Phytochemistry, 1980, Vol. 19, pp. 1213–1218. Pergamon Press Ltd. Printed in England

ANTICANCER INDOLE ALKALOIDS OF *ERVATAMIA HEYNEANA**

SARATH P. GUNASEKERA, GEOFFREY A. CORDELL and NORMAN R. FARNSWORTH

Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612, U.S.A.

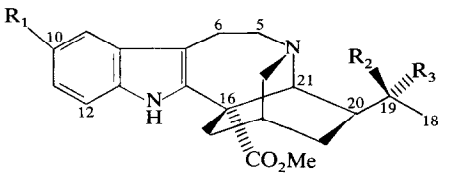
(*Revised received* 30 *August* 1979)

Key Word Index—*Ervatamia heyneana*; Apocynaceae; cytotoxic and antitumor activity; chemical correlation; indole alkaloids; triterpenes.

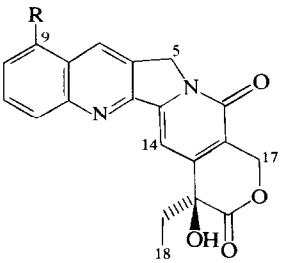
Abstract—The wood and stem bark of *Ervatamia heyneana* (Apocynaceae) yielded 14 indole alkaloids and 3 triterpenoids. Six of these isolates, camptothecin (**2**), 9-methoxycamptothecin (**3**), coronaridine (**1**), pericalline (**25**), heyneatine (**18**) and 10-methoxyeglandine-N-oxide (**4**) displayed cytotoxic activity. Three of the indole alkaloids **18**, **4** and 10-hydroxycoronaridine (**8**) are new members in the iboga series and their structures were determined by a combination of spectral interpretation and chemical correlation.

INTRODUCTION

The close botanical relationship of the genera *Tabernaemontana*, *Pagiantha* and *Ervatamia* has prompted numerous phytochemical investigations and the latter genus has yielded several types of indole alkaloids ([1–10]; Bruneton, J. *et al.*, unpublished results quoted in ref. [10]) and other constituents [8, 11–13]. Our interest in the genus *Ervatamia*, particularly the Indian plant *E. heyneana* (Wall.) T. Cooke (Apocynaceae), was prompted by the finding that an aqueous alcoholic extract of the roots of this plant displayed both cytotoxic and antitumor activity [14]. *Tabernaemontana divaricata* (L.) R. Br. (syn.: *E. coronaria* (Jacq.) Stapf) has been used as a cancer remedy in Taiwan [15] but did not display cytotoxic activity [16]. *Pagiantha dichotoma* (Roxb.) Mgf. (syn.: *E. dichotoma* (Roxb.) Burk) did show anticancer activity, and Kupchan and co-workers isolated coronaridine (**1**) as the active principle from the fruit



	R_1	R_2	R_3
1	H	H	H
5	OMe	H	H
8	OH	H	H
20	OMe	OH	H
22	OMe	=O	
23	H	OH	H



2 R = H
3 R = OMe

[2]. The aerial parts of *E. heyneana* were previously reported [17] as having marginal cytotoxic activity.

We have previously reported [18] on the major contributors to the anticancer activity of the wood and stem bark of *E. heyneana*, namely, camptothecin (**2**) and 9-methoxycamptothecin (**3**) and wish now to describe the isolation of several additional active compounds and the structure elucidation of 3 new iboga alkaloids.

RESULTS AND DISCUSSION

10-Methoxyeglandine-*N*-oxide (**4**) was obtained as a gum displaying a M^+ at *m/e* 398 analysing for $C_{22}H_{26}N_2O_5$ and a UV spectrum very similar to that of voacangine (**5**). The substitution of the aromatic nucleus at C-10 by a methoxy group (δ 3.84) at C-10 was substantiated by the classic pattern of signals at 6.80 ($J = 8.4$, 2.4 Hz), 6.88 ($J = 2.3$ Hz) and 7.14 ($J = 8.4$ Hz) for protons at C-11, C-9 and C-12, respectively. Other signals revealed in the NMR spectrum included an ethyl side chain (triplet at 0.89 ppm) and a carbomethoxy group (singlet at 3.68 ppm), and the latter was substantiated by an IR absorption at 1730 cm^{-1}.

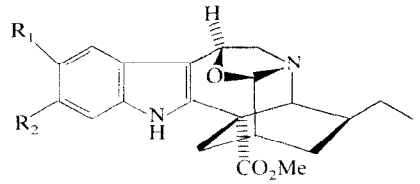
A loss of 16 amu from the M^+ was attributed to loss of an oxygen atom by the thermal decomposition from

* Part 15 in the series "Potential Anticancer Agents". For Part 14 see Gunasekera, S., Cordell, G. A. and Farnsworth, N. R. (1979) *J. Nat. Prod.* **42**, 658.

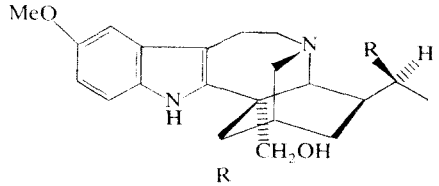
an *N*-oxide group [19] and the subsequent fragmentation was similar to that reported [20] for 11-methoxyeglandine (**6**). Substantiation of this skeletal assignment came from the observation of a doublet of doublets ($J = 8.8$, 7.2 Hz) at 4.11 ppm for the C_6 α proton [21]. $LiAlH_4$ reduction of the isolate afforded voacanginol identical (NMR, MS, TLC) to the semi-synthetic product derived from voacangine (**5**). The new isolate is therefore ascribed the structure 10-methoxyeglandine-*N*-oxide (**4**).

