along with C-7 and C-8′ were inferred by ^2J and ^3J correlations of H-5 and H-8 with C-6 and C-7 (δ_C 140.0 and 144.5, respectively), and ^3J correlation of H-5′ and H-1′ with C-7′ and C-8′ (δ_C 145.4 and 139.9, respectively). This leaves only the C-6′ as a possible position for hydroxyl group, that is also supported by ^2J correlation of H-5′ with C-6′ (δ_C 146.0) in the HMBC spectrum. The stereochemistry at C-1′ was deduced from specific rotation.[26] The positions of hydroxyl groups and ether linkages between two benzylisoquinline units were assigned largely on the basis of biogenetic considerations.[27,28] Alternate arrangements of ether linkages between C-6/C-8′, C-7/C-7′ and C-12/C-12′ have never been reported yet in the literature for bisbenzylisoquinoline alkaloids with three diaryl ether linkages. Thus the structure of compound **1** was established as kurramine 2′-β-*N*-oxide.

Compound **2** was isolated as a yellow powder. UV absorption maxima at 201, 228, 264, 290 and 333 nm, characteristic of a bisbenzylisoquinoline alkaloid.[10] The IR spectrum showed absorptions at 3395 (OH), 1575 (C=N) and 1507, 1457 (C=C) cm^{-1}. The mass spectrum of **2** was very similar to that of compound **1**. However, these two compounds showed considerable difference on TLC and *Rf* values. The HR-EI-MS of **2** showed the M^+ at *m/z* 548.2128 ($C_{33}H_{28}N_2O_6$, Calcd 548.2135) with a prominent $[M-16]^+$ ion resulting from the loss of an oxygen atom from the M^+, a characteristic feature of an *N*-oxide.[15—18]

The ^{13}C-NMR spectrum of compound **2** exhibited thirty-three carbon signals (Table 2). All the spectral characterization of compound **2** was very close to that of compound **1** except the protons of the methyl group linked to 2′-*N*-oxide, which resonated upfield more than in the case of **1**. Relative to the chemical shift of the 2′-*N*-methyl signal of kurramine (δ_H 2.57),[4] the corresponding signals in compounds **1** (δ_H 3.64) and **2** (δ_H 3.16) deshielded because of the 2′-*N*-oxide group. The presence of 2′-*N*-oxide moiety also caused a mark deshielding of C-1′ proton in both cases. The 2′-*N*-CH_3 position was inferred as β in compound **2** because of the smaller downfield shift of 2′-*N*-CH_3 in the ^{1}H-NMR spectrum, which showed the H-1′ was on the same side as the 2′-*N*-oxide oxygen atom.[21,24] H-1′ (δ_H 4.34) also showed ^3J HMBC correlations with C-3′ (Fig. 2). The positions of OH subsitituents and ether linkages were assigned on the basis of spectroscopic studies and biogenetic considerations.[27,28] The stereochemistry at C-1′ was also confirmed with the help of specific rotation measurement.[26] The structure of compound **2** was inferred as kurramine-2′-α-*N*-oxide.

Table 3. Summary of the *in Vitro* Anticholinesterase Activities of the Compounds **1**—**5**

Compound	IC_{50} (μM)±S.E.M.[a]	
	AChE	BChE
1	10.0±0.5	Inactive
2	150.0±2.5	Inactive
3	116.5±2.5	183.0±3.0
4	47.6±1.5	6.1±0.25
5	100.0±1.2	12.0±0.5
Galanthamine[b]	0.5±0.001	8.1±0.02
Eserine[c]	0.04±0.0001	0.857±0.001

a) Standard mean error of five assays. *b*, *c*) Standard inhibitors of acetyl- and butyrylcholinesterase.

Compounds **1** and **2** were separately subjected to *N*-oxide reduction (Zinc powder in 10% HCl, stirred at room temperature for 2 h). Resulting products were found to be identical in all respects (TLC and spectral data). This observation further supported the isomeric nature of two compounds.

The other known compounds, 1,2-dehydroapateline (**3**),[4] cocsoline (**4**)[4] and cocsuline (**5**),[4] have also been isolated from this plant and structurally characterized by comparison of their spectral data with the literature values.

Compounds **1**—**5** were screened for their anticholinesterase activity in a mechanism-based assay. Compounds **4** (IC_{50} 6.1 μM) and **5** (IC_{50} 12.0 μM) were found to be active against butyrylcholinesterase, while compounds **1** (IC_{50} 10.0 μM) and **4** (IC_{50} 47.6 μM) have inhibited acetylcholinesterase significantly. Galanthamine and eserine were use as standard inhibitors for comparison purpose (Table 3).

