Conomicidines A and B, Unusual Alkaloid – Hydroxycinnamyl Alcohol Conjugates from *Tabernaemontana corymbosa*

by **Kuan-Hon Lim** and **Toh-Seok Kam***

Department of Chemistry, University of Malaya, 50603 Kuala Lumpur, Malaysia
(phone: +603-79674266; fax: +603-9674193; e-mail: tskam@um.edu.my)

Conomicidines A and B (**1a** and **2a**, resp.) together with the diastereomeric isoconomicidines A and B (**1b** and **2b**, resp.) were isolated from *Tabernaemontana corymbosa* as unresolvable mixtures of the (1′*S*,2′*S*)- and (1′*R*,2′*R*)-diastereoisomers (*i.e.*, **1a** and **1b**; **2a** and **2b**). These novel natural products are constituted from the union of an iboga alkaloid, ibogaine, and hydroxycinnamyl alcohol moieties, and represent the first examples of such alkaloid – hydroxycinnamyl alcohol conjugates. The structures were determined by spectroscopic methods, including the extensive use of NOE experiments for the assignment of the configuration.

Introduction. – Plants of the genus *Tabernaemontana* are rich sources of structurally novel, as well as biologically active, indole and bisindole alkaloids [1–4]. In recent years a number of alkaloids of unusual structures have been reported from plants of this genus. The Malayan *T. corymbosa* for instance provided several new alkaloids which are characterized by novel molecular skeletons [5–7] in addition to biologically active indole and bisindole alkaloids [8–17]. In a recent study of a different sample of the same plant collected from a different location, we isolated a hexacyclic indole from the stem-bark extract, conolutinine, which was characterized by a novel ring system incorporating a diazaspiro center and fused oxadiazepine-tetrahydrofuran rings [18]. In addition, we also reported the structure of the unprecedented iboga – lignan conjugate, conoliferine, which was obtained as an unresolvable mixture of the (1′*S*,2′*S*)- and (1′*R*,2′*R*)-diastereomers (**3a** and **3b**, resp.) from the stem-bark extract [19]. We now report the isolation of additional new alkaloids, conomicidines A and B (**1a** and **1b**, and **2a** and **2b**, resp.) which represent the first examples of alkaloid – hydroxycinnamyl alcohol conjugates, which were isolated from the stem-bark extract of the same plant.

Results and Discussion. – Conomicidine A and isoconomicidine A (**1a** and **1b**, resp.) were obtained as an approximately 1:1 mixture of the (1′*S*,2′*S*)- and (1′*R*,2′*R*)-diastereoisomers, which, like the conoliferines (**3a** and **3b**), was intractable to further resolution by chromatography. The UV spectrum resembled that of the conoliferines with absorption maxima at 207, 232, 291, and 299 nm, while the IR spectrum showed bands due to OH (3534 cm^{-1}) and NH (3371 cm^{-1}) functions. The EI-MS showed a molecular ion at *m*/*z* 506 which indicated the molecular formula $C_{30}H_{38}N_2O_5$, requiring 13 degrees of unsaturation. Other significant fragment ions were observed at *m*/*z* 488 ($[M - H_2O]^+$), 445 ($[C_{28}H_{33}N_2O_3]^+$), and 309 ($[C_{20}H_{25}N_2O]^+$).

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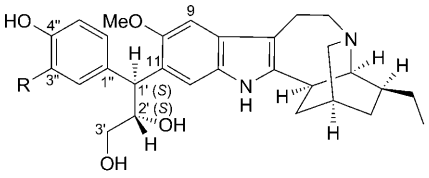

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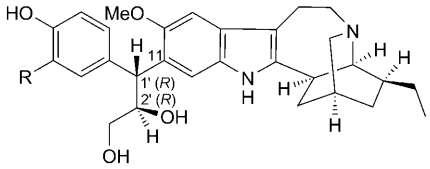
2a R = H



