219. Studies on Natural Products from Cuban Plants. Alkaloids from *Tabernaemontana citrifolia*

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Summary

A detailed study of the alkaloids from *Tabernaemontana citrifolia*, a species growing in Cuba is presented. Thirteen alkaloids were isolated and characterized. One of these, (–)-19-oxovoacristine (**7**), is a new compound isolated for the first time from a natural source.

Introduction. – The *Tabernaemontanae* tribe (*Apocynaceae* family) to which the genus *Tabernaemontana* belongs, has proven to be a rich source of indole alkaloids [1] and a potential source of pharmacologically active agents [2–4]. As a part of a general survey of *Tabernaemontana* species growing in Cuba [5–7], a detailed study of the alkaloid content of *T. citrifolia* was undertaken. The fruits of this species had previously been shown to contain (–)-coronaridine (**1**) and (–)-tabersonine (**9**) [8], and from the leaves the known alkaloids (–)-coronaridine (**1**), (–)-voacangine (**3**) and (–)-apparicine (**11**) have been identified [9].

The investigation detailed below concerns extractives of the root and stem bark of *T. citrifolia*, parts which had not been previously studied, as well as a re-examination of the fruits and leaves from which four additional alkaloids were isolated and identified. In the course of this study we isolated a new alkaloid from the stem bark of the plant, as well as a series of known compounds. The structure of the new compound was assigned on the basis of spectral data (UV., IR., ^{1}H-NMR. and MS.) and was confirmed chemically.

Results and Discussion. – The extraction and isolation of the alkaloids as performed on the stem bark portion of this plant is outlined in *Scheme 1*. The identical extraction procedure was employed for the other portions.

Crude alkaloid mixtures from the various plant tissues were separated by a combination of column chromatography, preparative thin layer chromatography

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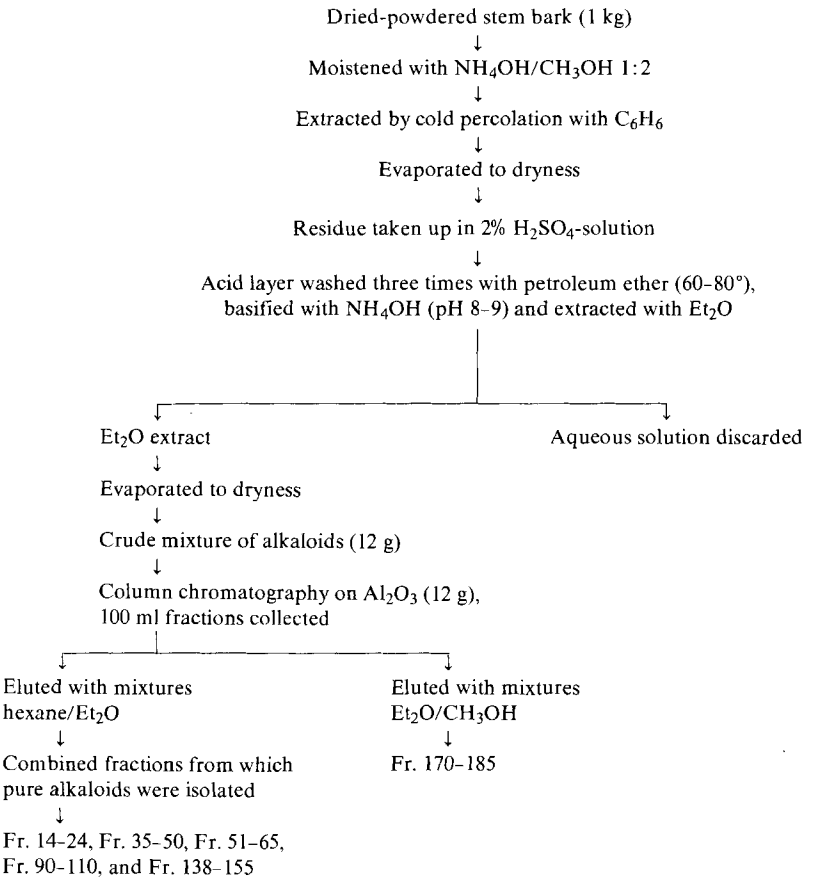
Scheme 1. *Isolation of alkaloids from stem bark of* T. citrifolia



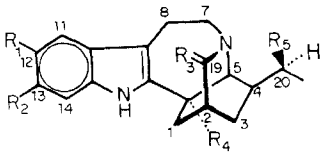
and fractional crystallization. The isolated alkaloids were then identified by spectral and physical constants, and by comparison with authentic samples when available. *Table 1* summarizes data on the alkaloids **1–13** isolated and characterized from the tissues of *T. citrifolia.*

