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HEYNEANINE HYDROXYINDOLENINE, A NEW INDOLE ALKALOID FROM *ERVATAMIA CORONARIA* VAR. *PLENA*

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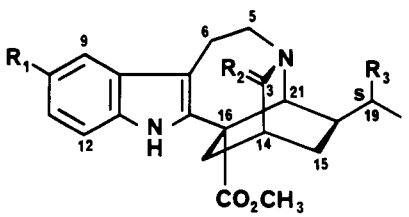
ABSTRACT.—The whole plant of *Ervatamia coronaria* var. *plena* obtained from Thailand has afforded a new indole alkaloid 19*S*-heyneanine hydroxyindolenine [**10**] whose structure was deduced through interpretation of spectral data. Nine known alkaloids, coronaridine [**1**], coronaridine hydroxyindolenine [**2**], voacangine [**3**], voacangine hydroxyindolenine [**4**], heyneanine [**5**], voacristine [**6**], 3-oxo-coronaridine [**7**], 3-oxo-voacangine [**8**], and voacristine hydroxyindolenine [**9**], and six common triterpenoids were also isolated. Coronaridine was the principal cytotoxic alkaloid obtained.

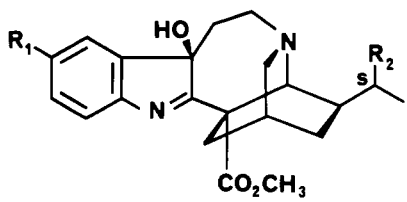
Ervatamia coronaria (Jacq.) Stapf. [syn. *Tabernaemontana divaricata* (L.) R.Br.] (1), has been used as a cancer remedy in Taiwan (2), although it was previously reported (3) that cytotoxic activity could not be established for the extracts of this plant. Members of the genus *Ervatamia* (Apocynaceae) are quite widely distributed and have been well studied for their alkaloid constituents (4–14). There is still some confusion as to whether the genus *Ervatamia* should exist per se or whether it should be subsumed into the genus *Tabernaemontana* (1).

The acidic and weakly basic fractions of the MeOH extract of the whole plant of *E. coronaria* var. *plena* obtained in Thailand afforded the terpenoids lupeol, lupeol acetate, α-amyrin acetate, β-sitosterol, β-sitosterol-β-D-glucoside, and ursolic acid, and the alkaloids coronaridine [**1**] (4), coronaridine hydroxyindolenine [**2**] (14,15), voacangine [**3**] (16), and voacangine hydroxyindolenine [**4**] (17). The basic alkaloid fraction afforded (−)-19*S*-heyneanine [**5**] (18), voacristine [**6**] (18), 3-oxo-coronaridine [**7**] (19), 3-oxo-voacangine [**8**] (20), voacristine hydroxyindolenine [**9**] (21,22), and a new alkaloid, heyneanine hydroxyindolenine [**10**]. The known compounds were identified either by comparison of their spectral data with those reported in the literature or by mmp, co-tlc, and superimposable ir and nmr spectra with authentic samples.

Heyneanine hydroxyindolenine [**10**] was obtained as an amorphous powder, displaying an $[M]^+$ at *m/z* 370 ($C_{21}H_{26}N_2O_4$) and a uv spectrum [λ max 223, 260, 282 (sh) and 290 nm] characteristic of an indolenine chromophore (24). Ir absorption bands



	R_1	R_2	R_3
1	H	H_2	H
3	OMe	H_2	H
5	H	H_2	OH
6	OMe	H_2	OH
7	H	O	H
8	OMe	O	H



	R_1	R_2
2	H	H
4	OMe	H
9	OMe	OH
10	H	OH

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at 3480 and 1770 cm^{-1} indicated the presence of hydroxyl and carbomethoxy groups, respectively. In the high-field ^{1}H-nmr spectrum four aromatic protons, a three-proton singlet for the carbomethoxy group and a -CH(OH)Me (d at 1.08 ppm, m at 4.08 ppm) were established. The mass spectral fragmentation displayed a series of ions representing losses of the functional groups, i.e. *m/z* 353 $[M-OH]^+$, 335 $[M-OH-H_2O]^+$, 325 $[M-MeCHOH]^+$, and 311 $[M-COOMe]^+$ followed by a series of ions analogous to those of other hydroxy indolenines of the *Iboga* series (*m/z* 230, 188, 146, and 132) (23).

