TWO NOVEL SARPAGINE-TYPE INDOLE ALKALOIDS FROM THE LEAVES OF *HUNTERIA ZEYLANICA* IN THAILAND

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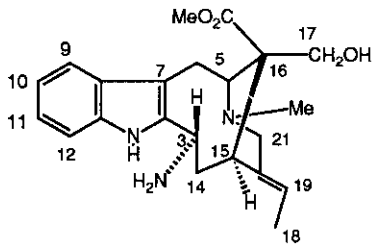
Abstract --- The chemical investigation of the leaves of *Hunteria zeylanica* (Retz) Gardn. ex Thw., collected in Thailand, resulted in the isolation of two new sarpagine-type indole alkaloids, N_b-methyl-3α-amino-*seco*-voacarpine and hunteriatryptamine, along with twelve known bases.

Hunteria zeylanica (Retz) Gardn. ex Thw. is a tropical plant of the family Apocynaceae. The latex of this plant has been traditionally used as a folk medicine for the treatment of sores of yaws.[1] By recent pharmacological studies of the crude alkaloidal fractions of the stem bark of this plant, their antinociceptive, antipyretic, and anti-inflammatory effects have been found.[2] In our previous paper we described the isolation and structure determination of the indole alkaloids from the stem bark[3] and the leaves[4] of this plant that was collected in Thailand. In our further studies on the alkaloidal constituents of the leaves of *Hunteria zeylanica* we have now isolated two new minor alkaloids and determined their structures.

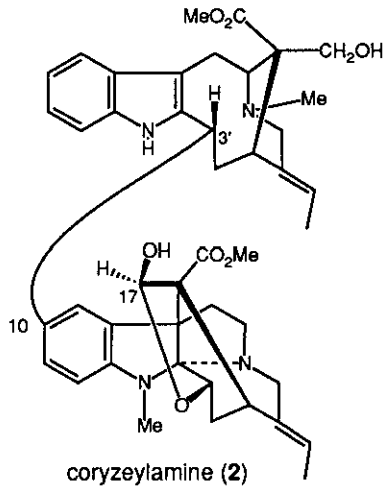
Seven alkaloids were isolated from the leaves of *Hunteria zeylanica* (Retz) Gardn. ex Thw., collected in Thailand in June 1992 (the first collection). They are corymine,[5] deformylcorymine,[6] coryzeylamine (**2**),[4a] deformylcoryzeylamine,[4a] fluorocarpamine,[7] lanceomigine,[8] and N_b-methyl-3α-amino-*seco*-voacarpine (**1**). Ten alkaloids were isolated from the leaves of the second collection in August, 1993 which included

corymine, deformylcoryzeylamine, coryzeylamine (**2**), N_a-demethylcorymine, N_a-demethyldeformylcorymine,[4b] pleiocarpamine,[9] tubotaiwine,[10] (17*S*)- and (17*R*)-17,4',5',6'-tetrahydrousambarenine,[11] and hunteriatryptamine (**3**). Among them, N_b-methyl-3α-amino-*seco*-voacarpine (**1**) and hunteriatryptamine (**3**) are new alkaloids.

The first new alkaloid (**1**) was obtained as a colorless solid, mp 154-155 °C. The uv spectrum (λ_{max} at 229.5 and 284.6 nm) displayed absorptions typical of an indole derivative. The ^{1}H nmr spectrum showed four aromatic adjacent signals, two 3H-singlet signals at δ 2.51 (N_bMe) and 2.38 (OMe), one singlet-methylene signal at δ 3.66 (H_2-17) and a signal due to one ethylidene side chain. Unambiguous assignments of all carbons and protons were obtained by using ^{1}H-^{1}H correlation spectroscopy (COSY), phase-sensitive heteronuclear single quantum coherence (PHSQC), and heteronuclear multiple bond connectivity (HMBC) spectra. The ^{13}C nmr spectrum of **1** showed almost superimposable peaks on those of the sarpagine-type moiety in coryzeylamine (**2**) (Table 1). The molecular formula of **1** ($C_{22}H_{29}N_3O_3$), obtained by HR-EIms (found 383.2208, required 383.2207, M^+) and HR-FABms (found 384.2288, required 384.2287, M^++H), showed the presence of an additional nitrogen atom in the molecule compared with the usual monoterpenoid indole alkaloids. The fact that this additional nitrogen atom is an -NH_2 group was evidenced by the fragment peak at 366.1942 (required 366.1942) in the HR-ms spectrum of **1**, resulting from a loses of an NH_3 molecule. The -NH_2 function should be located at the C-3 position, because the chemical shift of the methine carbon (C-3) in **1** is at δ 49.00, a shift position reasonable for a carbon having a primary amine substitution.[12] The stereochemistry of the C-16 position was proved to be *S* by a highly shifted methoxy group of the ester function (δ 2.38 ppm), which can be interpreted by the