10-Hydroxycoronaridine (**8**) was also obtained as an amorphous gum showing a M^+ at *m/e* 354 ($C_{21}H_{26}N_2O_3$). Two of the three oxygen atoms were traced to a carbomethoxy group (1715 cm^{-1}, δ 3.70), and the third to a phenolic group showing a green color with ethanolic $FeCl_3$ solution and a bathochromic UV shift on addition of base. Location of this group at C-10 was apparent from the characteristic signals for C-9 H, C-11 H and C-12 H at 6.87, 6.68 and 7.08 ppm, respectively. Typical losses in the MS of methyl, ethyl and carbomethoxyl radicals were followed by a series of ions at *m/e* 269 (**9**), 230 (**10**) and 170 (**11**) containing the aromatic moiety, and corresponding to the ions *m/e* 283 (**12**), 244 (**13**) and 184 (**14**) in the MS of voacangine (**5**) [22]. Ions at *m/e* 136 (**15**), 124 (**16**) and 122 (**17**) were also present in the spectrum of **8** and **5**. On this basis, the isolate was assigned the structure 10-hydroxycoronaridine (**8**).

The third new alkaloid, (−)-heyneatine (**18**) is a member of a new group of alkaloids within the iboga series as the data will indicate. A M^+ at *m/e* 382 ($C_{22}H_{26}N_2O_4$) and the observation of both aromatic methoxy δ 3.84) and carbomethoxy (δ 3.70) groups suggested that the final oxygen might be involved in an



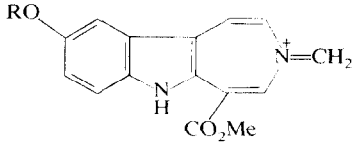
4 R_1 = OMe, R_2 = H; *N*-oxide
6 R_1 = H, R_2 = OMe



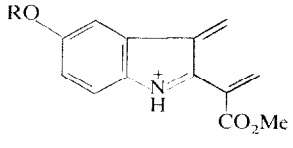
7 R = H
19 R = OH

ether moiety (no —OH, olefinic double bonds or ketonic CO) in order to account for the number of degrees of unsaturation indicated by the molecular formula. An *ortho*-coupled doublet ($J = 8.4$ Hz) at 7.12 ppm demonstrated that the methoxy group was located at C-10, and the similarity of the UV spectrum with voacangine (**5**) confirmed this. Ions at *m/e* 244 (**13**) and 184 (**14**) were analogous to those in the MS of **5** [23].

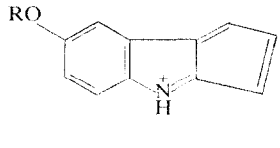
A 3-proton doublet at 1.12 ppm indicated that C-19 was substituted by oxygen and the observation of a singlet at 4.0 ppm integrating for one proton suggested that the ether bridge terminated at C-3. $LiAlH_4$ reduction afforded 19*S*-voacangarinol (**19**) and $NaBH_4$ reduction yielded 19*S*-voacangarine (**20**), confirming the carbinolamine ether substitution at C-3.