The new compound to which we assigned the structure of (−)-19-oxovoacristine (**7**) was present in fractions 170–185 (see *Scheme 1*) eluted with Et_2O/CH_3OH 98:2. The compound was purified by a combination of column chromatography on Al_2O_3 (act. II–III) and prep. TLC. (Et_2O/CH_3OH 9:1) after which it crystallized from methanol, m.p. 250–252°, $[\alpha]_D^{22} = -35.5°$ ($c = 0.1$, $CHCl_3$). Its UV. spectrum (λ_{max} at 222, 278, 298 and 310 *S* nm) was typical of an indole chromophore [10]. The IR. spectrum suggested the presence of OH and NH groups (3450 and 3360 cm^{-1}), a saturated ester (1740 cm^{-1}) and a lactam function (1680 cm^{-1}). The ^{1}H-NMR. of (−)-19-oxovoacristine (**7**) was very similar to that of (−)-19-oxovoacangine (**6**). It showed the HN resonance at 7.9 ppm, the typical 1,2,4 aromatic proton pattern between 7.9 and 6.8 ppm, as well as aromatic and ester methoxy groups at 3.82

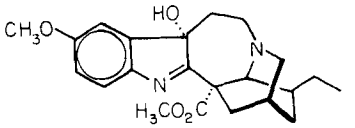
Table 1. *Alkaloids from* T. citrifolia

Alkaloid	Leaves		Stem Bark		Root Bark		Fruits	
	Amount [g]	Yield[a] [%]	Amount [g]	Yield[a] [%]	Amount [g]	Yield[a] [%]	Amount [g]	Yield[a] [%]
(−)-Coronaridine (**1**)	0.020	0.08	0.025	0.20	0.085	1.77	0.020	0.86
(−)-Ibogamine (**2**)	0.010	0.04	–	–	–	–	–	–
(−)-Voacangine (**3**)	0.032	0.13	0.25	2.08	0.075	1.56	–	–
(−)-Iboxygaine (**4**)	–	–	0.055	0.45	0.050	1.4	–	–
(−)-Voacristine (**5**)	–	–	0.085	0.70	–	–	–	–
(−)-19-Oxovoacangine (**6**)	–	–	–	–	0.015	0.31	–	–
(−)-19-Oxovoacristine (**7**)	–	–	0.038	1.31	–	–	–	–
(−)-Voacangine hydroxyindolenin (**8**)	0.016	0.06	–	–	–	–	–	–
(−)-Tabersonine (**9**)	–	–	–	–	–	–	0.016	0.69
(−)-Lochnericine (**10**)	–	–	–	–	–	–	0.009	0.39
(−)-Appericine (**11**)	0.072	0.30	0.016	0.13	–	–	–	–
(+)-Vallesamine (**12**)	–	–	0.140	1.1	–	–	–	–
Akuammidine (**13**)	0.021	0.08	–	–	–	–	–	–

[a]) The yields are based on the weight of the crude alkaloid mixture.



	R^1	R^2	R^3	R^4	R^5
1	H	H	H	$COOCH_3$	H
2	H	H	H	H	H
3	OCH_3	H	H	$COOCH_3$	H
4	OCH_3	H	H	H	OH
5	OCH_3	H	H	$COOCH_3$	OH
6	OCH_3	H	O	$COOCH_3$	H
7	OCH_3	H	O	$COOCH_3$	OH



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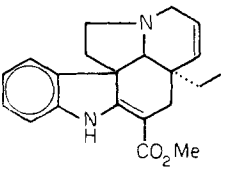

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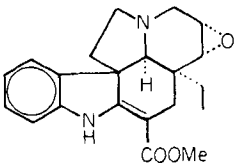

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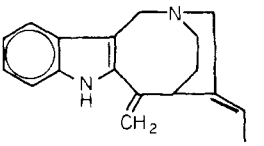

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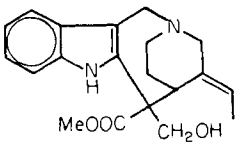

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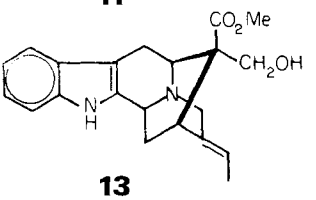

