Aust. J. Chem., 1975, **28**, 1813–23

Indole Alkaloids from *Ervatamia orientalis*. I Isolation of Alkaloids and Structural Identification of Two Dimers

John R. Knox and Jacob Slobbe

Department of Organic Chemistry, University of Western Australia, Nedlands, W.A. 6009.

Abstract

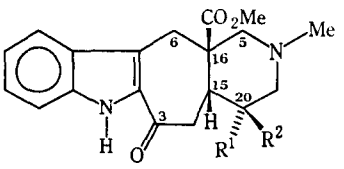
Ethanol extracts of *Ervatamia orientalis* have yielded the following known alkaloids: ibogaine, iboxygaine, voacristine, vobasine, dregamine, tabernaemontanine, apparicine, voacamine and 16-demethoxycarbonylvoacamine. In addition, two new dimeric alkaloids of the voacamine group and the novel 2-acylindoles ervatamine, 20-epiervatamine and 19-dehydroervatamine have been isolated from the extracts. The two new dimeric compounds have been identified by physical and chemical methods as 16-demethoxycarbonyldihydrovoacamine and 16-demethoxycarbonyl-20′-epi-dihydrovoacamine.

Plants classified in the Tabernaemontaneae tribe (Apocynaceae) have proved to be a rich source of indole alkaloids.[1] The tribe is the sole plant source of the iboga type alkaloids (with one exception[2]), the related voacamine group of dimeric alkaloids and the vobtusine group of dimeric alkaloids; it is also an important source of the 2-acylindole class and has afforded miscellaneous other types of indole alkaloids.[1,3,4] The second largest of the 20 genera in the tribe is *Ervatamia*[5] but relatively few of the 92–95 species in the genus have been examined for alkaloids. Iboga-type alkaloids have been obtained from *E. dichotoma* (coronaridine,[6] heyneanine,[7] voacristine hydroxyindolenine[8]), *E. coronaria* (coronaridine, voacangine[9]) and *E. pandacaqui* (coronaridine[10]), 2-acylindoles from *E. coronaria* (dregamine, tabernaemontanine[9]), *E. divaricata* (tabernaemontanine[11]) and *E. pandacaqui* (tabernaemontanine[10]) and aspidospermine types from *E. dichotoma* (tabersonine[12]) and *E. divaricata* (lochnericine, voaphylline[11]). *E. pandacaqui* has also afforded 20-epilochneridine and three dimeric alkaloids (ervafoline, ervafolidine, isoerva-

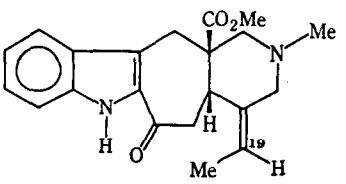
[1] Hegnauer, R., 'Chemotaxonomie der Pflanzen' Vol. 3, pp. 124–63, 632–7 (Birkhäuser: Basel 1964).
[2] Gorman, M., Neuss, N., and Cone, N. J., *J. Amer. Chem. Soc.*, 1965, **87**, 93.
[3] Hesse, M., 'Indole Alkaloide in Tabellen' (Springer: Berlin 1964 and supplement 1968).
[4] Manske, R. W. F., (Ed.) 'The Alkaloids' Vols 8, 11 (Academic Press: London 1965, 1968).
[5] Pichon, M., *Mem. Mus. Nat. Hist. Nat.*, 1948, **27**, 153.
[6] Kupchan, S. M., Bright, A., and Macko, E., *J. Pharm. Sci.*, 1963, **52**, 598.
[7] Kupchan, S. M., Cassady, J. M., and Telang, S. A., *Tetrahedron Lett.*, 1966, 1251.
[8] Schnoes, H. K., Thomas, D. W., Aksornvitaya, R., Schleigh, W. R., and Kupchan, S. M., *J. Org. Chem.*, 1968, **33**, 1225.
[9] Gorman, M., Neuss, N., Cone, N. J., and Deyrup, J. A., *J. Amer. Chem. Soc.*, 1960, **82**, 1142.
[10] Lathuilliere, P., Olivier, L., Levy, J., and Le Men, J., *Ann. Pharm. Fr.*, 1970, **28**, 57.
[11] Raj, K., Shoeb, A., Kapil, R. S., and Popli, S. P., *Phytochemistry*, 1974, **13**, 1621.
[12] King, M. L., *Diss. Abstr.*, 1965, **25**, 3854.

folidine) which appear to be of the vobtusine type.[10] The generic name *Tabernaemontana* is synonymous with *Ervatamia* for some of these plants.

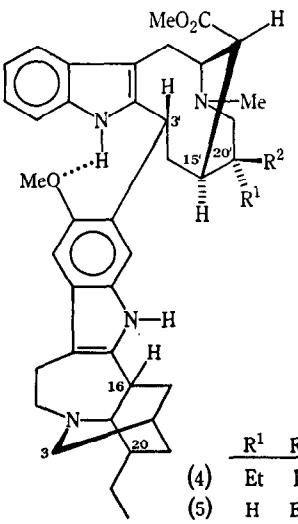
The presence of alkaloids in *E. orientalis* (R.Br.) Turtill was indicated in field tests by Webb[13] and more recently Jewers *et al.*[14] found traces of alkaloids only in the roots. This plant was originally classified in the *Tabernaemontana* genus (*T. orientalis* R.Br.)[15] but its classification in the *Ervatamia* genus has been accepted by Pichon.[5]



