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ANTIMICROBIALLY ACTIVE ALKALOIDS FROM *TABERNAEMONTANA PACHYSIPHON**

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Key Word Index—*Tabernaemontana pachysiphon*; Apocynaceae; indole alkaloids; antimicrobial activity.

Abstract—Twenty-three alkaloids and five steroids and triterpenes have been isolated and identified from the root bark and stem bark of a Nigerian *Tabernaemontana pachysiphon*. The following bases have not previously been obtained from this species: isositsirikine, 16-epiisositsirikine, normacusine B, 16-epiaffinine, anhydrovobasindiol, tubotaiwine, ibogaline, isovoacangine, voacamine, lochnericine, 3*R*-hydroxyconopharyngine, 3*S*-hydroxyconopharyngine and 11-demethylconoduramine, the last three being new alkaloids. The dimeric indole alkaloids and 3*R*/*S*-hydroxyconopharyngine were shown to possess strong antibacterial activity against Gram-positive bacteria and the dimers against Gram-negative bacteria also.

INTRODUCTION

Tabernaemontana pachysiphon Stapf is a glabrous shrub or medium-sized tree occurring in tropical West Africa. In a current revision of the genus *Tabernaemontana* by Leeuwenberg and coworkers this species was shown to possess several synonyms [2] such as *Conopharyngia angolensis*, *C. cumminsii*, *C. holstii*, *C. pachysiphon*, *T. angolensis*, *T. cumminsii* and *T. holstii*. Because of the frequent use of this plant in traditional medicine in several African countries, many phytochemical investigations prior to the one presented here have been carried out. The results of these previous studies are summarized in Table 1.

Some of the more interesting uses in traditional medicine are the use of the latex for healing wounds [14, 15] and for the treatment of sore eyes [15, 16]. Also, a decoction of the roots is said to be used against stomachache, constipation, flatulence, headache and as a hypnotic [15]. In a recent antimicrobial screening of 19 different *Tabernaemontana* species [1], it was found that the ethanolic extracts of the root bark and stem bark of *T. pachysiphon* showed strong antimicrobial activity against both Gram-positive and Gram-negative bacteria. This observation prompted a closer examination of this species in order to identify the compounds responsible for the antimicrobial activity. The results of this study are set out below. Also the present phytochemical findings are compared with the older studies and discussed.

RESULTS AND DISCUSSION

The root bark and stem bark were extracted with alcohol and the alkaloids separated by an acid–base extraction. As only the tertiary alkaloid fractions showed antimicrobial activity, only these fractions were investigated further, except for some steroids which precipitated in large amounts from the acidic ethyl acetate fraction. The tertiary alkaloids were separated and purified by means of LC and preparative TLC.

As the alkaloidal composition of the stem bark and root bark differed only quantitatively from each other—the root bark being somewhat richer in dimeric alkaloids—the alkaloids isolated from the root bark and stem bark were combined for greater yield. Table 2 lists the identified alkaloids from the root bark and stem bark, together with an indication of their relative abundance in the stem bark.

Of the 23 alkaloids isolated, 3 were new and 10 have not been isolated before from this species. The structural elucidation of the new alkaloids is described below.

3R- *and* 3S-*hydroxyconopharyngine* (**15** *and* **16**)

This alkaloid, which showed one spot on TLC in all the solvents used, gave a purple colour with the ferric chloride–perchloric acid spray reagent. The UV spectrum showed maxima at 223, 302 and 306 nm with shoulders at 273, 285 and 311 nm, which is fairly characteristic for a 10,11-disubstituted indole. The mass spectrum showed major peaks at *m/z* 414, 412, 398, 397, 396 (100%), 381, 337, 136 and 122, which is typical for 3-hydroxyiboga alkaloids [17, 18]. This suggested that the alkaloid might be 3-hydroxyconopharyngine. The typical mass spectral behaviour—$[M]^+$, $[M-2]^+$, $[M-16]^+$, $[M-18]^+$—is caused by thermal processes within the mass spectrometer [17]. In the ^{1}H NMR spectrum the most characteristic signals were: a broad singlet at δ 7.65 (NH); four singlets at δ 6.92, 6.91, 6.80 and 6.79, each integrating for *ca* half a proton (H-9 and H-12); a broad singlet ($W_{1/2}$ = 6 Hz) integrating for 0.4 proton at 4.43 (H-3); a doublet (J = 1.6 Hz) integrating for 0.6 proton at 4.12 (H-3); two singlets, each integrating for three protons at 3.92 and 3.89 (2 × OMe); two singlets, together integrating for three protons at 3.69 (COOMe); and a triplet (J = 7.5 Hz) integrating for three protons at 0.90 (H-18). From these data it follows that this base is present as a mixture of 3*R*-