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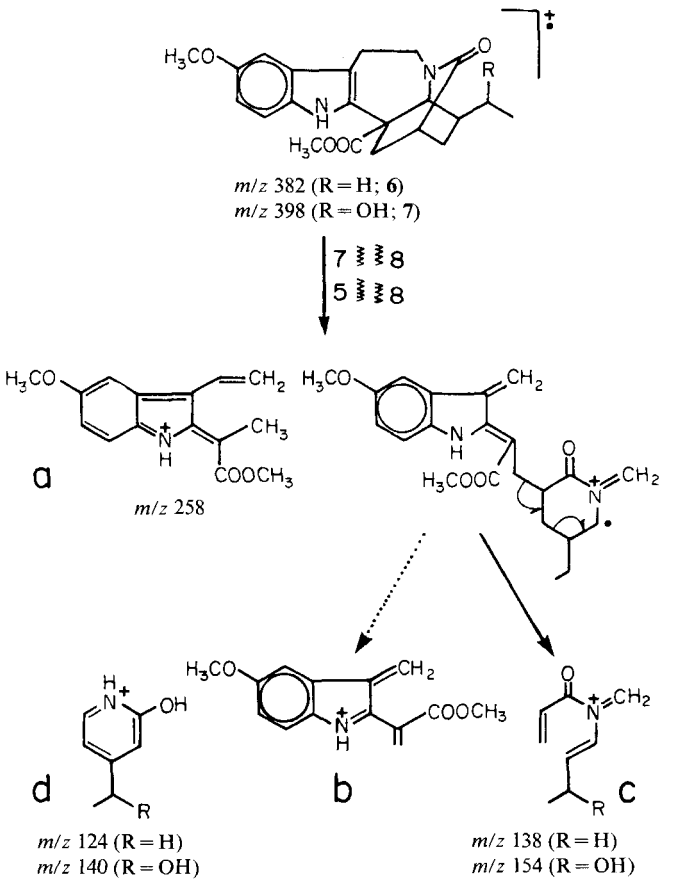
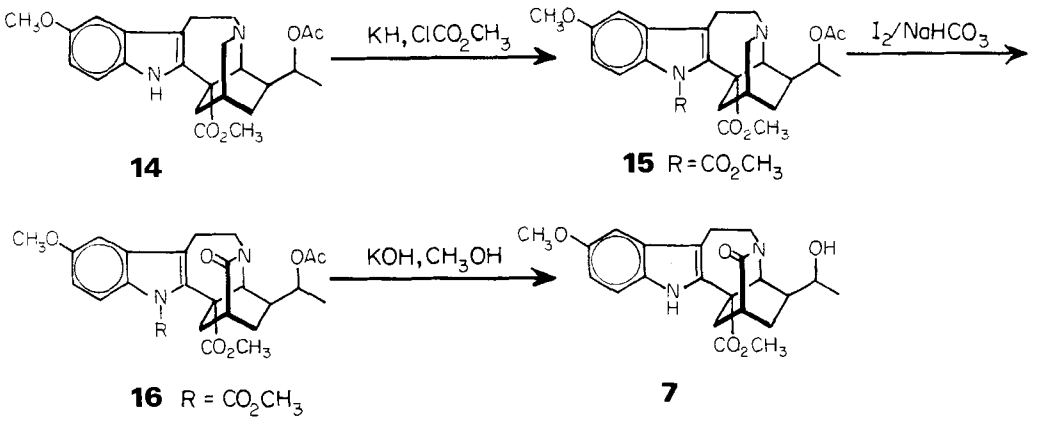
and 3.73 ppm. Also present was a two-proton signal centered at 4.30 ppm, which was interpreted as being a singlet superimposed on a broader one-proton resonance. This feature is typical of a carbonyl group at C(19) in the *Iboga* series [3] [11] [12], and the ^{1}H-NMR. signal was therefore assigned to the protons at C(5) and C(2) [11]. Unlike (−)-19-oxovoacangine (**6**) which shows a triplet at 0.93 ppm [13] due to protons on the terminal carbon atom of the ethyl side chain, (−)-19-oxovoacristine (**7**) exhibits a doublet integrating for three protons at 1.7 ppm, attributable to the methyl group of a hydroxyethyl residue [14–16]. The mass spectrum showed a molecular ion at *m/z* 398 (corresponding to $C_{22}H_{26}N_2O_5$), 16 units higher than that corresponding to (−)-19-oxovoacangine (**6**), for which the fragmentation pattern shown in *Scheme 2* has been described [13]. The occurrence in the spectra of both compounds of peaks at *m/z* 258 (**a**) and 244 (**b**) proved their similarity in the indole portion. However, the constant increment of 16 mass units for fragments **c** (*m/z* 154) and **d** (*m/z* 140) containing the side chain, confirmed the presence of a hydroxyethyl group in this new compound.