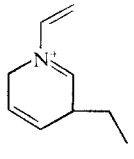
9 *m/e* 269 R = H
12 *m/e* 283 R = Me



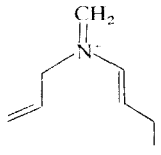
10 *m/e* 230 R = H
13 *m/e* 244 R = Me



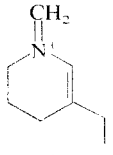
11 *m/e* 170 R = H
14 *m/e* 184, R = Me



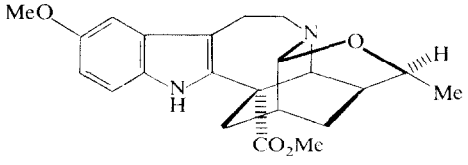
15 *m/e* 136



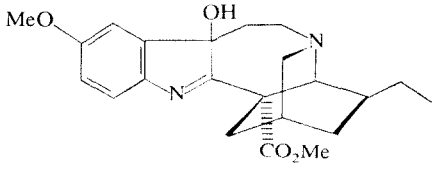
16 *m/e* 124



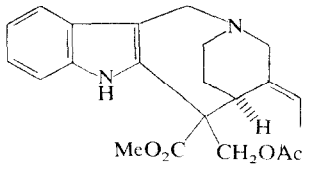
17 *m/e* 122



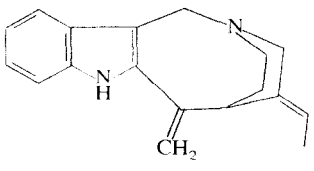
18



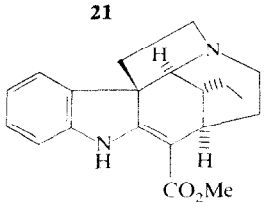
21



24



25



26

Table 1. ^{13}C NMR data for selected iboga alkaloids from *Ervatamia heyneana*

Carbon	Coronaridine (**1**)	19*S*-Heyneanine (**23**)	Voacangine (**5**)	Voacangarine (**20**)
2	137.4*	136.9*	138.2	138.0
3	53.6	53.1	53.4	53.1
5	52.2	52.3	52.0	52.0
6	22.2	22.1	22.2	22.2
7	110.9	110.9	110.6	110.7
8	n.o.	129.7	129.9	129.5
9	118.9	119.4	101.6	102.1
10	119.8	120.4	154.8	155.5
11	122.5	123.3	112.2	113.2
12	110.9	111.5	111.4	112.2
13	136.4*	136.6*	131.4	132.2
14	27.7	27.5	27.5	27.5
15	32.3	23.7	32.1	23.7
16	n.o.	n.o.	55.4	55.0
17	36.8	37.7	36.6	37.7
18	11.6	20.9	11.4	20.9
19	27.0	72.1	26.8	72.1
20	39.4	40.6	39.2	40.6
21	57.7	60.5	57.5	60.5
C=O	176.5	176.2	176.2	176.3
COOMe	52.5	53.4	52.3	53.3
ArOMe			56.2	56.8

n.o. Not observed.
*Indicates assignments may be reversed.

In the 19-hydroxycoronaridine series assignment of stereochemistry may be made [24] from the δ value of the C-18 doublet (19*S* 1.11 ppm; 19*R* 1.28 ppm) and the C-19 H (19*S* 4.13 ppm; 19*R* 3.81 ppm). (−)-Heyneatine displayed these signals at 1.12 and 4.08 ppm in agreement with a 19*S* stereochemistry determined by chemical correlation. (−)-Heyneatine is therefore (−)-3,19*S*-oxidovoacangine (**18**). This is the first iboga alkaloid to be isolated having a 3,19-ether bridge.

The basic alkaloid fraction also yielded the known alkaloids voacangine (**5**), coronaridine (**1**), voacangine hydroxyindolenine (**21**), voacryptine (**22**), 19*S*-heyneanine (**23**), 19*S*-voacangarine (**20**), *O*-acetyl vallesamine (**24**), pericalline (**25**), and 19,20-dihydrocondylocarpine (**26**).

From the neutral fractions, two alkaloids camptothecin (**2**) and 9-methoxycamptothecin (**3**) were isolated [18] together with the triterpenes ursolic acid (0.003%), β-amyrin acetate (0.02%) and β-amyrin (0.006%). The structures of these constituents were confirmed by comparison with authentic samples.

^{13}C NMR data were obtained for four of the alkaloids. The data for coronaridine (**1**), 19*S*-heyneanine (**23**) and voacangine (**5**) were in substantial agreement with those obtained previously [25] and the data for 19*S*-voacangarine (**20**) were deduced by analogy. These data are summarized in Table 1.

The anticancer activity data for the isolated alkaloids of *E. heyneana* are summarized in Table 2.

Table 2. Cytotoxic activity of alkaloids of *Ervatamia heyneana*

Compound	P-388 lymphocytic leukemia *in vitro*, ED_{50} (μg/ml)*	*in vivo* (T/C mg/kg)†
Voacangine (**5**)	42.0	
Coronaridine (**1**) (NSC-127490)	0.43	
Voacangine hydroxyindolenine (**21**)	26.0	
Voacryptine (**22**)	27.0	
19*S*-Heyneanine (**23**) (NSC-306218)	7.4	
19*S*-Voacangarine (**20**)	50.0	
10-Methoxyeglandine-*N*-oxide (**4**) (NSC-306223).	3.2	
10-Hydroxycoronaridine (**8**)	25.0	
Heyneatine (**23**) (NSC-312884)	1.6	
O-Acetyl vallesamine (**24**)	26.0	
Pericalline (**25**) (NSC-85631)	3.8	
Dihydrocondylocarpine (**26**)	23.0	
Camptothecin (**2**) (NSC-94600)	0.053	181% 1.56
9-Methoxycamptothecin (**3**) (NSC-176323)	0.0036	

* A compound is considered active if it displays an $ED_{50} \leqq 4.0$ μg/ml [14].
† A compound is considered active if it displays a T/C $\geqq$ 130% [14].

EXPERIMENTAL

Mps were determined by means of a Kofler hot plate and are uncorr. ^{1}H NMR spectra were recorded in $CDCl_3$ soln at 60 MHz. TMS was used as an int. standard and chemical shifts are reported in δ units. Column chromatography was carried out using Si gel PF-254.

Plant material. Wood stem and stem bark parts of *E. heyneana* (Wall.) T. Cooke (Apocynaceae) were collected in India in December 1976. Identification was made by the Economic Botany Laboratory, Plant Genetics and Germplasm Institute, U.S.D.A., Beltsville, MD., funded by the National Cancer Institute. A herbarium specimen is deposited at the Herbarium of the National Arboretum, Agricultural Research Service, U.S.D.A., Washington, D.C.

Extraction and fractionation. Wood stem and stem bark material (35 kg) of *E. heyneana* was extracted successively with petrol and MeOH. After evapn of extracts *in vacuo* the residues weighed 180 and 960 g, respectively. Partition of the MeOH soluble residue between H_2O (3 l.) and EtOAc (1.5 l. × 2) afforded after usual work-up an organic soluble residue weighed 115 g. This residue (115 g) was triturated with 5% tartaric acid (0.5 l. × 3) and the non-basic fraction after processing weighed 102.2 g. The basic alkaloid fraction on work-up weighed 12.2 g.

Separation of alkaloid fraction. The basic alkaloid fraction (4.47 g) was chromatographed on a column of Si gel (225 g) packed in petrol. A total of 37 fractions were collected as the solvent was progressively changed to increasingly polar mixtures of C_6H_6–$CHCl_3$, $CHCl_3$ and MeOH–$CHCl_3$.