*Part 7 in the series "Pharmacognostical Studies of *Tabernaemontana* Species". For Part 6 see ref. [1].

Table 1. Alkaloids previously isolated from *T. pachysiphon*

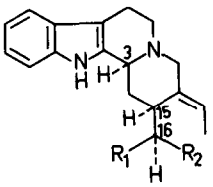
Alkaloid	Plant part*	Country of origin	Reference
Pericyclivine (**4**)	R	Kenya	[3]
Affinine (**5**)	SB	Nigeria	[4]
Perivine	R	Kenya	[3]
Vobasine (**7**)	R	Kenya	[3]
Apparicine (**9**)	L	Africa	[5, 6]
Tubotaiwine-*N*-oxide (**11**)	R	Kenya	[7]
Pachysiphine	Se	Nigeria	[4]
Conopharyngine (**14**)	L	Ghana	[8, 9]
Conopharyngine (**14**)	SB	Nigeria	[9]
19-Hydroxyconopharyngine (**17**)	L	Ghana	[4, 10]
Conopharyngine-hydroxy-indolenine (**22**)	L	Unknown	[11]
Coronaridine	R	Kenya	[3]
Coronaridine	SB	Nigeria	[10]
3-Oxocoronaridine	R	Kenya	[3]
Voacangine	Se; SB	Nigeria	[4, 10]
Conopharyngine pseudoindoxyl	L	Ghana	[8]
Decarbomethyoxy-15,20; 16,17-tetrahydrosecodine	L	Unknown	[12]
Conoduramine (**19**)	R	Kenya	[3]
Conodurine (**20**)	R	Kenya	[3]
3-Oxoconodurine	R	Kenya	[3]
3-(2′-Oxopropyl)conodurine	R	Kenya	[3]
Gabunine	R	Kenya	[3]
20α-Aminopregn-5-en-3β-yl β-D-glucoside	R	Principe Is.	[13]

*R = Root, SB = stem bark, L = leaves, Se = seeds.

Table 2. Alkaloids isolated from the root bark and stem bark of *T. pachysiphon*

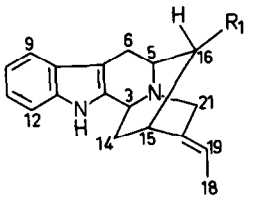
Alkaloid	Relative abundance*	Method of identification
Isositsirikine (**1**)	+ +	UV, MS, ^{1}H NMR, co-TLC
16-Epiisositsirikine (**2**)	+	UV, MS, ^{1}H NMR, co-TLC
Normacusine B (**3**)	+ +	UV, MS, ^{1}H NMR
Pericyclivine (**4**)	+	UV, MS, ^{1}H NMR, co-TLC
Affinine (**5**)	+	UV, MS
16-Epiaffinine (**6**)	+ +	UV, MS, ^{1}H NMR, co-TLC
Vobasine (**7**)	+ +	UV, MS, ^{1}H NMR, co-TLC
Anhydrovobasindiol (**8**)	+ +	UV, MS, ^{1}H NMR
Apparicine (**9**)	+ +	UV, MS, ^{1}H NMR, co-TLC
Tubotaiwine (**10**)	+ + +	UV, MS, ^{1}H NMR, ^{13}C NMR, co-TLC
Tubotaiwine-*N*-Oxide (**11**)	+	UV, MS
Ibogaline (**12**)	+	UV, MS
Isovoacangine (**13**)	+ +	UV, MS, ^{1}H NMR, co-TLC
Conopharyngine (**14**)	+ + +	UV, MS, ^{1}H NMR, ^{13}C NMR, co-TLC
3*R*-Hydroxyconopharyngine (**15**)	+ +	UV, MS, ^{1}H NMR, chcr†
3*S*-Hydroxyconopharyngine (**16**)	+ +	UV, MS, ^{1}H NMR, chcr
19*S*-Hydroxyconopharyngine (**17**)	+ +	UV, MS, ^{1}H NMR
11-Demethylconoduramine (**18**)	+ +	UV, MS, ^{1}H NMR
Conoduramine (**19**)	+ +	UV, MS, ^{1}H NMR, ^{13}C NMR, co-TLC
Conodurine (**20**)	+ +	UV, MS, ^{1}H NMR, co-TLC
Voacamine (**21**)	+	UV, MS, co-TLC
Conopharyngine-hydroxyindolenine (**22**)	+	UV, MS
Lochnericine (**23**)	+	UV, MS