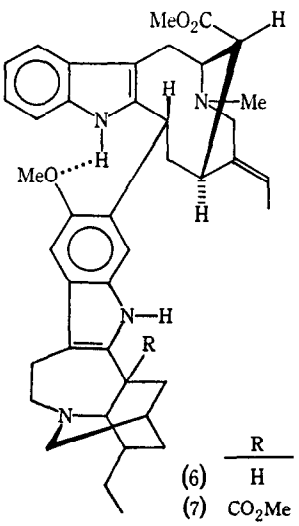
	R^1	R^2
(1)	H	Et
(2)	Et	H



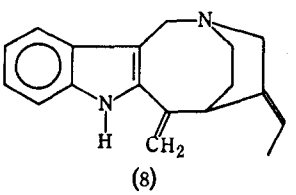
(3)



	R^1	R^2
(4)	Et	H
(5)	H	Et



	R
(6)	H
(7)	CO_2Me

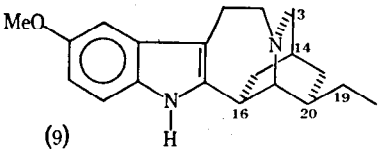


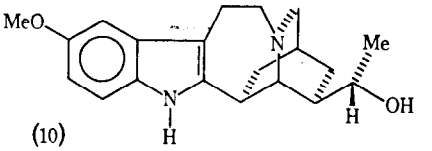
(8)

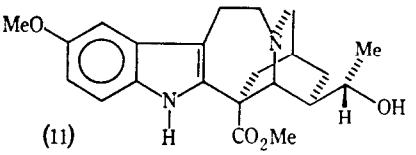
The plant grows as a shrub from 10 to 15 ft in height with white flowers and has an intensely bitter bark.[16,17] It has an extremely wide distribution, ranging from the high rainfall area of northern Western Australia,[16] through tropical Northern Territory and the Cape York region of Queensland, to New Guinea, the Malayan Archipelago, continental South East Asia and Polynesia.[18]

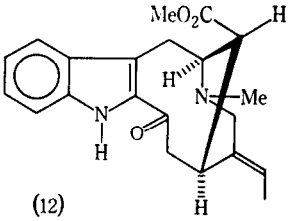
[13] Webb, L. J., 'Australian Phytochemical Survey' Part I (CSIRO Bulletin 241: 1949), Part II (CSIRO Bulletin 268: 1952).
[14] Jewers, K., Manchanda, A. H., and Wood, A. B., *Phytochemistry*, 1969, **8**, 2099.
[15] Shaw, F. H., 'A Phytochemical Register of Australian Plants' Vol. 1 (CSIRO: Melbourne 1959).
[16] Beard, J. S., 'Descriptive Catalogue of West Australian Plants' (Surrey Beatty & Sons: Sydney 1970).
[17] Maiden, J. H., 'The Useful Native Plants of Australia' (Turner & Henderson: Sydney 1889).
[18] Specht, R. L., and Mountford, C. P., (Eds) 'Records of the American–Australian Scientific Expedition to Arnhem Land' Vol. 3 (Melbourne University Press 1958).
[19] Knox, J. R., and Slobbe, J., *Tetrahedron Lett.*, 1971, 2149.

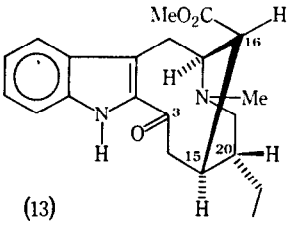
Collections of the leaves and the bark of the plant have been obtained from various sites in Queensland for the study that is described in this paper. Qualitative and quantitative differences in alkaloid composition have been found for the different collections and a total of 14 alkaloids have been isolated from the extracts by means of countercurrent distribution and chromatographic techniques. Five of these compounds have not been reported previously. These are ervatamine (1),

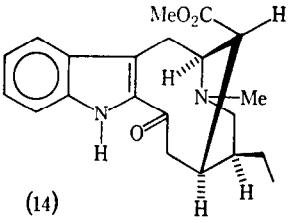

(9)

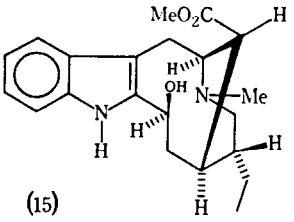

(10)

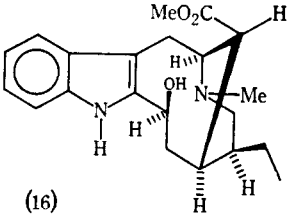

(11)


(12)


(13)


(14)


(15)


(16)

20-epiervatamine (2) and 19-dehydroervatamine (3), 16-demethoxycarbonyldihydrovoacamine (4), and 16-demethoxycarbonyl-20′-epidihydrovoacamine (5).* The structures of the latter two dimeric compounds are established by the evidence presented later in this paper whereas the structures of the former three 2-acylindoles (1), (2) and (3) are the subject of separate communications.[20,21]

* The numbering system employed in this paper follows that of Le Men and Taylor.[19]

[20] Knox, J. R., and Slobbe, J., *Aust. J. Chem.*, 1975, **28**, 1825.
[21] Le Men, J., and Taylor, W. I., *Experientia*, 1965, **21**, 508.

The nine known alkaloids have been identified by their spectral and physical characteristics and (with the exception of (6)), by comparison with authentic samples. These are apparicine (8), the iboga class alkaloids ibogaine (9), iboxygaine (10) and voacristine (11), the 2-acylindoles, vobasine (12), dregamine (13) and tabernaemontanine (14) and the dimeric alkaloids voacamine (7) and 16-demethoxycarbonylvoacamine (6).