(−)-*Voacangine* (**5**). Fractions 13 and 14 (508 mg) eluted with $CHCl_3$–C_6H_6 (2:1) were further separated by prep. TLC on Si gel eluting × 3 times with $CHCl_3$. Two bands were removed and processed. The more polar fraction afforded voacangine (**5**) (170 mg, 0.0013%) [22]: $[\alpha]_D^{26}$ −43.4° ($CHCl_3$) (lit. [22] $[\alpha]_D$ −42°). UV λ_{max}^{MeOH} nm: 224 (log ε 4.41), 285 (3.95), and 300 (3.91). IR ν_{max}^{KBr} cm^{-1}: 3340, 2815, 1710, 1625, 1590 and 1425. ^{1}H NMR ($CDCl_3$): δ 0.89 (*t*, 3H, J = 6.5 Hz, C-18 H_3), 3.7 (*s*, 3H, $-CO_2Me$), 3.84 (*s*, 3H, ArOMe), 6.80 (*dd*, 1H, J = 2.4 and 8.4 Hz, C-10 H), 6.87 (*d*, 1H, J = 2.4 Hz, C-9 H) and 7.1 (*d*, 1H, J = 8.4 Hz, C-12 H). MS: *m/e* 368 (M^+, 100%), 353 (14), 399 (4), 309 (4), 283 (10), 244 (15), 184 (24), 160 (12), 136 (40), 124 (15), and 122 (12). This compound was identical with an authentic sample (UV, IR, ^{1}H NMR, TLC).

(−)-*Coronaridine* (**1**). The less polar fraction from the above prep.-TLC yielded coronaridine (**1**) (202 mg, 0.0011%) [1], crystallizing as the HCl: mp 228–229°, $[\alpha]_D^{26}$ −11.1° (HCl, MeOH) [lit. [1] mp 230° (HCl) $[\alpha]_D$ −9° (HCl, MeOH)]. UV λ_{max}^{MeOH} nm: 244 (log ε 4.55), 284 (3.89) and 2.92 (3.83). IR ν_{max}^{KBr} cm^{-1}: 3360, 2840, 1715, 1435, 1220, and 740. ^{1}H NMR ($CDCl_3$): δ 0.89 (*t*, 3H, J = 7.2 Hz, C-18 H_3), 3.37 (*s*, 3H, $-CO_2Me$), 6.95–7.24 (*m*, 3H, C-9 H, C-10 H and C-11 H) and 7.43 (*m*, 1H, C-12 H). MS: *m/e* 338 (M^+, 100%), 323 (18), 309 (4), 279 (5), 253 (7), 214 (14), 208 (10), 169 (11), 154 (14), 136 (40), 124 (20) and 122 (17). This compound was identical with an authentic sample (UV, IR, ^{1}H NMR, TLC) [26].

Voacangine hydroxyindolenine (**21**). Combined fractions 15 and 16 obtained by further eluting the column with $CHCl_3$–C_6H_6 (2:1) were separated by prep.-TLC eluting with $CHCl_3$. A UV-visible band was separated and processed, and the residue crystallized from petrol to afford white crystals (40 mg, 0.0002%) of voacangine hydroxyindolenine (**21**) [27]: mp 134–135°, $[\alpha]_D$ +133.2° ($CHCl_3$) (lit. [26] mp 137°, $[\alpha]_D$ +137°). UV λ_{max}^{MeOH} nm: 226 (log ε 4.26), 272 (3.80), 286 (3.81), 292 (3.81), and 314 *sh* (3.70). IR ν_{max}^{KBr} cm^{-1}: 3490, 3440, 2940, 1725, 1608, 1475, 1440, 1245, 980, 870, and 812. ^{1}H NMR ($CDCl_3$): δ 0.86 (*t*, 3H, J = 6.5 Hz, C-18 H_3), 3.7 (*s*, 3H, $-CO_2Me$), 3.82 (*s*, 3H, ArOMe), 6.73 (*dd*, 1H, J = 2.5 and 8.5 Hz, C-11 H), 6.9 (*d*, 1H, J = 2.5 Hz, C-9 H) and 7.45 (*d*, 1H, J = 8.5 Hz, C-12 H). MS: *m/e* 384 (M^+, 100%), 367 (60), 355 (8), 337 (7), 325 (9), 260 (8), 218 (11), 190 (11), 176 (12), 162 (12) and 122 (10). The spectral and physical data were identical with those reported [27] for voacangine hydroxyindolenine (**21**).

Voacryptine (**22**). Fraction 17, obtained by further elution of the column with $CHCl_3$–C_6H_6 (4:1) was separated by prep.-TLC on Si gel. A UV-visible band was separated, processed and the residue crystallized from petrol to afford white crystals (11 mg, 0.00007%) of voacryptine (**22**) [28]: mp 175–176°, (lit. [28] mp 176°). UV λ_{max}^{MeOH} nm: 224 (log ε 4.44), 282 (3.97) and 300 *sh* (3.94). MS: *m/e* 382 (M+, 100%), 367 (4), 339 (14), 323 (6), 244 (35), 184 (16), 160 (12), 150 (25) and 94 (14). This compound was identical with an authentic sample.