Experimental

General Experimental Procedures The UV spectra were measured on a Hitachi U-3200 spectrophotometer and the IR spectra were recorded on a Jasco A-302 spectrophotometer in $CHCl_3$. Optical rotations were taken with a JASCO DIP-360 automatic digital polarimeter. The ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker AM 400 and 500 AM spectrometers using tetramethylsilane as an internal standard. The EI-MS was recorded with a Finnigan MAT-312 double focusing mass spectrometer. The HR-EI-MS were

recorded on Jeol JMS 600 and HX 110 mass spectrometers with the data system DA 5000. Column chromatography (CC) was carried out on silica gel, 70—230 mesh. TLC purification was carried out on pre-coated TLC plates (silica gel F-254, 0.5 mm, E·Merck). TLC plates were monitored under UV light at 254 and 366 nm, and by staining with Dragendorff's spraying reagent.

Plant Material The plant material of *Cocculus pendulus* (J. R. & G. FORST.) (38 kg) was collected in October 2001 from Karachi, Pakistan. A voucher specimen (under accession voucher number KUH 67989) was deposited in the herbarium of the Department of Botany, University of Karachi.

Extraction and Isolation The air-dried plant material (15 kg) was crushed and soaked in EtOH (50 l) for 30 d. The EtOH extract was evaporated and the residue (987 g) dissolved in distilled water (5.0 l) and extracted with petroleum ether (21.0 g; A), $CHCl_3$ at pH 3 (6.0 g; B), $CHCl_3$ at pH 7 (54.6 g; C) and pH 9 (63.6 g; D) and finally with EtOAc (35.0 g; E). The crude ethanolic extract of *C. pendulus* exhibited promising anticholinesterase activity (78.0%, inhibition against BChE and 53.0% inhibition against AChE). Based on these results, Fr. C and D were subjected to bioassay-guided repeated column chromatography (silica gel) and prep. TLC which resulted in the isolation of two new **1** and **2** and three known **3**—**5** alkaloids. Fraction D was loaded on a column packed with silica gel. Elution was carried out with increasing polarities of $CHCl_3$–MeOH to give eight fractions (Fr. D1—D8). Fraction D2—D5 exhibited 98.6% inhibition against BChE and 57.0% inhibition against AChE. These fractions were individually subjected to flash column chromatography (silica gel), using increasing polarities of $CHCl_3$–MeOH to semi-impure subfractions that were finally purified by prep. TLC (precoated silica gel glass plates, MeOH : $CHCl_3$: NH_4OH 2.5 : 6.9 : 0.6, MeOH : $CHCl_3$: NH_4OH 1.5 : 7.9 : 0.6) to afford two new compounds **1** and **2**, respectively.

Fraction C was also subjected to column chromatography and eluted with increasing polarities of $CHCl_3$–MeOH (0—100%) to afford ten fractions (Fr. C1—C10). These fractions showed significant inhibitory cholinesterase activity (90.0% inhibition against BChE and 56.5% inhibition against AChE) and were subjected to flash column chromatography [silica gel, $CHCl_3$–MeOH (0—100%)]. The resulting fractions were purified by prep. TLC [precoated silica gel glass plates (MeOH : $CHCl_3$: NH_4OH 0.5 : 9.45 : 0.05)] to obtain three known compounds **3**—**5**. The anticholinesterase activities of all the pure compounds were determined by the method describe below and results are summarized in Table 3.

***In Vitro* Cholinesterase Inhibition Assay** Electric-eel AChE (EC 3.1.1.7), horse-serum BChE (EC 3.1.1.8), acetylthiocholine iodide, butyrylthiocholine chloride, 5,5′-dithiobis[2-nitrobenzoic acid] (DTNB), eserine[(−)-physostigmine] and galanthamine were purchased from Sigma (St. Louis, MO, U.S.A.). Buffer and other chemicals were of analytical grades. Acetylcholinesterase and butyrylcholinesterase inhibiting activities were measured by slightly modifying the spectrophotometric method developed by Ellman *et al.*[29] Acetylthiocholine iodide and butyrylthiocholine chloride were used as substrates to assay AChE and BChE, respectively. The 5,5′-dithiobis[2-nitrobenzoic acid] (DTNB) was used for the measurement of cholinesterase activity. 140 μl of 100 mM sodium phosphate buffer (pH 8.0), 10 μl of DTNB, 20 μl of test-compound solution and 20 μl of AChE or BChE solution were mixed and incubated for 15 min at 25 °C. The reaction was then initiated by the addition of 10 μl acetylthiocholine or butyrylthiocholine, respectively. The hydrolysis of these substrates was monitored spectrophotometrically by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine and butyrylthiocholine, respectively, at a wavelength of 412 nm for 15 min. Test compounds and the control were dissolved in MeOH. All the reactions were performed in triplicate in a 96 well micro-plate on *SpectraMax 340* (Molecular Devices, U.S.A.).

Estimation of IC_{50} Values The concentrations of test compounds that inhibited the hydrolysis of substrate (acetylthiocholine) by 50% (IC_{50}) were determined by monitoring effects of increasing concentrations of these compounds in the assays on the inhibition values. The IC_{50} values were then calculated using with the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, U.S.A.).