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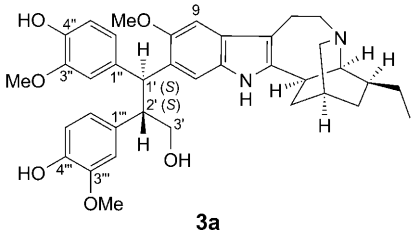
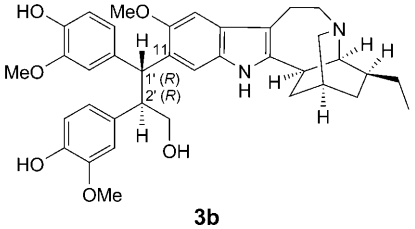


3a



3b

As in the case of the conoliferines (**3a** and **3b**), extensive overlap (complete coincidence) of most of the signals for both the diastereoisomers **1a** and **1b** was observed in the ^{1}H- and ^{13}C-NMR spectra. In addition, signals that were not completely coincident showed very similar chemical shifts (average $\Delta\nu$ for all paired ^{1}H and ^{13}C signals are 0.015 and 0.09 ppm, resp.) and were, therefore, not distinguishable. When variable-temperature NMR studies were carried out (^{1}H-NMR, (D_6)benzene, r.t. to 70°; (D_8)toluene, r.t. to 100°), the ^{1}H-NMR spectrum was essentially unchanged without any signs of coalescence at the higher temperatures applied.

The ^{1}H-NMR spectrum of **1** showed a general similarity with that of the conoliferines (**3**) [19], except for the absence of three aromatic H-atoms and an aromatic MeO group attributed to one of the 4-hydroxy-3-methoxyphenyl moieties. This was also consistent with the ^{13}C-NMR data, which showed the absence of six aromatic C-atoms (three CH groups, two O-bearing quaternary C-atoms, and one quaternary C-atom) and an aromatic MeO group when compared to **1**. The presence of an O-bearing CH (δ(C) 74.2) coupled with the observed downfield shift of H–C(2′) from δ(H) 3.65 in **3** to δ(H) 4.41–4.47 suggested that the 4-hydroxy-3-methoxyphenyl moiety attached to C(2′) in **3** has been replaced by a OH group in **1**. Apart from this, the structure is essentially similar to **3**. This is in agreement with the molecular formula of **1** as well as the HMBC data (*Fig.*), which showed heteronuclear correlations from H–C(1′) to C(10), C(11), C(12), C(2′), C(3′), C(1″), and C(6″). These observations are consistent with the presence of an ibogaine unit, substituted at C(11) by a modified phenylpropane unit *via* C(1′). This unit can be defined as 2,3-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)propyl. Other correlations are in agreement with the proposed structure. The structure is also consistent with the observed mass spectral fragments. The *m*/*z* 445 and 309 peaks are attributed to fragments arising from scission of the C(1′)–C(2′) and C(1′)–C(11) bonds, respectively.

In the case of **1** (and **2**), the observed J(1′,2′) coupling constant observed was about 8 Hz (*cf. ca.* 12 Hz in **3**). Such a magnitude for J(1′,2′) suggested three possibilities. First, there is no barrier to free rotation about the C(1′)–C(2′) bond, alternatively the

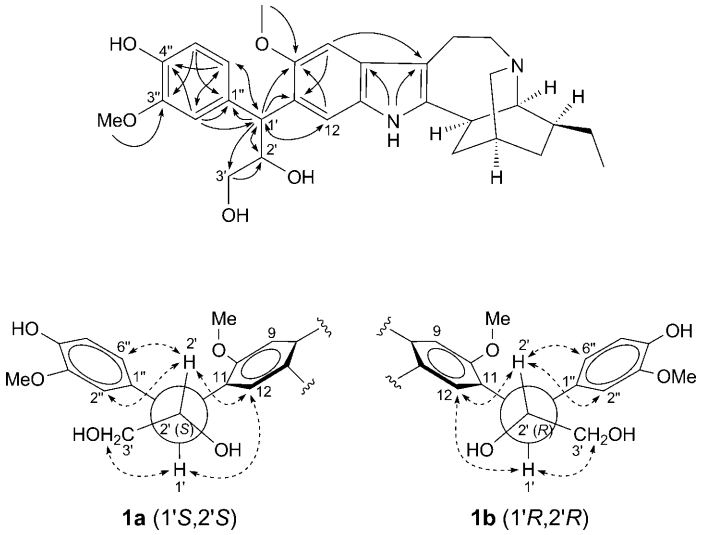


Figure. *Selected HMBCs and NOEs of* **1** (→ HMBC, ←- - -→ NOE)