Scheme 2. *Mass spectral fragmentation patterns of 19-oxovoacangine* (**6**) *and 19-oxovoacristine* (**7**)



Scheme 3. *The synthesis of (−)-19-oxovoacristine* (**7**) *from (−)-20-O-acetylvoacristine* (**14**)



The proposed structure **7** was unambiguously proven by its synthesis from (−)-voacristine (**5**) as shown in *Scheme 3*. (−)-Voacristine (**5**) was acetylated with pyridine/Ac_2O at room temperature to give its 20-*O*-acetyl derivative **14**. Deprotonation of **14** with potassium hydride in anhydrous THF at 0° to form the indole anion and subsequent reaction with methyl chloroformate provided the 20-*O*-acetyl-N_a-(methoxycarbonyl)voacristine (**15**). This latter compound upon reaction with iodine in basic medium, underwent a conversion to the corresponding lactam **16** which, upon alkaline hydrolysis, gave 19-oxovoacristine (**7**) with physical and spectral properties identical with those of the natural product.

In conclusion, it is seen that *T. citrifolia* is indeed a rich source of alkaloids.

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Experimental Part

General remarks. For thin layer chromatography (TLC.) as well as preparative TLC. (prep. TLC.) *Merck* silica gel G containing 2% fluorescent indicator was used (plates of 0.30 nm in both cases). Visualization was effected under UV. light and/or by colour reaction with Cer(III) sulfate spray reagent. For column chromatography *Merck* silica gel 60 (Fo-230 mesh) or *Merck* aluminium oxide 90 (neutral) was used. Melting points (m.p.) were determined on a *Kofler* block and are uncorrected. UV. spectra were performed on a *Cary* 15 spectrometer in either ethanol or methanol solution (absorption maxima in nm, log ε in parenthesis). IR. spectra were measured on a *Perkin-Elmer* model 710 or 457 spectrophotometer in either KBr disc or chloroform solution (absorption maxima in cm^{-1}). The ^{1}H-NMR. spectra were measured in deuteriochloroform ($CDCl_3$) solution at RT. on a *Varian* XL-100 and *Nicolet Oxford* ^{1}H-270 instrument. Chemical shifts are given in relative to tetramethylsilane (= 0 ppm) used as internal standard. Low resolution mass spectra were determined on either an *AEI*-MS-902 or an *Atlas* CH-4B spectrometer, high resolution mass spectra on an *AEI*-MS-902 instrument. Microanalyses were carried out by Mr. *P. Borda* of the Microanalytical Laboratory, University of British Columbia.

Plant material and extraction. – *T. citrifolia* for this study was collected in the zone of Imias, Guantanamo province (Cuba). This species is registered in the National Botanic Garden, Havana, Cuba, under No. 27433.

Various tissues of *T. citrifolia* (fruits, leaves, stem bark and root bark) were independently extracted. The dry powdered plant material was moistened with a solution of NH_4OH/CH_3OH 1:2 before extraction by cold percolation with benzene. The organic solution was evaporated *in vacuo* and the residue triturated with 2% H_2SO_4-solution. The acid layer was washed three times with petroleum ether (60–80°), basified with ammonia (pH 8–9) and extracted with diethyl ether. The organic solution was washed with water, dried over Na_2SO_4 and evaporated *in vacuo*. *Table 2* shows the yields of the crude alkaloid mixture obtained from various parts of *T. citrifolia*. The yields are based on the weight of dry plant material.

Table 2

Tissues	Weight Dry Material [g]	Weight Crude Alkaloid Mixture [g]	Yield [%]
Leaves	1803.0	23.8	1.3
Stem bark	1046.0	12.0	1.1
Root bark	187	4.8	2.5
Fruits	153.3	2.3	1.5

Alkaloids from the leaves. – Crude alkaloid mixture (23.8 g) was chromatographed on a column of Al_2O_3 (act. II–III, 714 g) with C_6H_6, C_6H_6/CH_2Cl_2, CH_2Cl_2 and CH_2Cl_2/CH_3OH. A total of 155 fractions (400 ml each) were collected.

(–)-Coronaridine (**1**). Fractions 1–8 (0.068 g; with C_6H_6) afforded, after crystallization from methanol, 20 mg of **1** · HCl, m.p. 201–204° (dec.) ([13]: m.p. 204° (dec.)). – IR. and MS.: identical with that of an authentic sample. – ^{1}H-NMR.: in accord with published data [13].