N_b-methyl-3α-amino-*seco*-voacarpine (**1**)



coryzeylamine (**2**)

Figure 1

anisotropic effect of the indole ring. The α-orientation of the amino group at the C-3 position was elucidated by the coupling constant of H-3 (*dd*, J = 12.3, 3.3 Hz) and by the differential NOE experiments which showed the correlation between H-3 and N_aH (1.3%), and between H-3 and H-15 (1.9%). The differential NOE experiment also showed the correlation between H-19 and H-21β (4.1%) which demonstrated the *E* configuration of the ethylidene side chain. Therefore, **1** was concluded to be N_b-methyl-3α-amino-*seco*-voacarpine.

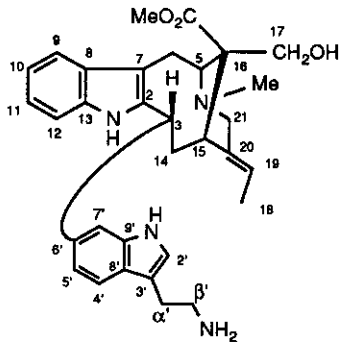
The second new alkaloid (**3**) was obtained as an amorphous powder. Its uv spectrum (222.1 and 285.1 nm) displayed absorptions typical of the indole nucleus. The ^{1}H nmr spectrum ($CDCl_3$ + 2 drops of CD_3OD) exhibited all the signals characteristic of the sarpagine-type unit in coryzeylamine (**2**), particularly two 3H-singlets at δ 2.55 and 2.35, one set of isolated methylene protons at δ 3.80 (*d*, J = 11.3 Hz) and 3.67 (*d*, J = 11.3 Hz), which are typical for NMe, CO_2Me and CH_2OH groups, respectively, in this series. In addition, signals accounting for nine other protons were observed, five of which were aromatic protons of the indole nucleus and the remaining four can be assigned to a -CH_2CH_2- chain. From these and ^{13}C nmr (*vide infra*) data, the presence of a second tryptamine unit was suggested. This information indicated that new alkaloid (**3**) should be a derivative of ceridimine (**4**).[13] Comparison of ^{1}H- and ^{13}C nmr spectra of **3** with those of **4**[13] indicated the presence of a CH_2OH function {δ 3.80 and 3.67 (H_2-17) and δ 70.41 (C-17)} in **3**. The molecular formula $C_{32}H_{38}N_4O_3$, obtained by HR-FABms (found: 527.3021, required: 527.3022), supported the above observation. Unambiguous assignment of all the carbons and protons was made by ^{1}H-^{1}H COSY, PHSQC, and HMBC spectra. The ^{13}C nmr signals of the second indole moiety appeared at δ 106.70 (C-3'), 110.84 (C-7'), 118.12 (C-4'), 119.33 (C-5'), 121.64 (C-2'), 127.97 (C-8'), 135.52 (C-9') and 139.55 (C-6'), which closely agreed with the chemical shifts of the 6-substituted indole derivatives.[13,14] The correlation between C-6' and H-3 in the HMBC spectrum of **3** suggested that C-3 of the sarpagine part and C-6' of the tryptamine unit should be connected. The tryptamine unit of the C-3 position could take only the α-orientation in order to avoid serious steric interference between the large tryptamine nucleus and the methoxycarbonyl function, which was supported by the coupling constant of H-3 (δ 5.01, br *d*, J = 10.7 Hz). The phase-sensitive rotating frame nuclear overhauser effect spectroscopy (PROESY) spectral data (between H-19 and H-21 and between H-18 and H-15) clarified the *E* stereochemistry at the C-19 position. Therefore, a new alkaloid (**3**), now named hunteriatryptamine, was characterized as 16-hydroxymethylceridimine.