H–C(1′)–C(2′)–H dihedral angle is either 0° or *ca.* 160°. The first alternative can be discounted since certain NOEs were observed (*vide infra*) which seemed to suggest the existence of a preferred conformation. The second requires an eclipsed conformation which is energetically unfavorable and which is also ruled out by the absence of an NOE between H–C(1′) and H–C(2′). This leaves the third possibility which best fits the NOE results. The observed coupling value, therefore, corresponds to a dihedral angle which is close to 180°, suggesting a virtually *anti*-arrangement of the two vicinal H-atoms. As in the case of the conoliferines (**3**), no NOE between H–C(1′) and H–C(2′) was observed, requiring these two H-atoms to be directed away from each other. Instead, reciprocal NOEs were observed between H–C(12) and H–C(1′), and between H–C(12) and H–C(2′), while reciprocal NOEs were not observed between H–C(12) and H–C(3′) (this is in contrast to **3** where the H–C(12) and H–C(3′) reciprocal NOEs were observed) (*Fig.*). These observations are consistent with structures **1a** (1′*S*,2′*S*) and **1b** (1′*R*,2′*R*), while ruling out structures **1c** (1′*S*,2′*R*) and **1d** (1′*R*,2′*S*). Conomicidine A and isoconomicidine A, therefore, correspond to the diastereoisomers **1a** and **1b**, respectively.

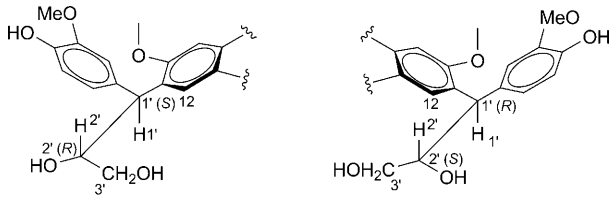


Table 1. *^{1}H-NMR Data for Compounds* **1a**, **1b**, **2a**, *and* **2b** (400 MHz, $CDCl_3$)[a]). δ in ppm, J in Hz.