* + + + = Major component, + + = minor component, + = trace component.
†chcr = Chemical correlation.



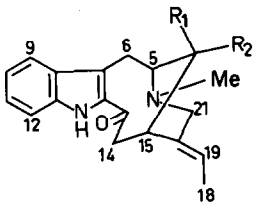
1 $R_1 = CH_2OH$, $R_2 = CO_2Me$

2 $R_1 = CO_2Me$, $R_2 = CH_2OH$



3 $R_1 = CH_2OH$

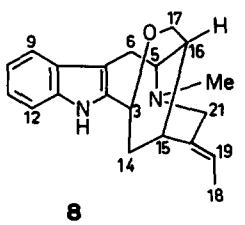
4 $R_1 = CO_2Me$



5 $R_1 = CH_2OH$, $R_2 = H$

6 $R_1 = H$, $R_2 = CH_2OH$

7 $R_1 = CO_2Me$, $R_2 = H$



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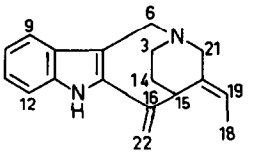

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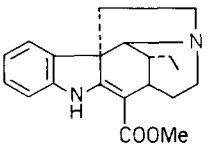

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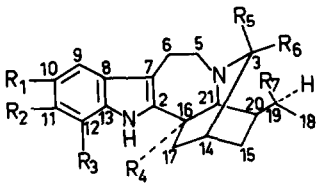
11 $N_4 \rightarrow O$

and 3*S*-hydroxyconopharyngine (**15** and **16**). From the integral of the two H-3 protons it can be concluded that the ratio between the 3*R*-isomer and the 3*S*-isomer is 3 to 2. As final proof for the correctness of these structures a reduction with sodium borohydride was carried out. Only conopharyngine (**14**), which has a different R_f value and gives a green colour with the ferric chloride–perchloric acid spray reagent, was obtained.

11-*Demethylconoduramine* (**18**)

The UV spectrum of this alkaloid, with maxima at 224, 286 and 294 nm and a shoulder at 303 nm indicated that it was a substituted indole. Upon basification, an extra maximum at 323 nm appeared suggesting the presence of a phenolic group. Additional evidence for the presence of a phenolic group was obtained by the strongly positive reaction of the alkaloid with Fast-blue salt. The mass spectrum with major peaks at *m/z* 690 $[M]^+$, 631, 510, 509, 508, 497, 496, 495, 379, 208, 194, 182, 180, 136 and 122 indicated that the alkaloid belonged to the dimeric voacamine type [19, 20]. The fragments at *m/z* 194, 182, 180 and 122 belonging to the vobasine half and the fragments at *m/z* 208, 148, 136 and 122 belonging to the iboga half did not shift in comparison with the mass spectrum of voacamine itself ($[M]^+$ *m/z* 704). This suggests that no changes had taken place in the aliphatic parts of both halves. The loss of 14 mass units ($[M]^+$ at *m/z* 690 instead of *m/z* 704) must therefore have occurred in the aromatic iboga part. This was confirmed by the fact that all the fragments possessing this part of the molecule had shifted 14 mass units (*m/z* 690, 631, 510, 509, 508, 497, 496, 495 and 379). The alkaloid was therefore thought to be either 10-demethylvoacamine, 10-demethylvoacamidine, 11-demethylconoduramine or 11-demethylconodurine. This would also explain the bathochromic shift in the UV spectrum upon basification (phenolic group). By means of the ^1H NMR spectrum, it was possible to distinguish between the four mentioned possibilities. The ^1H NMR spectrum of the unknown base was



