New Corymine-Related Indole Alkaloids from *Hunteria zeylanica* in Thailand

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Two new corymine-related indole alkaloids, N_a-demethylcorymine (2) and N_a-demethyldeformylcorymine (4), were isolated from the leaves of *Hunteria zeylanica* native to Thailand. Their structures were determined by spectroscopic analysis and chemical correlation.

Keywords indole alkaloid; corymine; N_a-demethylcorymine; N_a-demethyldeformylcorymine; *Hunteria zeylanica*; Apocynaceae

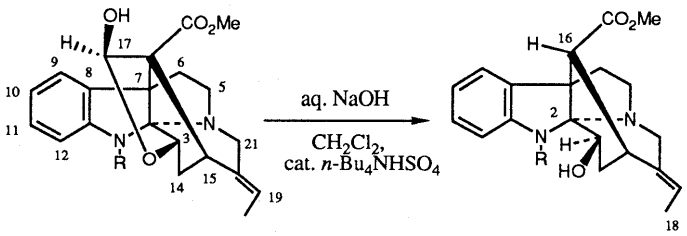
Hunteria zeylanica (RETZ) GARDN. ex THW., belonging to the family Apocynaceae, is native to tropical Africa and southeast Asia. Its latex has traditionally been used for smearing on the sores caused by yaws.[1] The previous research by many groups on the chemical components of *Hunteria* has clarified that this genus is a rich source of monoterpenoid indole alkaloids.[2] We have become interested in the alkaloidal constituents of *Hunteria zeylanica* native to Thailand, and have already isolated a biose-linked monoterpenoid indole alkaloid, named hunterioside, from the stem bark[3] and two novel dimeric indole alkaloids from the leaves of the plants.[4] Further investigation of the alkaloidal constituents of the leaves resulted in the isolation of corymine (**1**) and three corymine derivatives (**2**—**4**). In this report, studies on the structures of two of the new alkaloids (**2** and **4**) are described.

The first new alkaloid (**2**) was obtained as an amorphous powder, $[\alpha]_D^{28}$ +12.0° (c=1.25, $CHCl_3$). The ultraviolet (UV) spectrum (λ_{max} at 243 and 299 nm) is very similar to that of the main indole alkaloid of the leaves of this plant, corymine (**1**),[5] which has an indoline chromophore. The molecular formula $C_{21}H_{24}N_2O_4$ obtained from the high-resolution mass spectrum (MS) is less than that of corymine by a CH_2 fragment. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **2** in dimethyl sulfoxide-d_6 (DMSO-d_6) showed four aromatic adjacent protons, one methoxy group (δ 3.74), an ethylidene side chain (δ 5.31, q, J=6.6 Hz, H-19 and δ 1.64, 3H, dd, J=6.6, 1.8 Hz, H-18) and a hemiacetal proton (δ 4.94, d, J=4.6 Hz, H-17), which displayed very similar pattern to that of **1** except for the disappearance of an N_a-methyl signal. Unambiguous assignments of all the 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 carbon nuclear magnetic resonance (^{13}C-NMR) spectrum of **2** exhibited peaks almost superimposable on those of corymine (Table

TABLE I. ^{13}C-NMR Data for **1**, **2**, **3**, and **4**

Carbon	1[a]	2[a]	3[b]	4[b]
2	96.31	93.52	97.04	94.62
3	68.52	73.74	66.76	67.95
5	56.75	56.43	55.13	54.49
6	39.00	38.82	43.08	42.62
7	61.87	62.21	56.59	57.26
8	134.00	134.13	137.83	138.27
9	124.01	124.72	122.53	123.33
10	114.63	115.64	116.93	119.49
11	127.24	126.91	127.75	127.79
12	102.85	106.57	103.42	108.91
13	152.09	152.48	149.45	147.84
14	29.21	29.46	37.22	37.19
15	32.75	32.80	34.99	35.11
16	51.73	50.95	48.21	48.08
17	94.32	94.25	—	—
18	13.76	13.68	13.44	13.54
19	120.25	119.98	122.80	124.45
20	141.18	141.24	138.70	137.00
21	57.41	57.33	57.88	57.36
CO	172.63	172.67	173.66	173.40
OMe	51.73	51.61	51.62	51.74
NMe	27.10	—	26.40	—

a) In DMSO-d_6. *b*) In $CDCl_3$.