	1a	**1b**	**2a**	**2b**
CH_2(3)	2.94–3.00 (*m*), 3.01–3.07 (*m*)	2.94–3.00 (*m*), 3.01–3.07 (*m*)	2.94–2.98 (*m*), 3.00–3.06 (*m*)	2.94–2.98 (*m*), 3.00–3.06 (*m*)
CH_2(5)	3.09–3.15 (*m*), 3.30 (*dt*, *J* = 16.3, 4.4)	3.09–3.15 (*m*), 3.30 (*dt*, *J* = 16.3, 4.4)	3.07–3.15 (*m*), 3.30–3.38 (*m*)	3.07–3.15 (*m*), 3.30–3.38 (*m*)
CH_2(6)	2.55–2.62 (*m*), 3.34–3.40 (*m*)	2.55–2.62 (*m*), 3.34–3.40 (*m*)	2.54–2.60 (*m*), 3.25–3.35 (*m*)	2.54–2.60 (*m*), 3.25–3.35 (*m*)
H–C(9)	6.93 (*s*)	6.93 (*s*)	6.91 (*s*)	6.91 (*s*)
H–C(12)	7.17 (*s*)	7.17 (*s*)	7.17 (*s*)[b])	7.18 (*s*)[b])
H–C(14)	1.79–1.86 (*m*)	1.79–1.86 (*m*)	1.78–1.84 (*m*)	1.78–1.84 (*m*)
CH_2(15)	1.15–1.25 (*m*), 1.76–1.82 (*m*)	1.15–1.25 (*m*), 1.76–1.82 (*m*)	1.16–1.22 (*m*), 1.75–1.81 (*m*)	1.16–1.22 (*m*), 1.75–1.81 (*m*)
H–C(16)	2.84–2.90 (*m*)	2.84–2.90 (*m*)	2.80–2.88 (*m*)	2.80–2.88 (*m*)
CH_2(17)	1.57–1.63 (*m*), 1.97–2.06 (*m*)	1.57–1.63 (*m*), 1.97–2.06 (*m*)	1.49–1.55 (*m*), 1.96–2.06 (*m*)	1.49–1.55 (*m*), 1.96–2.06 (*m*)
Me(18)	0.88 (*t*, *J* = 7)[c])	0.89 (*t*, *J* = 7)[c])	0.875 (*t*, *J* = 7.1)[d])	0.881 (*t*, *J* = 7.1)[d])
CH_2(19)	1.44–1.50 (*m*), 1.53–1.59 (*m*)	1.44–1.50 (*m*), 1.53–1.59 (*m*)	1.44–1.50 (*m*), 1.56–1.62 (*m*)	1.44–1.50 (*m*), 1.56–1.62 (*m*)
H–C(20)	1.50–1.56 (*m*)	1.50–1.56 (*m*)	1.49–1.55 (*m*)	1.49–1.55 (*m*)
H–C(21)	2.81 (br. *s*)[e])	2.83 (br. *s*)[e])	2.79 (*s*)[f])	2.81 (*s*)[f])
10-OMe	3.852 (*s*)[g])	3.855 (*s*)[g])	3.819 (*s*)[h])	3.823 (*s*)[h])
NH	7.62 (br. *s*)[i])	7.63 (br. *s*)[i])	7.62 (br. *s*)[j])	7.64 (br. *s*)[j])
H–C(1′)	4.59 (*d*, *J* = 7.8)[k])	4.61 (*d*, *J* = 7.8)[k])	4.59 (*d*, *J* = 8.2)[l])	4.60 (*d*, *J* = 8.2)[l])
H–C(2′)	4.41–4.47 (*m*)	4.41–4.47 (*m*)	4.41–4.46 (*m*)	4.41–4.46 (*m*)
CH_2(3′)	3.50 (*dd*, *J* = 11.2, 6.4), 3.591 (*dd*, *J* = 11.5, 3.9)[m])	3.50 (*dd*, *J* = 11.2, 6.4), 3.595 (*dd*, *J* = 11.5, 3.9)[m])	3.48 (*dd*, *J* = 11, 7.6), 3.574 (*dd*, *J* = 11, 2.7)[n])	3.48 (*dd*, *J* = 11, 7.6), 3.582 (*dd*, *J* = 11, 2.7)[n])
H–C(2″)	6.86 (*d*, *J* = 1.7)[o])	6.88 (*d*, *J* = 1.7)[o])	7.16 (*d*, *J* = 8)	7.16 (*d*, *J* = 8)
H–C(3″)			6.67 (*d*, *J* = 8)	6.67 (*d*, *J* = 8)
H–C(5″)	6.779 (*d*, *J* = 8)[p])	6.784 (*d*, *J* = 8)[p])	6.67 (*d*, *J* = 8)	6.67 (*d*, *J* = 8)
H–C(6″)	6.84 (*dd*, *J* = 8, 1.7)[q])	6.85 (*dd*, *J* = 8, 1.7)[q])	7.16 (*d*, *J* = 8)	7.16 (*d*, *J* = 8)
3″-OMe	3.816 (*s*)[r])	3.824 (*s*)[r])		

[a]) Assignments based on COSY and HMQC. [b–r]) Assignments are interchangeable.

Conomicidine B and isoconomicidine B (**2a** and **2b**, resp.) were also obtained as a 1:1 mixture of two diastereoisomers which could not be separated by chromatography. The UV spectrum showed indole absorption maxima at 231, 289, and 300 nm, while the IR spectrum indicated the presence of OH/NH functions at 3393 and 3340 cm^{-1}. The EI-MS showed a molecular ion at *m/z* 476, consistent with the molecular formula $C_{29}H_{36}N_2O_4$, differing from **1** by 30 mass units. Other significant fragments in the mass spectrum were observed at *m/z* 458 ($[M - H_2O]^+$), 429 ($[M - H_2O - Et]^+$), 415 ($[M - CH(OH)CH_2(OH)]^+$), and 309 ($[C_{20}H_{25}N_2O]^+$). Comparison of the NMR data of **2** with those of **1** (*Tables 1* and *2*) revealed that the two compounds have essentially the same structure, except that the aromatic MeO group in the phenolic moiety at position 3″ in **1** was replaced by a H-atom in **2**. This was evident from the ^{1}H-NMR spectrum of **2**, which showed the presence of a pair of *AB doublet*s (each *doublet* integrating for 2 H) attributed to the chemically equivalent pairs H–C(2″)/H–C(6″) and H–C(3″)/