(–)-Ibogamine (**2**). Combined fractions 9–15 (0.430 g; with C_6H_6/CH_2Cl_2 95:5) were further separated by column chromatography on SiO_2 (15.4 g). Fractions 22–32 (0.129 g; with hexane/Et_2O 98:2) were separated by prep. TLC. on SiO_2 with petroleum ether (30–60°)/Et_2O 6:4. Two bands were removed, and the extract of the less polar fraction was crystallized from methanol to afford 10 mg of pure **2**, m.p. 159–160° ([17]: m.p. 160–161°), $[\alpha]_D^{20} = -56°$ ($c = 0.3$, ethanol; [17]: $[\alpha]_D^{20} = -54°$ (ethanol)). – IR.: identical with that of the original sample. – ^{1}H-NMR. and MS.: in agreement with published data [17] [18].

(–)-Voacangine (**3**). Fractions 16–25 (1.2 g; with C_6H_6/CH_2Cl_2 95:5) were rechromatographed on alumina (act. II–III, 100 g). Combined fractions 18–26 (with petroleum ether (60–80°)/Et_2O 85:5) afforded an amorphous residue which gave by crystallization from methanol pure **3** (0.032 g), m.p. 137–138°, identical chromatographycally, by mixt. m.p., IR. and MS. with an authentic sample.

(–)-9-Hydroxyvoacangine (indolenine) (**8**). Combined fractions 40–52 (0.93 g; with C_6H_6/CH_2Cl_2 4:6) were further purified by column chromatography on SiO_2. The residue (0.170 g) obtained after evaporation of fractions 15–20 (with C_6H_6/EtOAc 9:1) was further purified by prep. TLC. on SiO_2 with hexane/$CHCl_3$ 1:9. The more polar of the two bands yielded a white powder which crystallized from methanol to afford 16 mg of pure **8**, m.p. 135–136° ([19]: m.p. 135–137°). – UV., IR., ^{1}H-NMR. and MS.: in accord with published data [13] [19].

(–)-Apparicine (**11**). Fractions 78–94 (3.5 g; with C_6H_6/CH_2Cl_2 4:6) were further separated by column chromatography on Al_2O_3 (act. II–III, 105 g). Combined fractions 30–42 (with Et_2O) were purified by prep. TLC. on silica gel (Et_2O/CH_3OH 94:6). Three bands were separated, the most polar of which afforded a residue that after recrystallization from acetone gave **11** (72 mg), identical to an authentic sample by IR., ^{1}H-NMR. and MS., as well as mixt. m.p.

Akuammidine (**13**). Combined fractions 142–154 (2.8 g; with CH_2Cl_2/CH_3OH 99:1) were further separated by column chromatography on Al_2O_3 (act. II–III, 100 g). Fractions 50–65 (with Et_2O/CH_3OH 94:6) gave a residue (0.2 g) which was purified by prep. TLC. (Et_2O/CH_3OH 95:5). A UV.-visible band was separated and its residue crystallized from benzene to afford white crystals of **13** (21 mg), m.p. 246–247° ([20]: m.p. 249°). – IR., ^{1}H-NMR. and MS.: identical with those reported [14] [20].

Alkaloids from the stem bark. – Crude alkaloid mixture (12 g) was chromatographed on a column of alumina (act. II–III, 360 g) with hexane, hexane/Et_2O, Et_2O and Et_2O/CH_3OH. Fractions of 100 ml were collected.

(–)-Coronaridine (**1**). Fractions 14–24 (0.13 g; with hexane/Et_2O 9:1) yielded, after recrystallization from methanol, pure **1** · HCl (0.025 g), with m.p., IR., ^{1}H-NMR. and MS. identical to **1** isolated from the leaves.

(–)-Voacangine (**3**). Combined fractions 35–50 (0.5 g; with hexane/Et_2O 85:15) were further purified by prep. TLC. (petroleum ether/Et_2O 1:1). Two bands were processed. The more polar fraction afforded, after crystallization from methanol, white crystals of **3** (0.250 g), identical (m.p., IR., ^{1}H-NMR. and MS.) with **3** isolated from the leaves.

(–)-Apparicine (**11**). Fractions 51–65 (0.730 g; with hexane/Et_2O 75:25) were further purified on a column of alumina (act. II–III, 36 g). Fractions 50–68 (0.390 g; with petroleum ether (60–80°)/Et_2O 4:6) were purified by prep. TLC. (Et_2O/CH_3OH 97:3). Two bands were separated, and the less polar fraction gave, after recrystallization from acetone, white crystals of **11** (16 mg), identical (m.p., IR. and MS.) with **11** isolated from the leaves.