(−)-19S-*Heyneanine* (**23**). Combined fractions 18–20 obtained by elution of the column with $CHCl_3$–C_6H_6 (4:1) were further separated by prep.-TLC on Si gel eluting with $CHCl_3$–MeOH (98.5:1.5). A UV-visible band was separated, processed and the residue crystallized from Et_2O to afford white needles (181 mg, 0.0012%) of (−)-19S-heyneanine (**23**) [24]: mp 159–160°, $[\alpha]_D^{26}$ −28° ($CHCl_3$) (lit. [29] mp 162°, $[\alpha]_D$ −19°]. UV λ_{max}^{MeOH} nm: 224 (log ε 4.71), 284 (4.33), and 292 (4.27). IR ν_{max}^{KBr} cm^{-1}: 3102, 2840, 1710, 1420, 1210 and 710. ^{1}H NMR ($CDCl_3$): δ 1.10 (*d*, 3H, J = 6.5 Hz, C-18 H_3), 3.39 (*s*, 3H, $-CO_2Me$), 4.12 (*m*, 1H, C-19 H), 7.0–7.25 (*m*, 3H, C-9 H, C-10 H and C-11 H), and 7.43 (*m*, 1H, C-12 H). MS: *m/e* 354 (M^+, 100%), 339 (50), 336 (36), 310 (17), 295 (5), 214 (22), 152 (25) and 74 (52). This compound was identical with an authentic sample (UV, IR, ^{1}H NMR, MS) [30].

(−)-19S-*Voacangarine* (**20**). Combined fractions 21–23 obtained by elution of the column with $CHCl_3$ were further separated by prep.-TLC on Si gel eluting twice with $CHCl_3$–MeOH (45:1). A UV-visible band was separated, processed and residue crystallized from petrol to afford white crystals (140 mg, 0.0009%) of (−)-19S-voacangarine (**20**) [29]: mp 161–162°, $[\alpha]_D^{26}$ −28.7° ($CHCl_3$) (lit. [30] mp 165°, $[\alpha]_D$ −29° ($CHCl_3$)); UV λ_{max}^{MeOH} nm: 222 (log ε 4.45), 282 (3.98), 302 (3.90) and 314 sh (3.64). IR ν_{max}^{KBr} cm^{-1}: 3375, 3220, 2860, 1725, 1630, 1590, 1450, 1215, and 750. ^{1}H NMR ($CDCl_3$): δ 1.10 (*d*, 3H, J = 6.3 Hz, C-18 H_3), 3.72 (*s*, 3H, $-CO_2Me$), 3.84 (*s*, 3H, ArOMe), 4.11 (*m*, 1H, C-19 H), 6.8 (*dd*, 1H, J = 8.2 and 1.0 Hz, C-11 H), 6.9 (*d*, 1H, J = 8.3 and 1.0 Hz, C-12 H). MS: *m/e* 384 (M^+, 100%), 369 (33), 366 (54), 351 (4), 340 (14), 339 (14), 225 (5), 244 (28), 184 (18), 160 (20), 152 (30) and 140 (18). The data are identical with those reported [29].

10-*Methoxyeglandine*-N-*oxide* (**4**). Combined fractions 24-27 obtained by elution of the column with $CHCl_3$–MeOH (99:1) were further separated by prep.-TLC on Si gel eluting × 3 with $CHCl_3$–MeOH (24:1). Two bands were separated and extracted and the less polar fraction afforded a pale yellow gum (40 mg, 0.0002%) identified as 10-methoxyeglandine-*N*-oxide (**4**): $[\alpha]_D^{26}$ +86.6° (c 0.76, $CHCl_3$). UV λ_{max}^{MeOH}nm: 224 (log ε 4.46), 282 (3.98), 300 sh (3.96) and 312 sh (3.65). IR ν_{max}^{KBr} cm^{-1}: 3390, 2940, 2880, 1730, 1632, 1595, 1490, 1460, 1380, 1265, 1220, 1155, 1080, 972, 930, 832, 800 and 760. ^{1}H NMR ($CDCl_3$): δ 0.89 (*t*, 3H, J = 7.1 Hz, C-18 H_3), 3.68 (*s*, 3H, $-CO_2Me$), 3.84 (*s*, 3H, ArOMe), 4.11 (*dd*, 1H, J = 8.8 and 7.2 Hz, C-6 H), 6.8

(*dd*, 1H, $J = 8.4$ and 2.4 Hz, C-11 H), 6.88 (*d*, 1H, $J = 2.3$ Hz, C-9 H), 7.14 (*d*, 1H, $J = 8.4$ Hz, C-12 H), and 7.7 (*s*, 1H, N—H). MS: *m/e* 398 (M^+, 30%), 382 (50), 368 (100), 366 (29), 353 (17), 339 (10), 323 (10), 284 (13), 283 (11), 258 (11), 244 (18), 227 (14), 208 (15), 184 (30), 160 (17), 136 (46), and 124 (30). Mass measurement, Obs.: 398.1806; Calc. for $C_{22}H_{26}N_2O_5$ requires: 398.1841.