12 $R_1 = R_2 = OMe$, $R_3 = R_4 = R_5 = R_6 = R_7 = H$

13 $R_1 = R_3 = R_5 = R_6 = R_7 = H$, $R_2 = OMe$, $R_4 = CO_2Me$

14 $R_1 = R_2 = OMe$, $R_3 = R_5 = R_6 = R_7 = H$, $R_4 = CO_2Me$

15 $R_1 = R_2 = OMe$, $R_3 = R_5 = R_7 = H$, $R_4 = CO_2Me$, $R_6 = OH$

16 $R_1 = R_2 = OMe$, $R_3 = R_6 = R_7 = H$, $R_4 = CO_2Me$, $R_5 = OH$

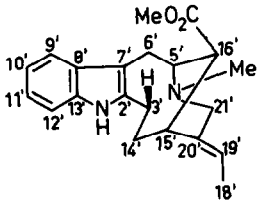
17 $R_1 = R_2 = OMe$, $R_3 = R_5 = R_6 = H$, $R_4 = CO_2Me$, $R_7 = OH$

18 $R_1 =$ 3'-vobasinyl, $R_2 = OH$, $R_3 = R_5 = R_6 = R_7 = H$, $R_4 = CO_2Me$

19 $R_1 =$ 3'-vobasinyl, $R_2 = OMe$, $R_3 = R_5 = R_6 = R_7 = H$, $R_4 = CO_2Me$

20 $R_1 = R_5 = R_6 = R_7 = H$, $R_2 = OMe$, $R_3 =$ 3'-vobasinyl, $R_4 = CO_2Me$

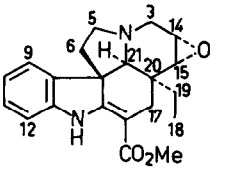
21 $R_1 = OMe$, $R_2 =$ 3'-vobasinyl, $R_3 = R_5 = R_6 = R_7 = H$, $R_4 = CO_2Me$



3'-Vobasinyl

superimposable only with the ^1H NMR spectrum of conoduramine, except for the fact that the signal for the 11-methoxy group at δ 3.97 was missing. The unknown alkaloid is thus 11-demethylconoduramine (**18**). To confirm this a chemical correlation with conoduramine was attempted.

However, after prolonged treatment with diazomethane, even in the presence of boron trifluoride, the starting material was recovered unchanged. This is probably caused by steric hindrance by the vobasine half.



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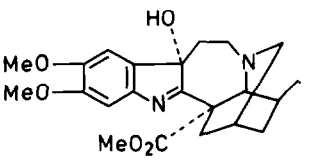
Phytochemistry and chemotaxonomy

If one compares the results of the present phytochemical study (Table 2) with those carried out previously (Table 1) it can be seen that the number and type of alkaloids found in the present investigations are similar to the number and type of alkaloids found in all the earlier studies together. The first fact is probably due to the better and more sensitive spectroscopic instruments and the availability of reference compounds. The fact that now in one species



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almost the same alkaloids are found as in all the previously studied species put together gives supporting evidence for the botanical revision by Leeuwenberg and coworkers [2] in which, most importantly, *T. holstii* was combined with *T. pachysiphon*. The part of the plant studied or the country of origin seems to play only a minor role in determining the type of alkaloids occurring in this species. From a chemotaxonomical view-point, *T. pachysiphon* is a typical example of the genus *Tabernaemontana* [2] because of its high content of corynanthean, ibogan and dimeric corynanthean–ibogan alkaloids as well as the presence of apparicine and tubotaiwine, which are also frequently encountered in *Tabernaemontana* species.