(–)-Voacristine (**5**). Combined fractions 90–110 (0.982 g; with hexane/Et_2O 6:4) were further separated by column chromatography on alumina (act. II–III, 68 g). Combined fractions 20–35 (632 mg; with C_6H_6/Et_2O 95:5) were further separated by prep. TLC. eluting 3 times with petroleum ether (60–80°)/Et_2O 2:8. Two bands were removed, and the residue (140 mg), after evaporation of the less polar fraction, was crystallized from methanol to afford **5** (0.085 g), m.p. 162–163° ([21]: m.p. 163–165°). – IR., ^{1}H-NMR. and MS.: identical with those reported [14] [22].

(–)-Iboxygaine (**4**). The more polar fraction of the above prep. TLC. gave, after recrystallization from methanol, white crystals of **4** (55 mg), m.p. 231–232° ([23]: m.p. 234°). – UV., IR. and MS.: in agreement with those published [23] [24].

(+)-Vallesamine (**12**). Combined fractions 138–155 (1.98 g; with Et_2O/hexane 9:1) were re-chromatographed on a column of Al_2O_3 (165 g). Fractions of 100 ml were collected. Combined fractions 32–46 (with C_6H_6/CH_2Cl_2 6:4) yielded a residue (0.5 g) which was further purified by prep. TLC. using CH_2Cl_2/CH_3OH 97:3. A UV.-visible band was separated, and its residue (0.230 g) crystallized from benzene to afford pure **12** (0.140 g), m.p. 162–163° ([25]: m.p. 165°). – UV., IR., ^{1}H-NMR. and MS.: in agreement with those published [25].

(–)-19-Oxovoacristine (**7**). Fractions 170–185 (2.3 g; with Et_2O/CH_3OH 98:2) were further separated by column chromatography on alumina (act. II–III, 100 g). Combined fractions 60–71 (0.85 g) were purified by prep. TLC. (Et_2O/CH_3OH 9:1). A UV.-visible band was processed to afford a residue which, after recrystallization from methanol, yielded **7** (38 mg), m.p. 251–252°, $[\alpha]_D^{22} = -35.5°$ ($c = 0.1$, $CHCl_3$). – UV. (CH_3OH): 222 (4.44), 278 (3.98), 298 (3.89), 310 *S* (3.61). – IR. ($CHCl_3$): 3450, 3360, 1740, 1680. – ^{1}H-NMR. ($CDCl_3$, 270 MHz): 1.3 (*d*, $J = 6.5$, 3 H, 3 H–C(21)); 3.73 (*s*, 3 H, $COOCH_3$); 3.82 (*s*, 3 H, $ArOCH_3$); 4.5 (*m*, 1 H, H–C(2)); 4.6 (*s*, 1 H, H–C(5)); 6.80 ($d \times d$, $J_1 = 8$, $J_2 = 2$, H–C(13)); 6.90 (*d*, $J = 2$, 1 H, H–C(11)); 7.13 (*d*, $J = 8$, 1 H, H–C(14)); 7.9 (*s*, 1 H, HN). – MS.: 398 (M^+), 287, 258, 244, 227, 184, 154, 140. – High resolution molecular weight determination 398.1847: ($C_{22}H_{26}N_2O_5$, Calc. 398.1841).

$C_{22}H_{26}N_2O_5$ Calc. C 66.31 H 6.57 N 7.03% Found C 66.64 H 6.55 N 7.01%

Alkaloids from the root bark. – The crude alkaloids (4.8 g) mixed with Al_2O_3 (act. II–III, 24 g) and CH_2Cl_2 (200 ml) were evaporated to dryness, the residue placed in an empty column and eluted with various solvent systems. Fractions 1–6 (with petroleum ether) were combined as A (2.2 g), fractions 7–12 (with petroleum ether/C_6H_6 1:1) as B (0.668 g) and fractions 13–18 (with C_6H_6) as C (0.250 g).

Separation of Fraction A. Fraction A (2.2 g) was rechromatographed on silica gel (40 g) with C_6H_6 and $C_6H_6/CHCl_3$. Fractions of 100 ml were collected. Fractions 10–18 (with $C_6H_6/CHCl_3$ 95:5) were combined as A_1, and fractions 25–32 (with $C_6H_6/CHCl_3$ 9:1) as A_2.

(–)-Coronaridine (**1**). A_1 (0.2 g) gave, after crystallization from methanol, 85 mg of **1** · HCl, with m.p., IR., ^{1}H-NMR. and MS. identical to **1** · HCl isolated from the leaves.