^{13}C-nmr data were obtained for the first time for 3-oxo-coronaridine [**7**], 3-oxo-voacangine [**8**], coronaridine hydroxyindolenine [**2**], voacangine hydroxyindolenine [**4**], and for heyneanine hydroxyindolenine [**10**] as shown in Table 1. All of the hydroxyindolenines display C-2 markedly shifted downfield to about 188 ppm, with C-7 shifted to about 87 ppm. These signals are, therefore, highly diagnostic for this skeleton. Heyneanine hydroxyindolenine [**10**] displayed these signals at 188.5 and 87.7 ppm, respectively. This evidence, together with the downfield shifts observed for C-18 (20.7 ppm) and C-19 (71.2 ppm) on comparison with **2** and **5**, and the characteristic ^{1}H nmr shifts of H_3-18 and H-19 for compounds in this series (24), established the isolate to be 19*S*-heyneanine hydroxyindolenine [**10**].

TABLE 1. Carbon-13 Nmr Data for Selected *Iboga* Alkaloids.

Carbon	Compound						
	1	**7**	**8**	**9**	**2**	**4**	**10**
2	136.5	135.5	134.5	186.0	189.1	188.1	188.5
3	53.1	172.8	172.8	47.7	48.5[a]	48.7[a]	47.7
5	51.5	42.6	42.6	48.0	48.9[a]	48.9[a]	48.1
6	22.2	20.9	21.0	33.0	33.6	32.2	32.7
7	110.3	109.1	108.8	86.7	88.1	87.3	87.7
8	128.8	127.6	127.9	143.0	142.4	144.9	141.8
9	118.3	118.1	100.2	107.9	120.6[b]	108.4	121.4
10	119.0	119.3	153.8	159.1	121.3[b]	158.4	120.7
11	121.8	122.1	112.3	113.8	129.1	113.3	129.4
12	110.3	110.4	111.2	121.4	126.6	121.6	126.9
13	135.6	133.8	130.7	143.6	151.0	143.9	151.1
14	27.2	35.3	35.2	26.3	26.8	28.4	26.3
15	32.0	30.8	30.8	22.8	31.8	30.5	22.8
16	55.1	55.4	55.4	55.6	58.5	57.5	57.5
17	36.3	35.6	35.7	33.0	34.7	36.7	35.5
18	11.6	11.2	11.8	20.1	11.4	11.7	20.7
19	26.6	27.5	27.5	71.1	26.3	27.1	71.2
20	38.9	38.0	38.0	38.1	37.4	38.9	38.1
21	57.2	55.9	55.8	59.9	58.2	55.5	59.8
-CO_2Me	175.9	175.6	175.6	172.9	173.5	172.6	172.7
-CO_2CH_3	52.4	52.9	52.8	53.4	53.1	52.5	53.4
ArOMe	—	—	55.9	55.6	—	54.9	—

[a,b]Values in the same column with the same superscript may be interchanged.

Although some alkaloids in the *Iboga* series have previously shown cytotoxic activity (25), the isolates **2**, **4**, **7**, **9**, and **10** were found to be inactive in both the KB and P-388 test systems in vitro (26). The activity observed in the basic fraction was, therefore, due to coronaridine [**1**] (5).

EXPERIMENTAL

Melting points were determined on a Kofler hot plate and are uncorrected. The uv spectra were recorded with a Beckman DU-7 model spectrophotometer. Ir spectra were determined as KB discs on a Nicolet MX-1 interferometer. ^{1}H-nmr and ^{13}C-nmr spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Nicolet WM 360 spectrometer. TMS was used as an internal standard; chemical shifts are reported in δ ppm units. Optical rotations were recorded with a Perkin-Elmer model 241 polarimeter. Mass spectra were obtained with a Varian MAT 112S double focusing mass spectrometer operating at 70eV. Si gel tlc plates (0.2 mm thick) supplied by Analtech were used for preparative tlc. Si gel was used for cc. Solvent systems used for preparative tlc were A (EtOAc-C_6H_6, 1:1) and B ($CHCl_3$-MeOH, 9:1).