Antimicrobial activity

Since it was found that only the fractions containing tertiary alkaloids showed antimicrobial activity, these fractions were studied further. This was done by using the biogram technique as a bioassay during the fractionation [21]. *Bacillus subtilis* and *Escherichia coli* were used as test-organisms. Using this method, 11-demethylconoduramine, conoduramine, conodurine and voacamine were shown to possess activity against both *B. subtilis* and *E. coli*, while 3*R*/*S*-hydroxyconopharyngine was shown to possess activity against *B. subtilis* only.

After purification of the alkaloids, the minimum inhibiting concentration (MIC value) and the antimicrobial spectrum of each of the alkaloids were determined by means of the agar diffusion method. As the alkaloids were insoluble in water, several other solvents were tested. It was found that a citrate–phosphate buffer of pH 4.0 gave optimum results for this type of alkaloid, and this solvent was consequently used in the testing. The results are presented in Table 3. The MIC value of 11-demethylconoduramine was not determined as too little was left for the testing after the structural elucidation of this alkaloid. It can be seen that the dimeric indole alkaloids of the voacamine type showed strong antimicrobial activity against the Gram-positive *B. subtilis* and *Staphylococcus aureus* and moderate activity against the Gram-negative *E. coli* and *Pseudomonas aeruginosa*. 3*R*/*S*-Hydroxyconopharyngine showed moderate activity against the Gram-positive bacteria only. None of the compounds showed activity against the yeast *Candida albicans* or the fungus *Aspergillus niger* at any of the concentrations tested.

Dimeric alkaloids of the voacamine type have not been previously reported as having antimicrobial activity. The fact that 3-hydroxyiboga alkaloids possess antimicrobial activity is already known [18]. As many *Tabernaemontana* species contain dimeric alkaloids of the voacamine type, this could in part explain the high percentage of activity against Gram-positive bacteria in a recent antimicrobial screening of *Tabernaemontana* species [1] and the use of many *Tabernaemontana* species all over the tropical world against several types of infections [2].

EXPERIMENTAL

Plant materials were collected in Nigeria and identified by A. J. M. Leeuwenberg. A voucher specimen has been deposited at the Laboratory for Plant Systematics, Wageningen, The Netherlands. ^{1}H NMR spectra were recorded in $CDCl_3$ at 100 or 300 MHz. ^{13}C NMR spectra were recorded in $CDCl_3$ at 25.2 MHz. MS were determined at 70 or 12 eV using a direct inlet system. The following TLC systems were used in combination with silica gel plates: A: toluene–EtOH satd with NH_3 (19:1)*; B: toluene–EtOH satd with NH_3 (9:1)*; C: toluene–EtOH satd with NH_3 (4:1)*; D: cyclohexane–$CHCl_3$–Et_2NH (6:3:1); E: petrol–*iso*-PrOH satd with NH_3 (9:1)*; F: EtOAc–*iso*-PrOH satd with NH_3 (17:3)*; G: $CHCl_3$–MeOH (13:2); H: $CHCl_3$–MeOH–25% NH_4OH (54:5:1) (*Prior to development the plates were left standing in an atmosphere of NH_3 for 20 min.)

After development the TLC plates were sprayed with 1% $Ce(SO_4)_2$ in 10% H_2SO_4 (Ce^{4+}) or with 0.2 M $FeCl_3$ in 35% $HClO_4$ (Fe^{3+}), followed by heating with hot air. The isolation procedure was the same for root bark and stem bark. Stem bark (2250 g) and root bark (150 g) were extracted for 15 hr with 96% EtOH in a Soxhlet apparatus working under a pressure of 0.25 atm. After cooling at −16° a ppt. was formed, which was collected (steroids). The remaining soln was evapd *in vacuo* to dryness. The extracts were partitioned between EtOAc and 2% HOAc. The aq. layer was collected and adjusted to pH 8 with NH_4OH and extracted with EtOAc. The EtOAc layer was collected, dried (Na_2SO_4) and evapd *in vacuo* (tertiary alkaloids). The yield for the stem bark was 13 g (0.58%) and for the root bark 1.1 g (0.73%). The extracts were separated on a silica gel column using first by system A, then B and lastly C as the mobile phase. The collected fractions were further separated and purified by means of prep. TLC (0.50 mm) with systems A–H. The alkaloids were identified by means of their spectral data, colour reactions and, if possible, by TLC comparison with authentic samples (Table 2).