(–)-Voacangine (**3**) *and (–)-voacristine* (**5**). A_2 (0.910 g) was rechromatographed on Al_2O_3 (100 g). Fractions of 50 ml were eluted with petroleum ether/Et_2O. Combined fractions 20–32 (with petroleum ether/Et_2O 85:15) yielded a residue (0.150 g) which afforded, after crystallization from methanol, pure **3** (0.075 g), identical by mixt. m.p., IR. and MS. with **3** isolated from the leaves. Combined

fractions 45–85 (with petroleum ether/Et_2O 7:3) yielded a residue (0.45 g) which gave, after crystallization from methanol, pure **5** (0.035 g), identical chromatographically, by mixt m.p., IR. and MS. with **5** from the steam bark.

Separation of Fraction B. (–)-Voacristine (**5**) *and (–)-19-oxovoacangine* (**6**). Fraction B (0.668 g) was further purified by prep. TLC. on silica gel with Et_2O/CH_3OH 99:1. Two bands were separated. The less polar fraction yielded a residue (0.060 g) which gave, after crystallization from methanol, pure **5** (0.015 g), identical to the alkaloid from the preceding fraction by mixt m.p. and MS. The more polar fraction afforded a residue (0.030 g) which crystallized from methanol to give **6** (0.005 g), identical to an authentic sample (mixt m.p., IR. and MS.).

Separation of Fraction C. Fraction C was rechromatographed on Al_2O_3 (66 g). Fractions of 50 ml were eluted with Et_2O/CH_3OH. *(–)-19-Oxovoacangine* (**6**). Combined fractions 50–65 (with Et_2O/CH_3OH 99:1) afforded a residue (0.088 g) which was further purified by prep. TLC. in the same system. The residue (0.055 g), of the main UV.-visible band crystallized from methanol to afford **6** (0.010 g), identical to **6** from the preceding fraction by mixt m.p. and MS.

Alkaloids from fruits. – The crude alkaloid mixture (2.3 g), $CHCl_3$ (100 ml) and SiO_2 (11.5 g) were evaporated to dryness, the residue placed in an empty column and eluted with various solvent systems. Fractions of 250 ml were collected. Fractions 1–4 (with petroleum ether) were combined as A (0.360 g) and fractions 5–9 (with petroleum ether/C_6H_6 1:1) as B (0.250 g).

Separation of Fraction A. Fraction A (0.360 g) was rechromatographed on silica gel (18 g). Fractions of 50 ml were eluted with benzene. *(–)-Coronaridine* (**1**). Combined fractions 1–20 yielded a residue (0.120 g) which was further purified by prep. TLC. on silica gel (petroleum ether/C_6H_6 6:4; two developments). Two bands were removed. The less polar fraction afforded an amorphous residue (0.028 g) which gave, after crystallization from methanol, pure **1** · HCl (0.20 g), with physical and spectroscopic data identical to **1** · HCl from the leaves.

(–)-Tabersonine (**9**). The more polar fraction from the above prep. TLC. yielded a residue (0.025 g) which gave, after crystallization from methanol, pure **9** · HCl (0.016 g), identical to an authentic sample (TLC, m.p., mixt m.p., IR. and MS.).

Separation of Fraction B. (–)-Lochnericine (**10**). Fraction B (0.250 g) was further purified by prep. TLC. on silica gel (petroleum ether/Et_2O 1:1). A UV.-visible band was separated and its residue crystallized from methanol to afford pure **10** (0.009 g), with identical physical ($[\alpha]_D$, m.p.) and spectral data (UV., IR., ^{1}H-NMR. and MS.) as those reported for **10** [22] [26].

Synthesis of (–)-19-oxovoacristine. – *Synthesis of 20-O-acetylvoacristine* (**14**). Voacristine (**5**) was acetylated with acetic anhydride (12 ml) and pyridine (0.2 ml) at RT. for 24 h. The mixture was treated with iced water, partly concentrated *in vacuo*, water added and the mixture basified with ammonia. The solution was extracted with CH_2Cl_2 and the extract washed with water, dried over Na_2SO_4 and evaporated *in vacuo* to give an amorphous solid (320 mg). The residues yielded, after crystallization from methanol **14** (260 mg), m.p. 189–190°. – IR. and MS.: in accord with the published data [14].