Table 2. *^{13}C-NMR Data for Compounds* **1a**, **1b**, **2a**, *and* **2b** (100 MHz, $CDCl_3$)[a]). δ in ppm, *J* in Hz.

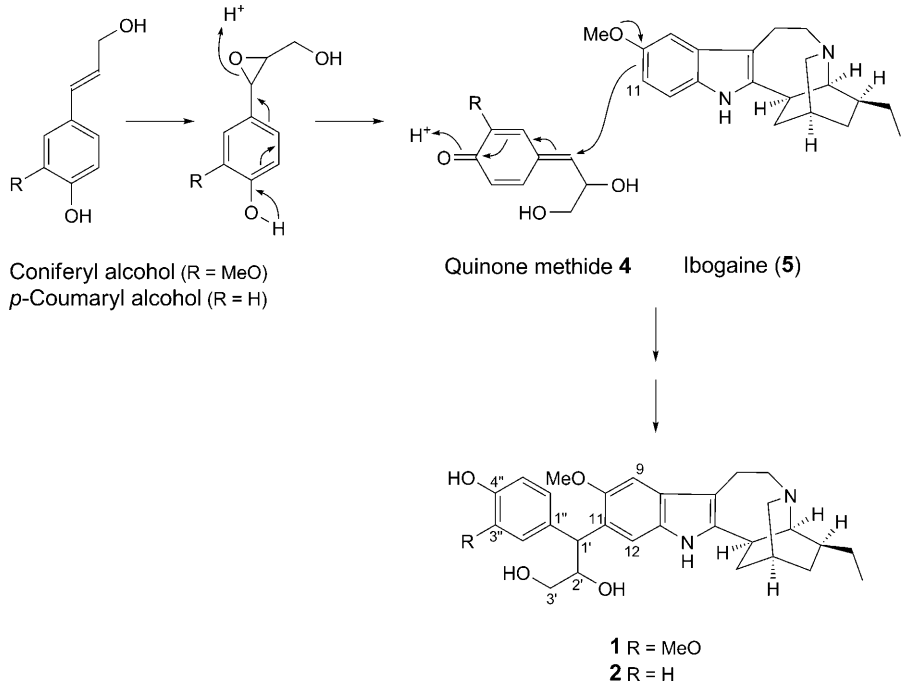
	1a	**1b**	**2a**	**2b**
C(2)	143.1	143.1	142.69[b])	142.80[b])
C(3)	50.0	50.0	49.80[c])	49.88[c])
C(5)	54.3	54.3	54.4	54.4
C(6)	20.8	20.8	20.6	20.6
C(7)	108.9	108.9	108.24[d])	108.29[d])
C(8)	128.7	128.7	128.2	128.2
C(9)	100.1	100.1	100.1	100.1
C(10)	152.1	152.1	152.0	152.0
C(11)	123.8	123.8	124.3	124.3
C(12)	110.71[e])	110.76[e])	110.5	110.5
C(13)	129.8	129.8	129.86[f])	129.90[f])
C(14)	26.5	26.5	26.3	26.3
C(15)	32.1	32.1	31.8	31.8
C(16)	41.4	41.4	41.0	41.0
C(17)	34.3	34.3	34.0	34.0
C(18)	12.0	12.0	12.0	12.0
C(19)	27.9	27.9	27.7	27.7
C(20)	42.0	42.0	41.9	41.9
C(21)	57.66[g])	57.73[g])	57.9	57.9
10-OMe	56.5	56.5	56.61[h])	56.63[h])
C(1′)	46.47[i])	46.65[i])	46.02[j])	46.14[j])
C(2′)	74.2	74.2	74.2	74.2
C(3′)	65.5	65.5	65.3	65.3
C(1″)	134.5	134.5	133.8	133.8
C(2″)	111.6	111.6	129.5	129.5
C(3″)	146.5	146.5	115.3	115.3
C(4″)	144.1	144.1	154.8	154.8
C(5″)	114.4	114.4		
C(6″)	121.05[k])	121.11[k])		
3″-OMe	56.0	56.0		

[a]) Assignments based on COSY and HMQC. [b–k]) Assignments are interchangeable.