Isositsirikine (1). TLC: R_f in system F 0.38; Ce^{4+}: yellow; Fe^{3+}: green-black; UV λ_{max}^{MeOH} nm: 224, 282, 290; for MS and ^{1}H NMR data, see ref. [22].

16-*Epiisositsirikine* (2). TLC: R_f in system F 0.33; Ce^{4+}: yellow; Fe^{3+}: green-black; UV λ_{max}^{MeOH} nm: 224, 282, 290; for MS and ^{1}H NMR data, see ref. [22].

Table 3. Minimum inhibitory concentration (MIC) as determined for some alkaloids isolated from *T. pachysiphon* and streptomycin

Compound	Solvent	MIC (μg/ml) against *B. subtilis*	*S. aureus*	*E. coli*	*Ps. aeruginosa*
Conoduramine	Buffer, pH 4	15	35	110	~400
Conodurine	Buffer, pH 4	4	50	70	~400
3-Hydroxyconopharyngine	Buffer, pH 4	60	140	>1000	>1000
Voacamine	Buffer, pH 4	20	20	160	~400
Streptomycin	Buffer, pH 4	1	5	80	7
Streptomycin	Distilled water	0.2	1	0.5	12

Normacusine B (**3**). TLC: R_f in system F 0.29, G 0.30; Ce^{4+}: purple; Fe^{3+}: brown-black; UV λ_{max}^{MeOH} nm: 225, 280, 290; MS (175°) *m/z* (rel. int.): 294 $[M]^+$ (87), 293 (81), 279 (10), 277 (11), 263 (38), 249 (12), 181 (25), 169 (100), 168 (79); ^1H NMR (300 MHz): δ 8.58 (*br s*, NH), 7.44 (*br d*, J = 7.5 Hz, H-12), 7.36 (*br d*, J = 7.5 Hz, H-9), 7.15 (*ddd*, J = 7.5, 7.5 and 1.2 Hz, H-11), 7.08 (*ddd*, J = 7.5, 75 and 1.2 Hz, H-10), 5.28 (*br q*, J = 7.0 Hz, H-19), 4.23 (*br d*, J = 10 Hz, H-3), 3.55–3.43 (*m*), 3.13 (*br dd*, J = 15.8 and 5.0 Hz, H-6a), 2.87 (*br dd*, J = 7 and 5.5 Hz), 2.78 (*br s*), 2.69 (*d*, J = 15.8 Hz, H-6b), 2.04 (*ddd*, J = 11, 10 and 1 Hz), 1.85 (*m*), 1.76 (*br d*, J = 12.5 Hz), 1.63 (*d*, J = 7.0 Hz, H-18).

Pericyclivine (**4**). TLC: R_f in system A 0.24, D 0.12; Ce^{4+}: purple-brown; Fe^{3+}: blue-grey; UV λ_{max}^{MeOH} nm: 226, 281, 289; MS *m/z* (rel. int.) 322 $[M]^+$ (67), 321 (52), 307 (19), 263 (27), 249 (16), 169 (100), 168 (95); ^1H NMR (300 MHz): δ 7.81 (*br s*, NH), 7.42 (*br d*, J = 7.5 Hz, H-12), 7.29 (*br d*, J = 7.5 Hz, H-9), 7.11 (*ddd*, J = 7.5, 7.5 and 1.3 Hz, H-11), 7.04 (*ddd*, J = 7.5, 7.5 and 1.3 Hz, H-10), 5.27 (*br q*, J = 6.9 Hz, H-19), 4.25 (*br d*, J = 10.1 Hz, H-3), 3.70 (*m*), 3.25 (*dd*, J = 15.8 and 1.9 Hz, H-6a), 3.06 (*s*, COOMe), 2.98 (*m*), 2.93 (*br dd*, J = 15.8 and 4.8 Hz, H-6b), 2.83 (*br dd*, J = 11.2 and 2.8 Hz), 2.60 (*ddd*, J = 13.0, 4.2 and 2.0 Hz), 1.78 (*m*), 1.62 (*ddd*, J = 6.9, 1.7 and 1.7 Hz, H-18).