The appearance of the new alkaloids (**1**) and (**3**) as well as coryzeylamine (**2**) in the same plant led us to

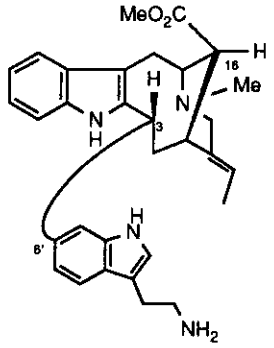
Table 1. ^{13}C-Nmr data of **1**, the sarpagine part in coryzeylamine (**2-s**), and **3**.

Carbon	1[a]	2-s[b]	3[a]
2	137.00	140.20	135.31
3	49.00	45.59	35.76
5	59.55	61.47	60.43
6	17.00	17.97	17.39
7	109.23	109.64	109.35
8	129.45	131.09	129.48
9	117.96	118.02	117.45
10	119.02	118.56	118.72
11	122.22	121.33	121.92
12	110.25	110.47	110.55
13	136.05	137.65	136.45
14	40.25	40.35	35.47
15	34.26	35.71	35.19
16	51.86	53.70	52.27
17	70.28	69.98	70.41
18	12.22	12.20	12.55
19	120.21	119.05	120.65
20	136.30	138.50	135.94
21	51.86	52.41	51.85
CO	174.16	173.42	174.00
OMe	50.27	49.55	50.40
NMe	41.96	42.40	42.04
2'	--	--	121.64
3'	--	--	106.70
4'	--	--	118.12
5'	--	--	119.33
6'	--	--	139.55
7'	--	--	110.84
8'	--	--	127.97
9'	--	--	135.92
α'	--	--	24.80
β'			40.95

Chemical shifts in ppm downfield from TMS, a) in $CDCl_3$, b) in pyridine-d_5



hunteriatryptamine (**3**)



ceridimine (**4**)

Figure 2

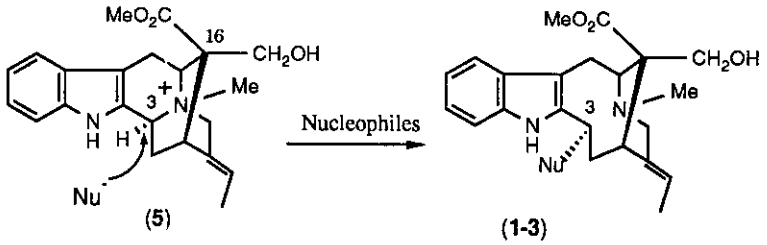


Figure 3

consider the existence of a quaternary alkaloid, 16-*epi*-macusine A (**5**) as a common biogenetic precursor of **1**, **3** and **2** (see Figure 3, nucleophiles: ammonia, tryptamine, corymine). However, we could not detect the quaternary alkaloid (**5**) or its analogue in the present investigation.

EXPERIMENTAL

Melting points were uncorrected. Uv spectra were recorded in MeOH using a Hitachi UV3400 spectrophotometer. Nmr spectra were recorded at 500 MHz for ^{1}H and at 125 MHz for ^{13}C nmr on a JEOL JNM-GSX 500A, ms were recorded on JEOL JMS-HX 101 and JMS-AM 20 spectrometers, and cd spectra were measured on JASCO J-500A in MeOH. Column chromatography, thin layer chromatography (tlc) and preparative tlc were carried out with silica gel 60 (Merck, 230-400 mesh), silica gel 60 F-254 (Merck, Pre-Coated Plates) and silica gel 60 GF-254 (Merck), respectively.