Attention should be drawn to the fact that the relative configurations of the ethyl side chain represented for dregamine (13) and tabernaemontanine (14) are the reverse of the original literature assignments.[22] The conclusive evidence pointing to the need for this reversal as well as some further chemistry of this group will be the subject of a separate publication.[23] These assignments have been confirmed by a recently published X-ray study.[24,25] The absolute configurations for ibogaine (9) and iboxygaine (10) have been demonstrated by circular dichroism studies of the iboga class of alkaloids[26] and the configurations of the iboga units of voacamine (7) and demethoxycarbonylvoacamine (6) follow from the same circular dichroism studies together with the method of their synthesis[27,28] and interrelation.[28] The absolute configuration for voacristine (11) has, however, not been demonstrated.

The co-occurrence of apparicine (8) with ervatamine (1), 20-epiervatamine (2) and 19-dehydroervatamine (3) is of interest because all four of these compounds lack the two-carbon link between N_b and the indole ring that is typical of most indole alkaloids; in apparicine the linkage is through a methylene group and in the other compounds it is through a three-carbon chain. It has been suggested that the two modified linkages are formed through related biosynthetic pathways involving cleavage of the bond between the α and β carbons of tryptamine-based structures.[24,29]

Apparicine has been isolated previously from several plants scattered through the Plumeroideae subfamily of the Apocynaceae[3,4] but the remaining eight known compounds obtained in this work are typical of the Tabernaemontaneae tribe.[1] It is noteworthy that these eight compounds [(6), (7), (9)–(14)] are representative of the iboga and 2-acylindole classes and of the dimeric class which has these units coupled together.

The previously undescribed dimeric alkaloids 16-demethoxycarbonyldihydrovoacamine (4) and 16-demethoxycarbonyl-20′-epidihydrovoacamine (5) isolated in this work are closely similar in their physical properties as would be expected from their epimeric structures. They may be distinguished by the fingerprint region of their i.r. spectra which are markedly different and by their differing mobility in thin-layer chromatography. As is typical of the class,[27] they lack characteristic melting points because decomposition occurs over a broad range. Their spectral properties are closely similar and have the expected relationships with the spectra of the respective monomeric units and of the two known alkaloids voacamine and decarbomethoxyvoacamine also obtained from the plant. Their u.v. spectra show

[22] Renner, U., Prins, D. A., Burlingame, A. L., and Biemann, K., *Helv. Chim. Acta*, 1963, **46**, 2186.
[23] Knox, J. R., and Slobbe, J., *Aust. J. Chem.*, 1975, **28**, 1843.
[24] Husson, A., Langlois, Y., Riche, C., Husson, H. P., and Potier, P., *Tetrahedron*, 1973, **29**, 3095.
[25] Riche, C., *Acta Crystallogr., Sect. B*, 1974, **30**, 610.
[26] Blaha, K., Koblicova, Z., and Trojanek, J., *Collect. Czech. Chem. Commun.*, 1974, **39**, 2258.
[27] Büchi, G., Manning, R. E., and Monti, S. A., *J. Amer. Chem. Soc.*, 1964, **86**, 4631.
[28] Thomas, D. W., and Biemann, K., *Lloydia*, 1969, **31**, 1.
[29] Ahond, A., Cave, A., Kan-Fan, C., Langlois, Y., and Potier, P., *Chem. Commun.*, 1970, 517.

normal indole chromophores while their i.r. spectra each show peaks for indole NH, methyl ester and *ortho*-disubstituted benzene groups.

The n.m.r. spectra of (4) and (5) each have broad C3 methine proton signals (at δ 4·93 and 5·03 for (4) and (5) respectively), a broadened signal for an aromatic methoxyl (at δ 3·91 and 3·94, $W_{h/2}$ *c.* 3 Hz) and peaks for shielded methoxycarbonyl and *N*-methyl groups (at δ 2·57 and 2·47 for (4) and at δ 2·52 and 2·44 for (5)). The reason for the broadening of the methoxyl signal is not clear, although it may be due to a slow rate of interconversion between conformations about the bond linking the monomeric units. The stability of such conformations and the shielding of the methoxyl protons would be influenced by hydrogen bonding between the methoxyl group and the indole NH of the 3,4-secosarpagine unit.[27] The extent of broadening seems too large for the alternative explanation of long-range coupling to an *ortho*-hydrogen as has been observed for methoxyl groups in some systems.[30,31] The mass spectra of the voacamine group of dimeric alkaloids[27,32,33] are complicated by the formation of ions of higher mass than the molecular ion through intramolecular reaction at the high temperature needed to volatilize these substances. The spectra of (4) and (5) are essentially identical and show a molecular ion (M^+) at *m/e* 648 and a weak M+14 ion at *m/e* 662. As these compounds lack a methoxycarbonyl group in the iboga unit, the M+14 ion is not as important as in voacamine and related compounds.[33] The remainder of the high mass region for (4) and (5) has relatively small peaks with the exception of significant peaks clustered around *m/e* 466 and 452. Equivalent peaks in the spectrum of voacamine (7) have been attributed to the loss of the piperidine ring system of the 3,4-secosarpagine unit.[33] Ions at *m/e* 182 and 196 are also present in the spectra of (4) and (5) due to retention of charge on the piperidine ring fragments; these ions are also important in the mass spectra of dregamine and tabernaemontanine. The ibogaine moiety of the dimers is responsible for the typical ions at *m/e* 122, 135, 136, 149 and 225.[34]