1: R=Me corymine
2: R=H demethylcorymine
3: R=Me deformylcorymine
4: R=H demethyldeformylcorymine

I), except for the loss of one methyl carbon and different chemical shifts of a few carbons. The signals of C-3 and C-12 in **2** are shifted to lower field compared with those of **1**. This can be interpreted in terms of the disappearance of the γ-steric interaction of the N_a-methyl group. The configuration of the ethylidene side chain and the stereochemistry of the C-17 position were determined from nuclear Overhauser effect (NOE) difference experiments. All of these facts led to the conclusion that **2** is N_a-demethylcorymine.

The second new alkaloid (**4**) was obtained as colorless crystals from acetone, mp 183—186 °C, $[\alpha]_D^{28}$ −142° (c=0.90, $CHCl_3$). The UV and ^{1}H-NMR spectra were very similar to those of deformylcorymine (**3**),[6] which was simultaneously obtained as a minor alkaloid from this plant. The high-resolution MS of **4** is consistent with the molecular formula $C_{20}H_{24}N_2O_3$, which is less than **2** and **3** by CO and CH_2 fragments, respectively. The disappearance of both the H-17 and C-17 signals due to the hemiacetal function from **2** was apparent in both the ^{1}H- and ^{13}C-NMR spectra of **4**. From these data, the alkaloid **4** was considered to be a deformyl derivative of **2**. The chemical transformation of corymine **1** to deformylcorymine **3** using aqueous KOH has been reported.[6] Thus, the alkaloid **2** was treated with alkali under modified conditions (10% aqueous NaOH/methylene chloride and tetra-*n*-butylammonium hydrogensulfate as a catalyst, at room temperature) to yield the alkaloid **4** in 61% yield. It was identical with the natural product on the basis of their chromatographic behavior, and UV, MS, and ^{1}H-NMR (500 MHz) spectral comparisons. The stereochemistry at the C-16 position was determined from the observation of NOE between the OMe group and C-18 protons. Therefore, the structure of the second new alkaloid was concluded to be N_a-demethyldeformylcorymine (**4**). The possibility that the alkaloids **2** and **4** are artefacts derived from compounds **1** and **3**, respectively, can not be excluded, because during the extraction steps strong ammonia water was used.

Experimental

The instruments used in this study were as follows; UV spectra were taken on a Hitachi UV3400 spectrophotometer; NMR spectra were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C on a JEOL JNM A500 spectrometer; MS were recorded on JEOL JMS-HX 101 and JMS-AM 20 spectrometers; optical rotation was measured on a JASCO DIP-140 polarimeter. Thin layer chromatography was performed on Merck Silica gel 60 GF_{254} plates. Column chromatography was carried out on Merck Silica gel 60, 230—400 mesh for flash chromatography and on a pre-packed column (Kusano CPS-HS-221-05) for medium-pressure column chromatography (MPLC). Abbreviations used are: singlet (s), doublet (d), triplet (t), multiplet (m), shoulder (sh).

Plant Material The leaves of *Hunteria zeylanica* (RETZ) GARDN. ex THW. were collected from Kao Pra Teaw Waterfall, Phuket Province, Thailand in August 1992 and identified by the Department of Biology, Faculty of Sciences, Prince of Songkla University. A voucher specimen has been deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkla, Thailand.

Extraction and Separation of the Alkaloidal Fraction Dried, coarsely powdered leaves (2.5 kg) were moistened with 25% ammonia water and allowed to stand overnight. They were then macerated with MeOH (12 l) for three days, three times, and filtered. The combined filtrate was concentrated to a syrupy mass under reduced pressure, mixed with 2% H_2SO_4 (3 × 250 ml) and filtered. The acidic filtrate was made basic (pH7) with 25% ammonia water and extracted with $CHCl_3$. The combined $CHCl_3$ extract was washed with water, dried over anhydrous Na_2SO_4 and evaporated to yield the crude alkaloids (19 g). A portion of the crude base (10.15 g) was 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 MeOH. The 5% MeOH/$CHCl_3$ eluate was subjected to SiO_2 flash column chromatography using $CHCl_3$/AcOEt and AcOEt/MeOH gradients. From the 5—10% MeOH/AcOEt fractions, alkaloids **1** and **3** were obtained. The 20% MeOH/AcOEt eluate was subjected again to SiO_2 flash column chromatography ($CHCl_3$/MeOH gradients) and then purified by preparative TLC (2% MeOH/$CHCl_3$ saturated with 25% ammonia water) to give 34.8 mg of **2** and 10.6 mg of **4**.