Synthesis of 20-O-acetyl-N$_a$-(methoxycarbonyl)voacristine (**15**). A suspension of potassium hydride (KH), oil dispersion (0.3 ml, 24%) in dry tetrahydrofuran (THF; 10 ml), was cooled to *ca.* –5° under N_2. Then **14** (250 mg, 0.58 mmol) in dry THF (2 ml) was added and the mixture stirred at –5° for 2 h. Methyl chloroformate (80 mg, 0.84 mmol) was added, and after 30 min, Al_2O_3 (*ca.* 0.6 g) to quench excess KH. The mixture was filtered and the filtrate evaporated *in vacuo*. The oily product was chromatographed on silica gel (20 g). Elution with petroleum ether/Et_2O 1:1 yielded pure **15** (240 mg, 84.5%) as a white foam, homogeneous by TLC. (petroleum ether/Et_2O 1:2), $[\alpha]_D^{22} = -25.8°$ ($c = 0.2$, $CHCl_3$). – UV. (CH_3OH): 232 (4.28), 268 (4.06), 297 *S* (3.66), 308 *S* (3.58). – IR. ($CHCl_3$): 2960, 2895, 1740. – ^{1}H-NMR. ($CDCl_3$, 270 MHz): 1.22 (*d*, $J = 6.5$, 3 H, 3 H–C(21)); 3.70 (*s*, 3 H, $COOCH_3$); 3.80 (*s*, 3 H, $ArOCH_3$); 3.9 (*s*, 3 H, N(a)–$COOCH_3$); 5.1 (*qa*, $J = 7$, 1 H, H–C(20)); 6.97 (*d* × *d*, $J_1 = 8$, $J_2 = 2$, 1 H, H–C(13)); 7 (*d*, $J = 2$, 1 H, H–C(11)); 8.04 (*d*, $J = 8$, 1 H, H–C(14)). – MS.: 484 (M^+), 426, 425, 424, 367, 366, 365, 182, 122. – High resolution molecular weight determination: 484.2214 ($C_{26}H_{32}N_2O_7$, Calc. 484.2209).

$C_{26}H_{32}N_2O_7$ Calc. C 64.44 H 6.65 N 5.78% Found C 64.80 H 6.64 N 5.69%

Synthesis of (−)-20-O-acetyl-N_a-methoxycarbonyl-19-oxovoacristine (**16**). Sodium hydrogencarbonate (1 g, 11.9 mmol), water (40 ml), **15** (200 mg, 0.41 mmol) and benzene (10 ml) were stirred at RT. A solution of iodine (520 mg, 20 mmol) in benzene (30 ml) was added and stirring continued for 1½ h. Sodium hydrogencarbonate (1.7 g) and sodium thiosulfate were added, the organic layer was separated and the aq. phase washed with dichloromethane (3 times 50 ml). The organic extracts were combined, dried over Na_2SO_4 and evaporated *in vacuo*. The amorphous residue (200 mg) was chromatographed on alumina (2 g, act. II–III). Elution with petroleum ether/Et_2O 3:7 gave **16** (133 mg), $[\alpha]_D^{22} = -78.8°$ ($c = 0.1$, $CHCl_3$). – UV. (CH_3OH): 243 (4.27), 268 (4.13), 298 (3.62), 310 (3.59). – IR. ($CHCl_3$): 2980, 1740, 1680, 1640. – ^{1}H-NMR. ($CDCl_3$, 270 MHz): 1.25 (*d*, $J = 6.5$, 3 H, 3 H–C(21)); 3.70 (*s*, 3 H, $COOCH_3$): 3.82 (*s*, 3 H, $ArOCH_3$); 3.9 (*s*, 3 H, N(a)–$COOCH_3$); 4.35 (*m*, 1 H, H–C(2)), 4.45 (*s*, 1 H, H–C(5)). – MS.: 498 (M^+), 438, 260, 185, 149. – High resolution molecular weight determination: 498.2008 ($C_{26}H_{30}N_2O_8$, Calc. 498.2002).

$C_{26}H_{30}N_2O_8$ Calc. C 62.63 H 6.06 N 5.62% Found C 62.58 H 6.08 N 5.40%

Synthesis of **7**. A solution of **16** (100 mg, 0.2 mmol) in 1% methanolic KOH-solution (5 ml) was stirred at RT. for 4 h, diluted with dichloromethane (10 ml) and filtered through a short plug of silica gel. Evaporation of the filtrate gave an amorphous powder (71 mg) which was further purified by prep. TLC. (Et_2O/CH_3OH 9:1). The residue, after recrystallization from methanol, afforded pure **7** (45 mg) identical with the natural sample (m.p., mixt m.p., IR., ^{1}H-NMR. and MS.).

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