These spectral data suggest that the structures of the two compounds are dimeric from combination of ibogaine with a dregamine unit on the one hand and with a tabernaemontanine unit on the other hand but the evidence did not permit a separate assignment of the two structures. Further support for this conclusion came from acidic treatment[27,35] of 16-demethoxycarbonyl-20′-epidihydrovoacamine (5) which caused fission of the bonding linkage of the dimer to give ibogaine in low yield. This degradative procedure does not allow a fragment for the other half of the molecule to be isolated.[27,35]

The structures of the two dimers were confirmed and separate assignment made by their partial synthesis from the respective monomeric units.[27] Thus dergaminol (15) and ibogaine were condensed by heating in dilute methanolic HCl solution to give a moderate yield of partially synthetic 16-demethoxycarbonyldihydrovoacamine (4) and this was found to be identical with the natural dimer having the lower

[30] Woods, M. C., Miura, I., Ogiso, A., Kurabayashi, M., and Mishima, H., *Tetrahedron Lett.*, 1968, 2009.
[31] Ridley, D. D., Ritchie, E., and Taylor, W. C., *Aust. J. Chem.*, 1970, **23**, 147.
[32] Budzikiewicz, H., Djerassi, C., Puisieux, F., Percheron, F., and Poisson, J., *Bull. Soc. Chim. Fr.*, 1963, 1899.
[33] Thomas, D. W., and Biemann, K., *J. Amer. Chem. Soc.*, 1965, **87**, 5447.
[34] Biemann, K., and Friedmann-Spiteller, M., *J. Amer. Chem. Soc.*, 1961, **83**, 4805.
[35] Winkler, W., *Naturwissenschaften*, 1961, **48**, 494.

chromatographic mobility. For the other synthesis, tabernaemontaninol (16) was prepared by borohydride reduction of tabernaemontanine (14) in methanol–ether solution. A band at 1700 cm^{-1} for hydrogen-bonded methyl ester in the i.r. spectrum allows the hydroxyl configuration for (16) to be written as β (cf. dregaminol[27]). This alcohol (16) was condensed with ibogaine under acidic conditions to give 16-demethoxycarbonyl-20′-epidihydrovoacamine (5) in low yield and the product was shown to be identical with the dimer having the higher chromatographic mobility.

Previous work on the positional isomers of voacamine (7)[27,36] clearly demonstrates that these syntheses will give products which have the position of linkage of the monomeric units as given in (4) and (5). In addition, the aromatic patterns of the n.m.r. spectra of the two dimers each have clearly discernible broadened singlets (δ 6·50 and 6·60 respectively); it follows that the iboga units have the two aromatic protons *para* to one another.[27,36]

The above evidence thus demonstrates that the two new dimers from *E. orientalis* have the structures (4) and (5).

Experimental

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Analyses were carried out by the Australian Microanalytical Service, Melbourne. Optical rotations were determined for chloroform solutions at room temperature. Infrared spectra were measured with a Perkin–Elmer Infracord on CS_2 solutions (quoted frequencies) or Nujol mulls (fingerprint comparisons). Ultraviolet absorption spectra were recorded with a Perkin–Elmer 137 spectrophotometer for ethanol solutions. Nuclear magnetic resonance spectra were measured with a Varian A60 spectrometer for $CHCl_3$ (or $CDCl_3$) solutions containing tetramethylsilane as an internal standard. Mass spectra were measured routinely with a Varian MAT CH 7 or an AEI MS9 spectrometer. The precise mass measurements were obtained with the latter machine.

Extraction of Alkaloids

(i) *Bark.*—The dried milled stembark of *E. orientalis* (herbarium voucher No. SN 8268; 12 kg) collected at Cannonvale, Queensland, in June 1967, was exhausted with cold ethanol. The solvent was concentrated to a small volume, the residue taken up in 5% aqueous citric acid solution and partitioned with ether. The ether layer was extracted exhaustively with 5% aqueous citric acid until it failed to give a positive test with Mayer's reagent. The aqueous layer was basified with ammonia solution and extracted three times with chloroform. This organic layer was washed again with citric acid which in turn was basified and extracted with chloroform. Removal of the solvent left a brown gum (160 g; 1·3%).

Further bark samples collected at Port Douglas Beach, Queensland, in June 1966 (IC 3043; 5 kg) and north of Ellis Beach, Cairns, Queensland, in July 1969 (IC 3749; 0·5 kg) yielded basic fractions (100 g, 2%; 7 g, 1·4% respectively) when extracted in the above manner.

(ii) *Leaves.*—Dried leaves collected from an unspecified locality in Queensland in July 1967 (IC 3450; 7 kg) and from the Port Douglas Beach collection (IC 3043; 27·5 kg) afforded crude alkaloid fractions (15·2 g, 0·22%; 35 g, 0·13% respectively) by a similar extraction procedure.