Plant material. The leaves of *Hunteria zeylanica* (Retz) Gardn. ex Thw. were collected from Ton Nga Chang Waterfall, Songkhla Province in June 1992 for the first collection and from Kao Pra Teaw Waterfall, Phuket Province, Thailand in August 1993 for the second collection. The plant materials were identified by the Department of Biology, Faculty of Science, Prince of Songkla University. The voucher specimens have been deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla, Thailand.

Extraction and isolation. The dried leaves of *H. zeylanica* were moistened with 25% aq. NH_4OH solution overnight. They were then macerated three times with MeOH for three days and filtered. The combined filtrate was evaporated under reduce pressure to give a syrupy mass. The mass was dissolved in 2% aq. H_2SO_4 solution, filtered and basified with 25% NH_4OH to pH 9. The basic solution was extracted with $CHCl_3$, which was then washed with purified water and evaporated to give a crude bases. The dried leaves, 1.2 kg (the first collection) and 2.5 kg (the second collection), gave crude alkaloids of 10.5 g and 19.0 g, respectively. The crude alkaloids of the first collection (10.5 g) were roughly separated by silica gel column chromatography. The column was eluted with 5% MeOH/$CHCl_3$, 10% MeOH/$CHCl_3$, 20% MeOH/$CHCl_3$, 30% MeOH/$CHCl_3$ and 50% MeOH/$CHCl_3$. Each fractions containing a mixture of alkaloids were purified by the combination of silica gel column chromatography, preparative tlc and medium pressure liquid silica gel chromatography. Deformylcorymine (174.2 mg), corymine (2105.6 mg), deformylcoryzeylamine (63.0 mg) and coryzeylamine (**2**) (17.9 mg) were isolated from the 10~20% MeOH/$CHCl_3$ eluate, fluorocarpamine (1.9 mg) and lanceomigine (19.4 mg) were isolated from the 20%

MeOH/$CHCl_3$ eluate, and N_b-methyl-3α-amino-*seco*-voacarpine (**1**) (43 mg) was isolated from the 30~50% MeOH/$CHCl_3$ eluate. The portions of crude base (10.15 g of the second collection) were roughly separated using silica gel column chromatography. The column was eluted with 5% MeOH/$CHCl_3$, 10% MeOH/$CHCl_3$, 20 % MeOH/$CHCl_3$, 30% MeOH/$CHCl_3$ and MeOH until no trace of the alkaloid could be detected. Corymine (1747.4 mg), deformylcoryzeylamine (25.2 mg), coryzeylamine (**2**) (101.6 mg) N_a-demethylcorymine (34.8 mg), N_a-demethyldeformylcorymine (10.6 mg), pleiocarpamine (13.1 mg) and tubotaiwine (14.0 mg) were isolated from the 5% MeOH/$CHCl_3$ eluate, (17*S*)-17,4',5',6'-tetrahydrousambarenine (45.8 mg), and (17*R*)-17,4',5',6'-tetrahydrousambarenine (69.3 mg) were isolated from the 10% MeOH/$CHCl_3$ eluate and hunteriatryptamine (**3**) (13.7 mg) was isolated from the 30% MeOH/$CHCl_3$ eluate.