Affinine (**5**). TLC: R_f in system A 0.10, D 0.10; Fe^{3+}: grey-black; UV λ_{max}^{MeOH} nm: 230 (sh), 317; MS (150°) *m/z* (rel. int.): 324 $[M]^+$ (57), 306 (12), 294 (16), 293 (28), 152 (95), 122 (100), 121 (56), 120 (55).

16-*Epiaffinine* (**6**). TLC: R_f in system A 0.17, D 0.22; Fe^{3+}: grey-black; UV λ_{max}^{MeOH} nm: 225 (sh), 317; MS *m/z* (rel. int.): 324 $[M]^+$ (1.5), 323 (1), 322 (2), 307 (14), 306 (46), 263 (7), 153 (12), 152 (100), 151 (12), 150 (13), 148 (10), 135 (11), 122 (13); ^1H NMR (300 MHz): δ 9.15 (*br s*, NH), 7.52 (*dd*, J = 8.2 and 1 Hz, H-9), 7.42 (*m*, H-11 and H-12), 7.18 (*ddd*, J = 8.2, 6.5 and 1.8 Hz, H-10), 5.51 (*br q*, J = 6.9 Hz, H-19), 3.73 (*br d*, J = 14.1 Hz, H-21a), 3.65 (*m*, H-17a), 3.60 (*m*, H-17b), 3.52 (*m*, H-14a), 3.47 (*m*, H-14b), 3.33 (*br t*, H-5 or H-15), 3.32 (*dd*, J = 13.1 and 12.0 Hz, H-6a), 3.09 (*br t*, H-15 or H-5), 3.08 (*d*, J = 14.1 Hz, H-21b), 2.67 (*dd*, J = 13.1 and 7.9 Hz, H-6b), 2.59 (*s*, NMe), 1.98 (*m*, $W_{1/2}$ = 13 Hz, H-16), 1.70 (*dd*, J = 6.9 and 2.0 Hz, H-18).

Vobasine (**7**). TLC: R_f in system A 0.33, D 0.34; Ce^{4+}: white; Fe^{3+}: grey-green with purple rim; UV λ_{max}^{MeOH} nm: 225 (sh), 313; MS (150°) *m/z* (rel. int.): 352 $[M]^+$ (15), 337 (2), 309 (3), 293 (5), 194 (6), 181 (18), 180 (100), 179 (30), 158 (6), 122 (12), 120 (18); ^1H NMR (100 MHz): δ 9.08 (*br s*, NH), 7.72 (*d*, J = 7.8 Hz, H-9), 7.1 (*m*, 3H), 5.48 (*q*, J = 6.8 Hz, H-19), 4.1–2.65 (*m*), 2.65 (*s*, NMe), 2.61 (*s*, CO_2Me), 1.72 (*dd*, J = 6.8 and 2.0 Hz, H-18).

Anhydrovobasindiol (**8**). TLC: R_f in system A 0.27, D 0.26; Fe^{3+}: after prolonged heating orange; UV λ_{max}^{MeOH} nm: 224, 280 (sh), 285, 293; MS (175°) *m/z* (rel. int.): 308 $[M]^+$ (72), 293 (20), 279 (7), 156 (8), 154 (10), 122 (100), 121 (70), 120 (30), 107 (18); ^1H NMR (100 MHz): δ 8.65 (*br s*, NH), 7.58 (*m*, 1H), 7.20 (*m*, 3H), 5.39 (*q*, J = 6.6 Hz, H-19), 5.15 (*d*, J = 8.8 Hz, H-3), 3.90 (*dd*, J = 11.7 and 11.7 Hz), 3.8–1.9 (*m*), 2.53 (*s*, NMe), 1.68 (*dd*, J = 6.6 and 1.7 Hz, H-18).