Countercurrent Distribution of the Extracts

Countercurrent distributions were conducted with a 100-tube Quickfit steady state distribution apparatus on the upper and lower phases (25 ml of each phase per tube) obtained from equilibrated mixtures of ethyl acetate and disodium hydrogen orthophosphate–citric acid buffers of various pH values.[37] Two procedures were used: (*a*) In the most frequently used procedure, the lower phase was introduced into each tube of the machine and the upper phase was introduced into the

[36] Renner, U., and Fritz, H., *Tetrahedron Lett.*, 1964, 283.
[37] Vogel, A. I., 'A Textbook of Quantitative Inorganic Analysis' p. 869 (Longmans: London 1961).

first ten tubes. The sample to be fractionated was then divided equally between the first three tubes and countercurrent distribution commenced. Transfer of 80 upper-phase aliquots was effected, followed by 100 lower-phase ones. Introduction of fresh upper and lower-phase solvent was then ceased and upper transfers were continued until this phase was removed totally from the apparatus. The machine was then emptied of the remaining lower-phase solution. (*b*) For some separations a gradient pH system was used. After introduction of the lower phase, the upper phase was introduced into the first 60 tubes. The sample to be fractionated was then divided between tubes 49–52 and the countercurrent distribution commenced. The pH of the lower phase was gradually changed by introducing 25 ml of lower pH buffer into the reservoir during each lower-phase transfer. Forty upper-phase transfers were conducted and then the introduction of fresh upper and lower phase was stopped and the machine emptied as before.

Table 1. Alkaloids from countercurrent distribution

UP, upper-phase; LP, lower-phase fraction

Bark SN 8268; *pH 5·4*			
UP 12–23	—(pH 4·6)→ LP 80–140	tabernaemontanine (14)	
	UP 50–90	ervatamine (1)	
UP 24–40	tabernaemontanine (14)		
	mother liquors —(pH 4·8)→ LP 1–50		16-demethoxycarbonyl-20′-epidihydrovoacamine (5)
	LP 100–190		tabernaemontanine (14)
			20-epiervatamine (2)
UP 54–100	dregamine (13)		
	mother liquors —(pH 5·0)→ LP 24–84		16-demethoxycarbonyl-20′-epidihydrovoacamine (5)
LP 125–180	16-demethoxycarbonyldihydrovoacamine (4)		
Bark IC 3749; *gradient pH*			
LP 147–155	demethoxycarbonylvoacamine (6)		
Bark IC 3043; (i) *pH 5·2*			
UP 14–30	ervatamine (1)		
UP 31–60	19-dehydroervatamine (3)		
UP 61–90	vobasine (12)		
Bark IC 3043; (ii) *gradient pH*			
LP 131–147	voacamine (7)		
LP 175–200	voacristine (11)		
Leaves IC 3043; *pH 5·4*			
UP 8–26	ervatamine (1)		
UP 27–72	19-dehydroervatamine (3)		
UP 73–100	apparicine (8)		
Leaves IC 3450; *pH 5·4*			
UP 12–24	ervatamine (1)		
UP 27–50	19-dehydroervatamine (3)		
UP 51–70	ibogaine (9)		
UP 71–100	apparicine (8)		
LP 40–66	iboxygaine (10)		

In this way a total of 200 lower-phase and 100 upper-phase (including early equilibrating upper-phase fractions) 25-ml fractions were collected, numbered in the order of appearance from the machine. Separation of the alkaloids was monitored by determining the u.v. spectra of individual fractions. Some of the fractions required further separation through countercurrent distribution at a different buffer pH to isolate the individual alkaloids. The fractions were combined to give the various alkaloids as summarized in Table 1.

(i) *Bark SN 8268.*—Part of the alkaloid fraction (78 g) was partitioned between ethyl acetate and a solution of disodium hydrogen phosphate and citric acid buffered to pH 4·4. Recovery of the separate fractions in the usual manner afforded ethyl acetate soluble bases (50 g), buffer solubles (18 g) and an insoluble material (6 g) which collected at the interface of the partition. The latter two fractions were not investigated further.

The ethyl acetate soluble alkaloids (12·5 g) were subjected to countercurrent distribution using an ethyl acetate–pH 5·4 buffer system. This separation was repeated a further three times and the combined fractions 24–40 (6·1 g) crystallized directly from ethyl acetate as needles of tabernaemontanine (14), m.p. and m.m.p. 219–220°, $[\alpha]_D$ −42° (lit.[22] m.p. 215–216°, $[\alpha]_D$ −58°); M^+ 354. The i.r. spectrum was identical to that of an authentic sample.

The semicrystalline material (9·7 g) from fractions 54–100 of the upper phase crystallized from ethyl acetate as solvated prisms of dregamine (13), m.p. 122–127° then 137–141°. A further crystallization from ether deposited small rods, m.p. and m.m.p. 137–141°, $[\alpha]_D$ −77° (lit.[22] m.p. 137–140°, $[\alpha]_D$ −90°); M^+ 354. The i.r. spectrum was identical to that of an authentic sample.

The glassy material (850 mg) recovered from the lower-phase tubes 125–180 was applied to a column of alumina (40 g) in light petroleum–benzene (1 : 4). Elution with this solvent produced *16-demethoxycarbonyldihydrovoacamine* (4) (400 mg) which on crystallization from benzene–light petroleum deposited as a white powder that decomposed without melting at 215°, $[\alpha]_D$ +22° (*c*, 1·7). λ_{max} 228, 290 and 298 nm (log ε 4·38, 3·82, 3·86); ν_{max} 3460 (indole NH), 1712 (methyl ester) and 750 cm^{-1} (*ortho*-disubstituted benzene); n.m.r. spectrum (δ): 4·93 (1H, m, $W_{h/2}$ 20 Hz, C3 methine); 3·91 (3H, broad s, methoxyl); 2·57 and 2·47 (6H, 2s, CO_2CH_3 and NCH_3); mass spectrum: *m/e* 648 (M^+, 23%), 662 (M+14, 10), 616 (16), 466 (85), 453 (98), 335 (32), 326 (39), 310 (65), 225 (38), 196 (22), 182 (45), 149 (61), 136 (100), 135 (89), 122 (65).