H–C(5″). The proposed structure is in full agreement with the HMBC data. Conomicidine B and isoconomicidine B showed similar $J(1',2')$ values (8.2 Hz) as well as NOEs as those observed for **1** and were, therefore, assigned the structures **2a** (1′*S*,2′*S*) and **2b** (1′*R*,2′*R*).

Conomicidines A and B (**1** and **2**, resp.) represent the first examples of monoterpene indole alkaloids linked to hydroxycinnamyl alcohol moieties, and are related to the iboga–lignan conjugates **3**, which were also isolated from this plant. Hydroxycinnamyl alcohols are a group of naturally occurring compounds that are often associated with the biosynthesis of lignins in plants. A possible biogenetic origin of **1** and **2** is shown in the *Scheme* and involves a nucleophilic attack by ibogaine (**5**) on the quinone methide **4**, derived from ring opening of an oxirane, in turn formed through oxidation of the coniferyl/*p*-coumaryl alcohol precursor.

Scheme. *A Possible Biogenetic Origin of* **1** *and* **2**



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Experimental Part

1. *General.* UV Spectra: *Shimadzu UV-3101PC* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Perkin-Elmer RX1* FT-IR spectrophotometer in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Jeol JNM-LA-400* spectrometer at 400 and 100 MHz, resp.; in $CDCl_3$ solns.; with Me_4Si as internal standard; δ in ppm, J in Hz. MS measurements were carried out at OIC Organic Mass Spectrometry, University of Tasmania, Tasmania, Australia.

2. *Plant Material*. The plant material was collected in Pahang, Malaysia (June, 2003) and identification was confirmed by Dr. *K. M. Wong*, Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia. Herbarium voucher specimens (K 667) are deposited at the Herbarium, University of Malaya.

3. *Extraction and Isolation*. Extraction of the stem-bark material was carried out in the usual manner by partitioning the conc. EtOH extracts with dil. acid as have described in [20]. The alkaloids were isolated by initial CC (SiO_2; CH_2Cl_2 with increasing proportions of MeOH), followed by rechromatography of the appropriate partially resolved fractions using CC or prep. centrifugal TLC. Initial CC of the basic fraction from the stem-bark extract provided essentially nine fractions. Conomicidine A and isoconomicidine A (**1a** and **1b**, resp.; 3 mg kg^{-1}) were obtained from *Fr. 7* after CC (SiO_2; CH_2Cl_2/MeOH) and prep. centrifugal TLC (SiO_2; AcOEt/hexanes, 0.5% NH_3). Conomicidine B and isoconomicidine B (**2a** and **2b**, resp.; 0.5 mg kg^{-1}) were obtained from *Fr. 8* following CC (SiO_2; CH_2Cl_2/MeOH) and two successive prep. centrifugal TLC (SiO_2; AcOEt/hexanes, 0.5% NH_3).

Conomicidine A and Isoconomicidine A (*= (2S,3S)-3-(4-Hydroxy-3-methoxyphenyl)-3-[(2α)-12-methoxyibogamin-13-yl]propane-1,2-diol*; **1a**, *and (2R,3R)-3-(4-Hydroxy-3-methoxyphenyl)-3-[(2α)-12-methoxyibogamin-13-yl]propane-1,2-diol*; **1b**). Colorless oil. UV (EtOH): 207 (4.47), 232 (4.40), 291 (4.01), 299 (4.01). IR (dry film): 3534, 3371. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 506 (82, M^+), 488 (5, $[M - H_2O]^+$), 472 (14), 445 (82, $[M - CH(OH)CH_2(OH)]^+$), 387 (12), 360 (21), 309 (12, $[M - C_{10}H_{13}O_4]^+$), 223 (12), 149 (52), 136 (100), 122 (13), 57 (23). HR-EI-MS: 506.2774 ($C_{30}H_{38}N_2O_5^+$; calc. 506.2781).