Kurramine-2′-β-*N*-oxide (**1**): Yellow amorphous powder (4.7 mg, 4.76×10^{-4}% yield); $[\alpha]_D^{25}$ +60° (c=0.01, MeOH); IR ($CHCl_3$) cm^{-1}: 3620 (OH), 1575 (C=N), 1507 (C=C) and 1458 (C=C) cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 203 (4.61), 235 (4.41), 263 (4.02), 291 (3.81), 341 (3.52); *Rf*: 0.17 (MeOH : $CHCl_3$: NH_4OH, 2.5 : 6.9 : 0.6); ^{1}H- and ^{13}C-NMR data (CD_3OD): Tables 1 and 2; HR-EI-MS *m/z*: 548.2130 (M^+) (Calcd for $C_{33}H_{28}N_2O_6$: 548.2135); EI-MS *m/z* (rel. int., %): 548 (7), 532 (80), 531 (100), 319 (18), 107 (4).

Kurramine-2′-α-*N*-oxide (**2**): Yellow amorphous powder (4.5 mg, 4.55×10^{-4}% yield); $[\alpha]_D^{25}$ +50° (c=0.012, MeOH); IR ($CHCl_3$) cm^{-1}: 3395 (OH), 1575 (C=N), 1507 and 1457 (C=C); UV λ_{max} (MeOH) nm (log ε): 201 (4.58), 228 (4.45), 264 (4.10), 290 (3.80), 333 (3.66); *Rf*: 0.21 (MeOH : $CHCl_3$: NH_4OH, 1.5 : 7.9 : 0.6); ^{1}H- and ^{13}C-NMR data (CD_3OD): Tables 1 and 2; HR-EI-MS *m/z*: 548.2128 (M^+) (Calcd for $C_{33}H_{28}N_2O_6$: 548.2135); EI-MS *m/z* (rel. int., %): 548 (8), 532 (83), 531 (100), 319 (12), 107 (5).

1,2-Dehydroapateline (**3**): Yellow amorphous powder (5.2 mg, 5.26×10^{-4}% yield); $[\alpha]_D^{25}$ +128° (c=0.42, MeOH); IR ($CHCl_3$) cm^{-1}: 3340 (OH), 1578 (C=N), 1508 and 1455 (C=C); UV λ_{max} (MeOH) nm (log ε): 230 (4.23), 270 (4.12), 288 (3.34), 333 (3.42); *Rf*: 0.18 ((MeOH : $CHCl_3$: NH_4OH 0.5 : 9.45 : 0.05); ^{1}H- and ^{13}C-NMR data ($CDCl_3$+CD_3OD): Tables 1 and 2; HR-EI-MS *m/z*: 546.3525 (M^+) (Calcd for $C_{34}H_{30}N_2O_5$: 546.3530); EI-MS *m/z* (rel. int., %): 546 (77), 545 (100), 335 (6), 273 (11), 175 (3), 107 (6).

Cocsoline (**4**): White amorphous powder (51.0 mg, 5.16×10^{-3}% yield); $[\alpha]_D^{25}$ +205° (c=0.15, $CHCl_3$); IR ($CHCl_3$) cm^{-1}: 3470 (OH), 1501 and 1451 (C=C); UV λ_{max} (MeOH) nm (log ε): 233 (4.35), 235 (4.34), 274 (3.52), 288 (3.51); *Rf*: 0.26 (MeOH : $CHCl_3$: NH_4OH, 0.5 : 9.45 : 0.05); ^{1}H- and ^{13}C-NMR data ($CDCl_3$+CD_3OD): Tables 1 and 2; HR-EI-MS *m/z*: 548.2128 (M^+) (Calcd for $C_{34}H_{32}N_2O_5$: 548.2135); EI-MS *m/z* (rel. int., %): 548 (19), 335 (34), 83 (100), 168 (25).

Cocsuline (**5**): Yellow amorphous powder (14.0 mg, 1.41×10^{-3}% yield); $[\alpha]_D^{25}$ +275° (c=0.30, MeOH); IR ($CHCl_3$) cm^{-1}: 3552 (OH), 1510 and 1452 (C=C); UV λ_{max} (MeOH) nm (log ε): 230 (4.30), 239 (4.41), 278 (3.58), 288 (3.56); *Rf*: 0.28 (MeOH : $CHCl_3$: NH_4OH, 0.5 : 9.45 : 0.05); ^{1}H- and ^{13}C-NMR data ($CDCl_3$+CD_3OD): Tables 1 and 2; HR-EI-MS *m/z*: 562.2541 $[M]^+$ (Calcd for $C_{35}H_{34}N_2O_5$: 562.2467); EI-MS *m/z* (rel. int., %): 562 (20), 349 (100), 175 (85), 83 (5).

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