The combined upper-phase fractions 12–23 (7·5 g) were reapplied to the countercurrent apparatus with the buffer solution now at pH 4·6. From lower-phase tubes 80–140 a fraction (1 g) was obtained which crystallized from ethyl acetate as further tabernaemontanine (14). The oily material recovered from upper-phase tubes 50–90 (1·1 g) was chromatographed on alumina (50 g). Elution with light petroleum–benzene (1 : 4) afforded a colourless oil (700 mg) which was homogeneous by t.l.c. and n.m.r. A portion (40 mg) was characterized as the picrate. Crystallization from acetone yielded light yellow plates of *ervatamine picrate* (50 mg), m.p. 236–237° (dec.) (Found: C, 56·0; H, 5·1; N, 12·0. $C_{21}H_{26}N_2O_3,C_6H_3N_3O_7$ requires C, 55·6; H, 5·0; N, 12·0%).

The free alkaloid was recovered from the picrate (70 mg) by shaking with saturated Na_2CO_3 solution and ether until dissolution had occurred. The ether was removed to give a white froth (42 mg). Crystallization from a small quantity of methanol gave solvated prisms of *ervatamine* (1) (25 mg), m.p. 92–98°, $[\alpha]_D$ −3·7° (*c*, 2·1). The solvation was supported by the n.m.r. spectrum which showed a peak for one molecule of methanol at δ 3·46 (Found: C, 68·6; H, 7·9; N, 7·2. $C_{21}H_{26}N_2O_3,CH_3OH$ requires C, 68·4; H, 7·8; N, 7·5%). λ_{max} 238 and 312 nm (log ε 4·14, 4·18); ν_{max} 3450 (indole NH), 1730 (methyl ester), 1640 (2-acylindole) and 750 cm^{-1} (*ortho*-disubstituted benzene); n.m.r. spectrum (δ): 9·4 (1H, broad s, indole NH, exchanges with D_2O); 7·0–7·8 (4H, m, ArH); 3·69 (2H, s, C5 or C6 H_2); 3·48 (3H, s, CO_2CH_3) and 2·28 (3H, s, NCH_3); mass spectrum: M^+ at 354·1949 (calc. for $C_{21}H_{26}N_2O_3$: 354·1943); low resolution *m/e* 354 (63%), 322 (10), 295 (34), 224 (14), 210 (47), 196 (14), 195 (16), 182 (100), 130 (19). Metastable at 149·4 (295 → 210).

The mother liquors (5 g) of upper-phase fractions 54–100 after removal of dregamine were applied to the countercurrent machine with the buffer solution at pH 5·0. From the lower-phase tubes 24–84 a fraction (250 mg) was collected and applied to a column of alumina (8 g). Elution with light petroleum–benzene (1 : 4) gave a fraction (130 mg) which crystallized from benzene–light petroleum as a powdery solid, yielding *16-demethoxycarbonyl-20′-epidihydrovoacamine* (5), decomposition without melting from 180°, $[\alpha]_D$ +18° (*c*, 0·75). λ_{max} 233, 291, 297 nm (log ε 4·52, 4·02, 4·04); ν_{max} 3450 (indole NH), 1735 (methyl ester) and 760 cm^{-1} (*ortho*-disubstituted benzene); n.m.r. spectrum (δ); 5·03 (1H, m, $W_{h/2}$ 17 Hz; C3 methine proton); 3·94 (3H, broad s, methoxyl); 2·52 and 2·44 (6H, 2s, NCH_3 and CO_2CH_3); mass spectrum: *m/e* 648 (M^+, 48%), 662 (M+14, 10), 616 (31), 466 (100), 453 (32), 335 (24), 196 (35), 182 (48), 149 (25), 136 (51), 135 (26), 122 (28).

The combined mother liquors (6 g) of upper-phase fractions 24–40 after removal of tabernaemontanine were also reapplied to the countercurrent (pH 4·8). Further 16-demethoxycarbonyl-20′-epidihydrovoacamine (5) (500 mg) was obtained from lower-phase tubes 1–50 in the manner described above. Crystallization (Et_2O) of the solid material (1·9 g) obtained from lower-phase

tubes 100–190 afforded further tabernaemontanine (14). Chromatography of the mother liquors on alumina (30 g) and elution with light petroleum–benzene (1 : 4) afforded a colourless oil (1·0 g) which crystallized from ether as colourless prisms, m.p. 167–175°. Part of this material (50 mg) was further purified by preparative t.l.c. to remove residual tabernaemontanine. The homogeneous upper band (42 mg) thus obtained crystallized from ether–light petroleum as colourless prisms of *20-epiervatamine* (2), m.p. 185–187° (dec.), $[\alpha]_D$ −22° (*c*, 1·1) (Found: C, 71·1; H, 7·6; N, 7·9. $C_{21}H_{26}N_2O_3$ requires C, 71·2; H, 7·4; N, 7·9%). λ_{max} 239 and 318 nm (log ε 4·00, 4·11); ν_{max} 3445, 3280 (indole NH), 1735 (methyl ester), 1655 (2-acylindole) and 750 cm^{-1} (*ortho*-disubstituted benzene); n.m.r. spectrum (δ): 3·58 (3H, s, CO_2CH_3) and 2·31 (3H, s, NCH_3); mass spectrum M^+ at 354·1950 (calc. for $C_{21}H_{26}N_2O_3$: 354·1943); low-resolution mass spectrum *m/e* 354 (72%), 322 (25), 295 (14), 224 (12), 210 (12), 182 (100), 180 (20), 130 (12).