$LiAlH_4$ *reduction of 10-methoxyeglandine-N-oxide* (**4**). 10-Methoxyeglandine-*N*-oxide (**4**, 5 mg) was treated with $LiAlH_4$ in dry THF under reflux for 3 hr [20, 21]. Standard work-up procedures afforded a crude product which on prep-TLC yielded voacanginol (**7**) as a pale gum (3 mg). ^1H NMR ($CDCl_3$): δ 0.92 (*t*, 3H, $J = 6.3$ Hz, C-18 H_3), 3.84 (*s*, 3H, $ArOCH_3$), 6.76 (*dd*, 1H, $J = 8.4$ and 2.3 Hz, C-11 H), 6.91 (*d*, 1H, $J = 2.3$ Hz, C-9 H), 7.18 (*d*, 1H, $J = 8.4$ Hz, C-12 H), 7.5 (*s*, 1H, N—H). MS: *m/e* 340 (M^+, 100%), 325 (23), 309 (16), 255 (18), 216 (21), 136 (54), and 122 (36). The reduction product was identical (^1H NMR, M and TLC) to an authentic sample of 19*S*-voacanginol (**7**) prepared by the $LiAlH_4$ reduction of 19*S*-voacangine (**5**).

10-*Hydroxycoronaridine* (**8**). The polar fraction from the separation of **4** on further purification by prep.-TLC on Si gel eluting with $CHCl_3$–MeOH (19:1) afforded a pale yellow gum (5 mg, 0.00003%) identified as 10-hydroxycoronaridine (**8**), which gave a characteristic color reaction for a phenolic group with $FeCl_3$ soln. UV λ_{max}^{MeOH} nm: 218 (log ε 4.33), 282 (3.80), and 304 (3.78). IR ν_{max}^{KBr} cm^{-1}: 3380, 2930, 2860, 1715, 1630, 1590, 1458, 1340, 1252 and 760. ^1H NMR ($CDCl_3$): δ 0.89 (*t*, 3H, $J = 5.8$ Hz, C-18 H_3), 3.7 (*s*, 3H, CO_2Me) 6.68 (*dd*, 1H, $J = 8.3$ and 2 Hz, C-11 H), 6.87 (*d*, 1H, $J = 2$ Hz, C-9 H), 7.08 (*d*, 1H, $J = 8.3$ Hz, C-12 H) and 7.62 (*s*, 1H, N-H). MS: *m/e* 354 (M^+, 100%), 339 (20), 325 (4), 295 (8), 294 (9), 269 (12), 230 (20), 170 (20), 136 (92), 124 (44) and 122 (88).

(−)-*Heyneatine* (**18**). Fraction 30 obtained by elution of the column with $CHCl_3$–MeOH (99:1) was further purified by prep.-TLC on Si gel eluting with $CHCl_3$–MeOH (19:1). A UV-visible band was separated, processed and the residue crystallized from EtOH–H_2O (9:1) to afford a white powder (9 mg, 0.00006%) identified as (−)-heyneatine (**18**): $[\alpha]_D^{26}$ −6.0° (*c* 0.14, $CHCl_3$). UV λ_{max}^{MeOH} nm: 220 (log ε 4.50), 280 (4.02) and 300 (3.93). IR ν_{max}^{KBr} cm^{-1}: 3380, 2930, 2873, 1730, 1625, 1590, 1485, 1455, 1370, 1260, 1215, 1165, 1148, 1115, 1065, 1030, 836 and 750. ^1H NMR ($CDCl_3$): δ 1.12 (*d*, 3H, $J = 6.3$ Hz, C-18 H_3), 3.7 (*s*, 3H, $-CO_2Me$), 3.84 (*s*, 3H, ArOMe), 4.0 (*s*, 1H, C-3 H), 4.08 (*m*, 1H, C-19 H), 6.8 (*dd*, 1H, $J = 8.4$ and 2 Hz, C-11 H), 6.89 (*d*, 1H, $J = 2$ Hz, C-9 H), 7.15 (*d*, 1H, $J = 8.4$ Hz, C-12 H) and 7.73 (*s*, 1H, N—H). MS: *m/e* 382 (M^+, 100%), 367 (12), 352 (14), 338 (28), 323 (27), 300 (37), 259 (32), 244 (62), 225 (14), 212 (18) and 184 (56). Mass measurement, Obs.: 382.1926; Calc. for $C_{22}H_{26}N_2O_4$ requires 382.1892.

$LiAlH_4$ *reduction of heyneatine* (**18**). Heyneatine (**18**, 4 mg) was treated with $LiAlH_4$ (10 mg) in dry Et_2O (5 ml) under reflux for 6 hr. Standard work-up procedures afforded a gum which on prep.-TLC afforded 19*S*-voacangarinol (**19**). IR $\nu_{max}^{NaCl\ plate}$ cm^{-1}: 3400, 2940, 2875, 1635, 1600, 1495, 1465, 1380, 1222, 1103 and 762. ^1H NMR ($CDCl_3$): δ 1.14 (*d*, 3H, $J = 6.5$ Hz, C-18 H_3), 3.85 (*s*, 3H, Ar-OMe), 4.16 (*m*, 1H, C-19 H), 6.82 (*dd*, 1H, $J = 8.2$ and 2.3 Hz, C-11 H), 6.89 (*d*, 1H, $J = 2.3$ Hz, C-9 H), 7.19 (*d*, 1H, $J = 8.2$ Hz, C-12 H) and 7.84 (*s*, 1H, N—H). MS: *m/e* 356 (M^+, 100%), 341 (48), 338 (49), 325 (25), 311 (24), 308 (22), 298 (16), 281 (16), 255 (18), 239 (15), 236 (17), 216 (28), 183 (36), 160 (32), 152 (52), 125 (39) and 123 (41). The reduction product was identical (IR, ^1H NMR, MS and TLC) with an authentic sample of 19*S*-voacangarinol (**19**) prepared from 19*S*-voacangarine (**20**) [20, 21].