Conomicidine B and Isoconomicidine B (*= (2S,3S)-3-(4-Hydroxyphenyl)-3-[(2α)-12-methoxyibogamin-13-yl]propane-1,2-diol*; **2a**, *and (2R,3R)-3-(4-Hydroxyphenyl)-3-[(2α)-12-methoxyibogamin-13-yl]propane-1,2-diol*; **2b**). Colorless oil. UV (EtOH): 231 (4.59), 289 (4.14), 300 (4.17). IR (dry film): 3393, 3340. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 476 (17, M^+), 458 (8, $[M - H_2O]^+$), 429 (20, $[M - H_2O - Et]^+$), 415 (50, $[M - CH(OH)CH_2(OH)]^+$), 357 (12), 330 (16), 309 (11, $[M - C_9H_{11}O_3]^+$), 252 (25), 237 (13), 208 (13), 149 (55), 136 (100), 122 (38), 107 (22). HR-EI-MS: 476.2668 ($C_{29}H_{36}N_2O_4^+$; calc. 476.2675).

REFERENCES

[1] T. A. Van Beek, R. Verpoorte, A. Baerheim Svendsen, A. J. M. Leeuwenberg, N. G. Bisset, *J. Ethnopharmacol.* **1984**, *10*, 1.
[2] B. Danieli, G. Palmisano, in 'The Alkaloids', Ed. A. Brossi, Academic Press, Orlando, 1986, Vol. 27, Chap. 1, pp. 1–130.
[3] T.-S. Kam, Y.-M. Choo, in 'The Alkaloids', Ed. G. A. Cordell, Academic Press, Amsterdam, 2006, Vol. 63, Chap. 4, pp. 181–337.
[4] T.-S. Kam, in 'Alkaloids: Chemical and Biological Perspectives', Ed. S. Pelletier, Pergamon, Amsterdam, 1999, Vol. 14, Chap. 2, pp. 285–435.
[5] T.-S. Kam, K.-M. Sim, T.-M. Lim, *Tetrahedron Lett.* **1999**, *40*, 5409.
[6] T.-S. Kam, K.-M. Sim, T.-M. Lim, *Tetrahedron Lett.* **2000**, *41*, 2733.
[7] T.-S. Kam, K.-M. Sim, T.-M. Lim, *Tetrahedron Lett.* **2001**, *42*, 4721.
[8] T.-S. Kam, K.-M. Sim, H.-S. Pang, T. Koyano, M. Hayashi, K. Komiyama, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4487.
[9] T.-S. Kam, K.-M. Sim, H.-S. Pang, *J. Nat. Prod.* **2003**, *66*, 11.
[10] T.-S. Kam, K.-M. Sim, *Helv. Chim. Acta* **2003**, *86*, 122.
[11] T.-S. Kam, K.-M. Sim, *Phytochemistry* **2003**, *63*, 625.
[12] T.-S. Kam, K.-M. Sim, *J. Nat. Prod.* **2002**, *65*, 669.
[13] T.-S. Kam, K.-M. Sim, *Helv. Chim. Acta* **2002**, *85*, 1027.
[14] T.-S. Kam, K.-M. Sim, *Heterocycles* **2002**, *57*, 2137.
[15] T.-S. Kam, K.-M. Sim, *Heterocycles* **2001**, *55*, 2405.
[16] T.-S. Kam, K.-M. Sim, T. Koyano, M. Toyoshima, M. Hayashi, K. Komiyama, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1693.

[17] T.-S. Kam, K.-Y. Loh, *Phytochemistry* **1993**, *32*, 1357.
[18] K.-H. Lim, T. Etoh, M. Hayashi, K. Komiyama, T.-S. Kam, *Tetrahedron Lett.* **2009**, *50*, 752.
[19] K.-H. Lim, T.-S. Kam, *Tetrahedron Lett.* **2009**, *50*, 3756.
[20] T.-S. Kam, P.-S. Tan, *Phytochemistry* **1990**, *29*, 2321.

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