(ii) *Bark IC 3749*.—The crude alkaloid fraction (7 g) was subjected to a gradient countercurrent distribution changing the pH of the buffer solution from 7 to approximately 4 during the course of the separation. This gradient was achieved by adding a 0·2M citric acid solution (25 ml per transfer) to the 7 l. of pH 7 buffer solution in the reservoir.

The combined lower-phase fractions 147–155 (244 mg) were chromatographed on alumina (5 g). Elution with benzene–chloroform 19 : 1 gave a pale brown solid (106 mg). Recrystallization from methanol gave demethoxycarbonylvoacamine (6) (25 mg), m.p. 219–223° (lit.[28] 225–230°). The i.r. and m.s. data corresponded well with those published[28] and the n.m.r. and u.v. spectra were consistent with the structure assigned.

(iii) *Bark IC 3043*.—(A) Part of the alkaloid fraction (20 g) was applied to the countercurrent machine and separation was effected using buffer pH 5·2. Further ervatamine (1) was obtained from upper-phase fractions 14–30 (2·0 g) by column chromatography followed by fractional crystallization of the appropriate fractions from methanol.

The material collected from the machine immediately after this fraction (tubes 31–60, 4·1 g) was subjected to column chromatography on alumina (180 g). Elution with light petroleum–benzene (1 : 4) gave a colourless oil (1·6 g) which crystallized from ether to give prisms of *19-dehydroervatamine* (3), m.p. 198–200° (dec.), $[\alpha]_D$ +53° (*c*, 1·0) (Found: C, 71·6; H, 7·0; N, 7·8. $C_{21}H_{24}N_2O_3$ requires C, 71·6; H, 6·9; N, 8·0%). λ_{max} 242 and 315 nm (log ε 4·11, 4·28); ν_{max} 3450 (indole NH), 1740 (methyl ester), 1650 (2-acylindole) and 750 cm^{-1} (*ortho*-disubstituted benzene); n.m.r. spectrum (δ): 5·44 (1H, q, *J* 6·5 Hz, vinylic proton); 3·56 (3H, s, CO_2CH_3); 2·27 (3H, s, NCH_3) and 1·58 (3H, d, *J* 6·5 Hz, vinylic methyl); mass spectrum: *m/e* 352 (M^+, 93%), 337 (15), 323 (16), 293 (30), 194 (38), 180 (100), 172 (21), 166 (16), 130 (20).

The adjoining fraction (tubes 61–90, 1·7 g) was applied to a column of alumina (34 g) . Elution with benzene–light petroleum (4 : 1) afforded a fraction (1·15 g) part of which (200 mg) was converted into the hydrochloride. Crystallization from methanol gave prisms of vobasine hydrochloride, m.p. and m.m.p. 255–260° (lit.[22] 245–248°). The i.r. spectrum was identical to that of an authentic sample. Recovery of the free alkaloid by shaking between ether and saturated Na_2CO_3 solution, and removal of the ether layer by evaporation, gave a colourless oil which by slow ether evaporation could be induced to deposit a few crystals of vobasine (12), m.p. 110–115° (lit.[22] 111–113°), M^+ 352. The vobasine sample was noticeably unstable.

(B) The crude alkaloids from the Port Douglas Beach bark collection (IC 3043, 4 g) were also subjected to countercurrent distribution using a gradient pH which changed from pH 5·4 to approximately 3·6 in the course of separation.

The combined lower-phase fractions 131–147 (453 mg) were chromatographed on alumina (10 g). Elution with benzene–chloroform (1 : 1) gave a fraction (220 mg) which was crystallized from benzene–light petroleum and then from aqueous methanol to give voacamine (7), m.p. 215–225° (dec.) (lit.[27] 227–229° (dec.)). The t.l.c. behaviour of this compound was identical with that of an authentic specimen and the m.p. was not depressed by admixture of the two samples. The u.v., m.s. and n.m.r. spectra were fully in accord with the assigned structure.

Chromatography of the combined lower-phase fractions 175–200 (890 mg) with benzene as the eluant gave voacristine (11) which crystallized from benzene–light petroleum (180 mg); m.p. 90–95°, $[\alpha]_D$ −27° (lit.[38] 92–95° and 166–167°). The i.r. spectra and t.l.c. behaviour were identical with those of an authentic specimen. The u.v., m.s. and n.m.r. spectra were fully in accord with the assigned structure.

[38] Joule, J. A., Monteiro, H., Durham, L. J., Gilbert, B., and Djerassi, C., *J. Chem. Soc.*, 1965, 4773.

(iv) *Leaves IC 3043.*—The crude alkaloids (14 g) were separated on the countercurrent instrument, the buffer being at pH 5·4. By the procedures described above upper-phase tubes 8–26 (3 g) and 27–72 (2·5 g) afforded further ervatamine (1) and 19-dehydroervatamine (3) respectively.

The material (1·2 g) from upper-phase tubes 73–100 was crystalline but unstable. Chromatography of a portion (400 mg) on alumina (20 g) and elution with benzene afforded crystals (220 mg) which after recrystallization from benzene gave flat needles of apparicine (8), m.p. and m.m.p. 192–194°; $[\alpha]_D$ −187° (lit.[38] m.p. 192–194°, $[\alpha]_D$ −177°), M^+ 264. The infrared spectrum was identical to that of an authentic sample.

(v) *Leaves IC 3450.*—The crude alkaloids (15 g) were subjected to countercurrent separation under the same conditions as used for (iv). As before ervatamine (1), 19-dehydroervatamine (3) and apparicine (8) were isolated in the pure state from upper-phase tubes 12–24 (0·5 g), 27–50 (1 g) and 71–100 (2 g) respectively.