$NaBH_4$ *reduction of heyneatine* (**18**). Heyneatine (**18**, 2 mg) was treated with $NaBH_4$ (5 mg) in MeOH (1 ml) at room temp. overnight. Work-up in the usual way afforded voacangarine (**20**) identical (MS, TLC) with an authentic sample [20, 21].

O-*Acetyl vallesamine* (**24**). Fraction 31 obtained by further elution of the column with $CHCl_3$–MeOH (99:1) was further purified by prep.-TLC on Si gel eluting with $CHCl_3$–MeOH (19:1). A UV-visible band was separated, processed and the residue crystallized from EtOH–H_2O (9:1) to afford a white powder (18 mg, 0.00012%) identified as *O*-acetyl vallesamine (**24**): $[\alpha]_D^{25}$ +151° ($CHCl_3$) (lit. [30] +155°). UV λ_{max}^{MeOH} nm: 222 (log ϵ 4.62), 283 (3.91) and 292 (3.86). IR $\nu_{max}^{NaCl\ plates}$ cm^{-1}: 3510, 2915, 2860, 1745, 1622, 1465, 1450, 1435, 1388, 1365, 1235, 1110, 1040 and 740. ^1H NMR ($CDCl_3$): δ 1.75 (*d*, 3H, $J = 6.9$ Hz, C-18 H_3), 2.08 (*s*, 3H, −OCOMe), 2.78–3.06 (*m*, 5H), 3.79 (*s*, 3H, $-CO_2Me$), 4.11 (*d*, 1H, $J = 17.9$ Hz, C-16 H), 4.29 (*d*, 1H, $J = 10.5$ Hz, C-6 H), 4.51 (*d*, 1H, $J = 10.5$ Hz, C-6 H), 4.78 (*d*, 1H, $J = 17.9$ Hz, C-16 H), 5.62 (*q*, 1H, $J = 6.9$ Hz, C-19 H), 6.8–7.6 (*m*, 4H, Ar-H) and 8.64 (*s*, 1H, N—H). MS: *m/e* 382 (M^+, 100%), 367 (6), 353 (3), 339 (14), 326 (80), 323 (30), 309 (34), 280 (10), 273 (10), 263 (27), 214 (50), 194 (30), 154 (55) and 122 (51). Physical and spectral data are in agreement with those reported for *O*-acetyl vallesamine [30].

Pericalline (**25**). Fraction 32 obtained by elution of the column with $CHCl_3$–MeOH (49:1) was separated by prep.-TLC on Si gel eluting with $CHCl_3$–MeOH (17:3). A UV-visible band was removed, processed and crystallized from petrol–$CHCl_3$ (4:1) to afford white crystals (18 mg, 0.00012%) identified as pericalline (**25**): mp 188–190°, $[\alpha]_D^{26}$ −176.5° ($CHCl_3$) (lit. [31] mp 196–202°, $[\alpha]_D$ −183°). UV λ_{max}^{MeOH} nm: 218 (log ε 4.71), 230 (4.69) and 307 (4.62). IR ν_{max}^{KBr} cm^{-1}: 3400, 3120, 2920, 2885, 1662, 1621, 1601, 1442, 1370, 1322, 1302, 1222, 1115, 1078, 880, and 735. ^1H NMR ($CDCl_3$): δ 1.45 (*dd*, 3H, $J = 6.8$, 1.7 Hz, C-18 H_3), 2.0 (*m*, 2H, C-14 H), 2.79–3.48 (*m*, 4H, C-3 H_2 and C-21 H_2), 3.92 (*m*, 1H, C-15 H), 4.18 (*d*, 1H, $J = 17.9$ Hz, C-6 H), 4.56 (*d*, 1H, $J = 17.9$ Hz, C-6 H), 5.24 (*q*, 1H, $J = 6.8$ Hz, C-19 H), 5.24 and 5.38 (*s*, 1H each, C-16 H_2), 6.90–7.48 (*m*, 4H, ArH) and 7.94 (*s*, 1H, N—H). MS: *m/e* 264 (M^+, 100%), 249 (18), 235 (17), 222 (35), 208 (37), 194 (20), 180 (16), 167 (15), 154 (16), 130 (8), and 108 (10). This compound was identical with an authentic sample (mmp, IR, TLC).

(+)-19,20-*Dihydrocondylocarpine* (**26**). Fraction 35 obtained by eluting the column with $CHCl_3$–MeOH (19:1) was separated by prep.-TLC eluting with $CHCl_3$–MeOH (17:3). A UV-visible band was processed to afford an amorphous gum (15 mg, 0.0001%) identified as (+)-19,20-dihydrocondylocarpine (**26**) [32]: $[\alpha]_D^{26}$ +538° ($CHCl_3$) (lit. [33] $[\alpha]_D$ +550°). UV λ_{max}^{MeOH} nm: 228 (log ε 4.21), 298 (3.91), and 329 (4.04). IR ν_{max}^{NaCl} cm^{-1}: 3378, 2960, 2935, 2880, 1680, 1610, 1480, 1460, 1440, 1282, 1240, 1155 and 755. ^1H NMR ($CDCl_3$): δ 3.76 (*s*, 3H, $—CO_2Me$), 6.7–7.2 (*m*, 4H, Ar–H) and 8.84 (*s*, 1H, N—H). MS: *m/e* 324 (M^+, 85%), 309 (8), 293 (14), 267 (35), 253 (25), 240 (15), 229 (100), 197 (24), 194 (35), 182 (40), 181 (35), 180 (75), 168 (25) and 167 (55). The physical and chemical data are in accord with those reported [26] for **26**.