N_a-Demethylcorymine (2) Amorphous powder. $[\alpha]_D^{28}$ +12.0° (c=1.25, $CHCl_3$). UV λ_{max}^{MeOH} nm : 243, 299. EI-MS m/z (%): 368 (M^+, 100), 351 (27), 324 (4), 184 (6), 157 (6), 130 (18). ^{1}H-NMR (DMSO-d_6) δ: 7.15 (1H, d, J=7.5 Hz, H-9), 6.81 (1H, td, J=7.6, 1.0 Hz, H-11), 6.64 (1H, d, J=4.6 Hz, 17-OH), 6.38 (1H, td, J=7.5, 1.0 Hz, H-10), 6.32 (1H, d, J=7.6 Hz, H-12), 5.89 (1H, s, NH), 5.31 (1H, q, J=6.6 Hz, H-19), 4.94 (1H, d, J=4.6 Hz, H-17), 4.17 (1H, d, J=4.2 Hz, H-3), 3.90 (1H, br d, J=15.9 Hz, H-21α), 3.74 (3H, s, OMe), 3.67 (1H, d, J=7.3 Hz, H-15), 3.16 (1H, td, J=12.0, 7.5 Hz, H-5), 2.88 (1H, d, J=15.9 Hz, H-21β), 2.74 (1H, dd, J=12.0, 7.3 Hz, H-5), 2.61 (1H, dd, J=14.2, 7.3, H-14), 2.56 (1H, td, J=12.4, 7.3 Hz, H-6), 1.84 (1H, dd, J=14.2, 4.2 Hz, H-14), 1.64 (3H, dd, J=6.6, 1.8 Hz, H-18), 1.41 (1H, dd, J=12.4, 7.5 Hz, H-6). Differential NOE data: irradiation of H-19 led to enhancement (3.4%) of H-21 (δ 2.88), and irradiation of H-17 led to enhancement (5.0%) of H-9. ^{13}C-NMR: see Table I. HR-MS Calcd for $C_{21}H_{24}N_2O_4$: 368.1736. Found 368.1743.

N_a-Demethyldeformylcorymine (4) Colorless crystals. mp 183—186 °C (acetone). $[\alpha]_D^{28}$ −142° (c=0.90, $CHCl_3$). UV λ_{max}^{MeOH} nm: 242, 295. EI-MS m/z (%): 340 (M^+, 100), 323 (98), 296 (30), 281 (9), 237 (8), 208 (9), 157 (26), 130 (31), 115 (16). ^{1}H-NMR ($CDCl_3$) δ: 7.22 (1H, dd, J=7.6, 0.9 Hz, H-9), 7.00 (1H, td, J=7.6, 1.2 Hz, H-11), 6.68 (1H, br t, J=7.6 Hz, H-10), 6.60 (1H, d, J=7.6 Hz, H-12), 5.47 (1H, q, J=6.8 Hz, H-19), 4.45 (1H, br d, J=7.9 Hz, H-3), 3.81 (3H, s, OMe), 3.77 (1H, d, J=4.0 Hz, H-15), 3.75 (1H, br d, J=15.1 Hz, H-21α), 3.51 (1H, m, H-5), 3.21 (1H, s, H-16), 3.00 (1H, d, J=15.1 Hz, H-21β), 2.81 (1H, dd, J=12.0, 8.5 Hz, H-5), 2.72 (1H, ddd, J=14.0, 11.8, 8.5 Hz, H-6), 2.27 (1H, br dd, J=14.2, 7.9 Hz, H-14), 2.06 (1H, dd, J=14.2, 4.0 Hz, H-14), 2.03 (1H, dd, J=14.0, 6.8 Hz, H-6), 1.61 (3H, dd, J=6.8, 1.7 Hz, H-18). ^{13}C-NMR: see Table I. HR-MS Calcd for $C_{20}H_{24}N_2O_3$: 340.1785. Found 340.1796.

Conversion of 2 into 4 Compound **2** (5.2 mg) was dissolved in methylene chloride (0.5 ml) and 10% aqueous NaOH solution (0.25 ml) and then tetra-*n*-butylammonium hydrogensulfate (1.0 mg) was added. The reaction mixture was vigorously stirred at room temperature for 2 h, then diluted with water, and the whole was extracted with methylene chloride. The organic layer was washed with brine, dried over magnesium sulfate, and evaporated. The residue was crystallized from acetone to give 2.9 mg (yield 61%) of **4** (mp 188—189 °C), which was identical with the natural product on the basis of TLC behavior and UV, MS, and ^{1}H-NMR (500 MHz) spectral comparisons.

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