An intermediate fraction from tubes 51–70 (2·5 g) was applied to a column of alumina (125 g). Elution with benzene–light petroleum (4 : 1) afforded an oily fraction (1·8 g) which crystallized from methanol as thick needles of ibogaine (9), m.p. and m.m.p. 151–153°, $[\alpha]_D$ −45° (lit.[39] 152–153°), M^+ 310; the i.r. spectrum was identical to that of an authentic sample.

Application of the fraction (1·2 g) obtained from lower-phase tubes 40–66 to a column of alumina (60 g) and elution with benzene–chloroform (1 : 1) afforded solid material (250 mg) which crystallized from methanol as solvated rods of iboxygaine (10) m.p. and m.m.p. 234–236°, $[\alpha]_D$ −3·5° (lit.[40] m.p. 234°, $[\alpha]_D$ −5°); M^+ 326; the i.r. spectrum was identical to that of an authentic sample.

Partial Synthesis of 16-Demethoxycarbonyldihydrovoacamine (4)

Dregaminol (15) (150 mg) was prepared from dregamine (13) (250 mg) as described by Renner *et al.*[22] A mixture of dregaminol (106 mg) and ibogaine (9) (100 mg) in 2% methanolic HCl (10 ml) was heated under reflux for 1 h (N_2). On cooling, water (15 ml) was added followed by solid sodium carbonate to neutralization. The white precipitate was extracted with methylene chloride which on removal left a froth (200 mg).

Filtration through alumina (9 g) with light petroleum–benzene (1 : 9) afforded a fraction (120 mg) which on crystallization from benzene–light petroleum yielded synthetic 16-demethoxycarbonyldihydrovoacamine (4) as a powder, decomposition without melting from 212°. This material was identical (i.r. spectra, t.l.c.) to the naturally occurring compound.

Acid Cleavage of 16-Demethoxycarbonyl-20′-epidihydrovoacamine (5)

The alkaloid (5) (200 mg) obtained from *E. orientalis* was heated under reflux with methanol (10 ml) and conc. HCl (5 ml) in a nitrogen atmosphere for 24 h. On cooling, the solution was basified with saturated Na_2CO_3 solution. Ether extraction of the precipitate and evaporation of the solvent left a froth (130 mg). Application of this material to a column of alumina (7 g) and elution with benzene–light petroleum gave a fraction (15 mg) which on crystallization from methanol yielded ibogaine (9), m.p. and m.m.p. 150–152°.

Partial Synthesis of 16-Demethoxycarbonyl-20′-epidihydrovoacamine (5)

(i) *Reduction of tabernaemontanine* (14).—Tabernaemontanine (250 mg) in methanol–ether (4 : 1, 5 ml) was treated with sodium borohydride (120 mg) with stirring for 8 h. The solution was poured into water and the semicrystalline product (250 mg) recovered by ether extraction. Crystallization from aqueous ethanol yielded needles of *tabernaemontaninol* (16), m.p. 186–187°, $[\alpha]_D$ +110° (*c*, 1·4) (Found: C, 70·5; H, 7·8; N, 7·5. $C_{21}H_{28}N_2O_3$ requires C, 70·7; H, 7·9; N, 7·9%). ν_{max} 1700 cm^{-1} (hydrogen-bonded methyl ester); n.m.r. spectrum (δ): 5·14 (1H, m, $W_{h/2}$ 10 Hz, C3 methine proton); 2·49 and 2·37 (6H, 2s, NCH_3 and CO_2CH_3) and 0·95 (3H, t, *J* 7 Hz, primary methyl).

(ii) *Condensation of ibogaine and tabernaemontaninol.*—Ibogaine (100 mg) and tabernaemontaninol (100 mg) were heated with 2% methanolic HCl (10 ml) under reflux for 1 h (N_2). The product (175 mg) was recovered by ether extraction of the basified solution and chromatographed on alumina

[39] Dickel, D. F., Holden, C. L., Maxfield, R. C., Paszek, L. E., and Taylor, W. I., *J. Amer. Chem. Soc.*, 1958, **80**, 123.
[40] Goutarel, R., Percheron, F., and Janot, M. M., *C. R. Acad. Sci.*, 1958, **246**, 279.

(8 g). Elution with benzene–light petroleum (4 : 1) gave a fraction (50 mg) which crystallized from light petroleum–benzene as powdery 16-demethoxycarbonyl-20′-epidihydrovoacamine (5), m.p. > 180° (dec.). The synthetic material was identical to the natural compound (i.r. spectra, t.l.c.).

Acknowledgments

One of us (J.S.) is indebted to the CSIRO for the award of a postgraduate studentship. The CSIRO is also thanked for the collection of the plant material. We are grateful for the precise mass measurements made by the Mass Spectrometry Unit, University of Sydney.

The following are warmly acknowledged for generous donations of samples: Professor C. Djerassi for (−)-apparicine; Dr U. Renner for vobasine hydrochloride, dregamine and tabernaemontanine; Dr W. I. Taylor for ibogaine hydrochloride; Dr B. Hwang for iboxygaine and voacristine; Professor E. Ritchie for voacamine and Professor J. Pecher for voacristine, voacamine and for spectral data of 16-demethoxycarbonylvoacamine.

Mr S. Gianatti is acknowledged for his asistance in the isolation and purification of voacristine, voacamine and 16-demethoxycarbonylvoacamine.

Manuscript received 17 March 1975