Acknowledgements—This work was carried out under contract CM-67090 with the Division of Cancer Treatment, Drug Research and Development Branch of the National

Cancer Institute, Department of Health, Education and Welfare, Bethesda, MD. The authors thank the Economic Botany Laboratory, Plant Genetics and Germplasm Institute, United States Department of Agriculture, Beltsville, MD., funded by the NCI, for the provision and identification of the plant material.

REFERENCES

1. Gorman, M., Neuss, N., Cone, N. J. and Deyrup, J. A. (1960) *J. Am. Chem. Soc.* **82,** 1142.
2. Kupchan, S. M., Bright, A. and Macko, F. (1963) *J. Pharm. Sci.* **52,** 598.
3. Willaman, J. J. and Li, H. L. (1970) *Lloydia* **33S,** 1.
4. Knox, J. R. and Slobbe, J. (1971) *Tetrahedron Letters* 2149.
5. Delle Monache, G., D'Albuquerque, I. L., Delle Monache, F. and Marini-Bettolo, G. B. (1972) *Atti Accad. Naz. Lincei. Cl. Sci. Fis. Mat. Nat. Rend.* **52,** 375.
6. Knox, J. R. and Slobbe, J. (1975) *Aust. J. Chem.* **28,** 1813.
7. Knox, J. R. and Slobbe, J. (1975) *Aust. J. Chem.* **28,** 1825.
8. Talapatra, B., Patra, A. and Talapatra, S. K. (1975) *Phytochemistry* **14,** 1652.
9. Gonzalez, C. G. and Martinez, J. (1976) *Rev. Cubana Farm* **10,** 45.
10. Bruneton, J., Cave, A., Hagaman, E. W., Kunesch, N. and Wenkert, E. (1976) *Tetrahedron Letters* 3567.
11. Anjaneyulu, B., Rao, V. B., Ganguly, A. K., Govindachari, T. R., Joshi, B. S., Kamat, V. N., Manmade, A. H., Mohamed, P. A., Rahimtula, A. D., Saksena, A. K., Varde, D. S. and Viswanathan, N. (1965) *Indian J. Chem.* **3,** 237.
12. Talapatra, S. K., Sen Gupta, S. and Talapatra, B. (1967) *J. Indian Chem. Soc.* **44,** 416.
13. Jewers, K., Manchanda, A. H. and Wood, A. B. (1969) *Phytochemistry* **8,** 2099.
14. Geran, R. I., Greenberg, N. H., MacDonald, M. M., Schumacher, A. M. and Abbott, B. J. (1972) *Cancer Chemother. Rep.* **3,** 1.
15. Hsu, Y. T. (1967) *J. Southeast. Asian Res.* **3,** 63.
16. Dhar, M. L., Dhar, M. N., Dhawan, B. N., Mehrotra, B. N. and Ray, C. (1968) *Indian J. Exp. Biol.* **6,** 232.
17. Dhar, M. L., Dhar, M. N., Dhawan, B. N., Mehrotra, B. N., Srimal, R. C. and Tandon, J. S. (1973) *Indian J. Exp. Biol.* **11,** 43.
18. Gunasekera, S. P., Badawi, M. M., Cordell, G. A., Farnsworth, N. R. and Chitnis, M. (1979) *J. Nat. Prod.* **42,** 475.
19. Phillipson, J. D. and Hemingway, S. R. (1973) *Phytochemistry* **12,** 1481.
20. Panas, J. M., Richard, B., Potron, C., Razafindramboa, R. S., Debray, M. M., Le Men-Olivier, L. and Le Men, J. (1975) *Phytochemistry* **14,** 1120.
21. Le Men, J., Potier, P., Le Men-Olivier, L., Panas, J. M., Richard, B. and Potron, B. (1974) *Bull. Soc. Chim. Fr.* 1369.
22. Walls, F., Collera, O. and Sandoval, A. (1958) *Tetrahedron* **2,** 173.
23. Budzikiewicz, H., Djerassi, C. and Williams, D. H. (1964) *Structure Elucidation of Natural Products by Mass Spectrometry, Volume I. Alkaloids.* p. 68. Holden Day, San Francisco.
24. DeBellefon, M., Debray, M. M., Le Men-Olivier, L. and Le Men, J. (1975) *Phytochemistry* **14,** 1649.
25. Wenkert, E., Cochran, D. W., Gottlieb, H. E., Hagaman, E. W., Braz Filho, R., Abreu Matos, F. J. de and Machado Madruga, M. I. L. (1976) *Helv. Chim. Acta* **59,** 2437.
26. Gabetta, B. and Mustich, G. (1975) *Spectral Data of Indole Alkaloids.* Elli & Pagani, Milan.
27. Thomas, D. W. and Biemann, K. (1968) *Tetrahedron* **24,** 4223.
28. Renner, U. (1959) *Experientia* **15,** 185.
29. Govindachari, T. R., Joshi, B. S., Saksena, A. K., Sathe, S. S. and Viswanathan, N. (1965) *Tetrahedron Letters* 3873.
30. Walser, A. and Djerassi, C. (1964) *Helv. Chim. Acta* **47,** 2072.
31. Farnsworth, N. R. and Fong, H. H. S. (1969) *J. Pharm. Sci.* **58,** 280.
32. Walser, A. and Djerassi, C. (1965) *Helv. Chim. Acta* **48,** 391.