PLANT MATERIAL.—Whole plants of *E. coronaria* var. *plena* were collected in Ayudhya, Thailand in March, 1982. A voucher specimen is deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

PREPARATION OF ALKALOID FRACTIONS.—The powdered, air-dried whole plant (8.0 kg) was extracted with petroleum ether (24 liters × 4) for 24 h at room temperature. After drying, the plant material was extracted with MeOH (24 liters × 4) at room temperature. The petroleum ether and MeOH extracts were concentrated in vacuo to afford 48.3 and 630 g of residue, respectively. The MeOH extract was treated with 2% tartaric acid and partitioned with $CHCl_3$ (200 ml × 4). Work-up in the normal way afforded a neutral/acidic fraction (Fraction A, 57.7 g). After basification of the aqueous phase with Na_2CO_3 to pH 8.5 and extraction with EtOAc (200 ml × 5), the EtOAc fraction was washed, dried (Na_2SO_4), and concentrated in vacuo to afford a basic fraction (Fraction B, 16.5 g, 0.21% yield).

ISOLATION OF CONSTITUENTS.—A sample (30.0 g) of fraction A was subjected to cc over Si gel (900 g) eluting with $CHCl_3$ and $CHCl_3$/MeOH mixtures of increasing polarity, collecting 40 fractions. Lupeol acetate, α-amyrin acetate, lupeol, β-sitosterol, β-sitosterol-β-D-glucoside, (−)-coronaridine [**1**] (200 mg, 0.005%), and coronaridine hydroxyindolenine [**2**] (30 mg, 0.0007%) were eluted successively with $CHCl_3$. Voacangine [**3**] (50 mg, 0.0012%), voacangine hydroxyindolenine [**4**] (6 mg, 0.0001%), and ursolic acid were then eluted with $CHCl_3$-MeOH (99:1) and separated by preparative tlc on Si gel eluting with system A.

Fraction 62 eluted with $CHCl_3$-MeOH (49:1) was subjected to preparative tlc in solvent system A and rechromatographed in solvent system B to afford (−)-19*S*-heyneanine [**5**] (17 mg, 0.0004%). A less polar fraction from fraction 62 yielded a pale yellow solid (12 mg, 0.0003%) identified as heyneanine hydroxyindolenine [**10**]: uv λ max (MeOH) 223 and 282 nm; ir ν max ($CHCl_3$) 3480, 2920, 2210, 1770, 1600, 1430, 1280, 1200, 1050 cm^{-1}; ^{1}H nmr (200 MHz, $CDCl_3$) 1.075 (d, 3H, J = 6.4 Hz, 18-H_3), 3.70 (s, 3H, CO_2Me), 4.08 (q, 1H, J = 6.4 Hz, H-19), 7.23–7.38 (m, 3H, H-9, H-10, H-11), 7.47 (d, 1H, J = 7.5 Hz, H-12); ms *m/z* (rel. int.) $[M]^+$ 370 (89%), $[M-OH]^+$ 353 (100), $[M-OH-H_2O]^+$ 335 (15), 325 (27), $[M-CO_2Me]^+$ 311 (11), 309 (16), 254 (13), 196 (12), 188 (68), 184 (12), 170 (23), 160 (49), 136 (10), 122 (15).

Fraction 63, a mixture of five compounds, was subjected to preparative tlc in solvent system A eluting twice to afford voacristine [**6**] (10 mg, 0.0003%), 3-oxo-coronaridine [**7**] (60 mg, 0.0015%), 3-oxo-voacangine [**8**] (20 mg, 0.0005%), voacristine hydroxyindolenine [**9**] (46 mg, 0.0011%), and heyneanine [**5**] (30 mg, 0.0007%).

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