N_b-Methyl-3α-amino-seco-voacarpine (**1**). A colorless solid, mp. 154-155° C, cd $\Delta\varepsilon^{23}$ (nm): +1.17 (200), +14.68 (210), 0 (222), -2.33 (229), 0 (237), +3.03 (243), +0.23 (266), +3.50 (290), +4.19 (294), +0.47 (312), 0 (332), (c = 0.000260, MeOH). Uv $\lambda_{max}{}^{MeOH}$ nm: 229.5, 284.6. EIms *m/z* (rel. int.): 383 (M^+, 12), 366 (12), 335 (37), 307 (6), 180 (100), 152 (26), 130 (26). HR-EIms: Found 383.2208, requires 383.2207 corresponding to the chemical formula $C_{22}H_{29}N_3O_3$ (M^+). HR-FABms: Found 384.2288, requires 384.2287 corresponding to the chemical formula $C_{22}H_{30}N_3O_3$ (M+H). ^1H Nmr ($CDCl_3$) : δ 8.60 (1H, *s*, NH), 7.50 (1H, *d*, *J* = 7.8 Hz, H-9), 7.20 (1H, *d*, *J* = 7.8 Hz, H-12), 7.13 (1H, *td*, *J* = 7.8, 1.2 Hz, H-11), 7.06 (1H, *td*, *J* = 7.8, 1.2 Hz, H-10), 5.44 (1H, *q*, *J* = 6.9 Hz, H-19), 4.40 (1H, *dd*, *J* = 12.3, 3.3 Hz, H-3), 3.79 (1H, *t*, *J* = 8.8 Hz, H-5), 3.66 (2H, *s*, H-17), 3.58 (1H, br *d*, *J* = 13.7 Hz, H-21), 3.41 (1H, *dd*, *J* = 12.3, 7.6, Hz, H-15), 3.36 (1H, *dd*, *J* = 14.9, 8.8 Hz, H-6), 3.15 (1H, *dd*, *J* = 14.9, 8.8 Hz, H-6), 2.95 (1H, *d*, *J* = 13.7 Hz, H-21), 2.56 (1H, *dd*, *J* = 14.8, 12.3 Hz, H-14), 2.51 (3H, *s*, NMe), 2.38 (3H, *s*, OMe), 1.95 (1H, *ddd*, *J* = 14.8, 7.6, 3.3 Hz, H-14), 1.69 (3H, *dd*, *J* = 6.9, 2.0 Hz, H-18)

Hunteriatryptamine (**3**). A colorless amorphous powder, cd $\Delta\varepsilon^{25.5}$ (nm): -3.99 (200), -69.34 (220), 0 (229), +45.43 (235), +3.19 (250), 0 (263), -4.47 (282), 0 (292), +3.19 (295), 0 (297), -2.87 (300), 0 (310), (c = 0.000190, MeOH). Uv $\lambda_{max}{}^{MeOH}$ nm: 222.1, 285.1. EIms *m/z* (rel int.): 526 (M^+, 15), 316 (2), 269 (13), 212 (13), 180 (100), 152 (13), 122 (15). Positive ion HR-FABms: Found 527.3021, requires 527.3022 corresponding to the chemical formula $C_{32}H_{39}N_4O_3$ $(M+H)^+$. ^1H Nmr ($CDCl_3$ + 2 drops of CD_3OD): δ 10.64 (1H, *s*, NH), 7.61 (1H, *s*, NH'), 7.53 (1H, *d*, *J* = 8.5 Hz, H-9), 7.47 (1H, *d*, *J* = 7.6 Hz, H-4'), 7.13 (1H, *d*, *J* = 6.8 Hz, H-12), 7.06-7.00 (4H, *m*, H-10, H-11, H-2', H-7'), 7.00

(1H, *d*, *J* = 7.6 Hz, H-5'), 5.40 (1H, *q*, *J* = 6.4 Hz, H-19), 5.01 (1H, *d*, *J* = 10.7 Hz, H-3), 3.86 (1H, *t*, *J* = 8.8 Hz, H-5), 3.80 (1H, *d*, *J* = 11.3 Hz, H-17), 3.67 (1H, *d*, *J* = 11.3 Hz, H-17), 3.61 (1H, *d*, *J* = 13.7 Hz, H-21α), 3.60 (1H, *m*, H-15), 3.54 (1H, *td*, *J* = 14.9, 8.8 Hz, H-6β), 3.36 (1H, *m*, H-β'), 3.29 (1H, *dd*, *J* = 14.9, 8.8 Hz, H-6α), 3.22 (1H, *m*, H-β'), 3.18 (2H, *m*, H-α'), 2.97 (1H, *d*, *J* = 13.7 Hz, H-21β), 2.79 (1H, *ddd*, *J* = 10.7, 13.2, 13.2 Hz, H-14), 2.55 (3H, *s*, NMe), 2.35 (3H *s*, OMe), 2.04 (1H, *m*, H-14), 1.66 (3H, *d*, *J* = 6.4 Hz, H-18).

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