Apparicine (**9**). TLC: R_f in system A 0.28, D 0.36; Fe^{3+}: purple; UV λ_{max}^{MeOH} nm: 207, 230 (sh), 303, 312 (sh); MS (75°) *m/z* (rel. int.): 264 $[M]^+$ (100), 249 (32), 235 (41), 222 (58), 221 (49), 220 (43), 208 (85), 207 (33), 206 (43), 194 (34), 180 (29), 167 (26), 154 (32); ^1H NMR (100 MHz): δ 7.92 (*br s*, NH), 7.46–6.97 (*m*, H-9/H-12), 5.41 (*s*, H-22a), 5.31 (H-19), 5.27 (*s*, H-22b), 4.55 (*d*, J = 17.9 Hz, H-6a), 4.24 (*d*, J = 17.9 Hz, H-6b), 3.92–1.85 (*m*), 1.47 (*dd*, J = 6.8 and 1 Hz, H-18).

Tubotaiwine (**10**). TLC: R_f in system A 0.31, D 0.35; Ce^{4+}: blue; Fe^{3+}: blue, UV λ_{max}^{MeOH} nm: 228, 297, 327; MS (100°) *m/z* (rel. int.): 324 $[M]^+$ (71), 309 (4), 293 (8), 267 (38), 253 (22), 229 (100), 182 (34), 181 (32), 180 (51), 167 (40); ^1H NMR (300 MHz): δ 8.85 (*br s*, NH), 7.15 (*br d*, J = 7.5 Hz, H-12), 7.11 (*ddd*, J = 7.5, 7.5 and 1.2 Hz, H-10), 6.87 (*ddd*, J = 7.5, 7.5 and 1.2 Hz, H-11), 6.81 (*br d*, J = 7.5 Hz, H-9), 3.85 (*br s*, H-21), 3.77 (*s*, CO_2Me), 3.10–2.82 (*m*, 5H), 2.48 (*m*, 1H), 1.99 (*m*, H-20), 1.88–1.75 (*m*, 3H), 0.88–0.77 (*m*, H-19), 0.70 (*t*, J = 7.1 Hz, H-18); ^{13}C NMR: δ 170.5 ($\underline{C}O_2Me$), 168.8 (C-2), 143.6 (C-13), 137.0 (C-8), 127.1 (C-11), 121.0 (C-9), 119.5 (C-10), 109.6 (C-12), 95.6 (C-16), 65.5 (C-21), 55.0 (C-7), 53.8 (C-5), 51.1 ($CO_2\underline{Me}$), 45.2 (C-6), 43.9 (C-3), 41.1 (C-20), 30.8 (C-15), 28.4 (C-14), 23.8 (C-19), 11.6 (C-18).

Tubotaiwine-N-*oxide* (**11**). TLC: R_f in system B 0.03; Fe^{3+}: blue; UV λ_{max}^{MeOH} nm: 208, 224, 293, 327; MS *m/z* (rel. int.): 340 $[M]^+$ (20), 324 (92), 322 (68), 309 (4), 293 (40), 267 (28), 229 (100), 194 (48), 180 (88), 167 (80); ^1H NMR (100 MHz): δ 8.75 (*br s*, NH), 7.3–6.7 (*m*, 4H), 3.79 (*s*, CO_2Me), 0.80 (*t*, H-18). This alkaloid is probably an artefact formed during the isolation procedure.

Ibogaline (**12**). TLC: R_f in system A 0.48, D 0.33; Fe^{3+}: purple; UV λ_{max}^{MeOH} nm: 225, 280 (sh), 300 (sh), 303; MS (110°) *m/z* (rel. int.): 340 $[M]^+$ (100), 339 (13), 325 (8), 311 (1.5), 255 (26), 216 (6), 204 (5), 170 (11), 149 (27), 136 (60), 135 (27), 122 (19).

Isovoacangine (**13**). TLC: R_f in system A 0.67, D 0.47; Ce^{4+}: orange-brown; Fe^{3+}: gold; UV λ_{max}^{MeOH} nm: 227, 280 (sh), 298; MS (200°) *m/z* (rel. int.): 368 $[M]^+$ (100), 353 (11), 339 (3), 309 (6), 283 (8), 244 (13), 208 (12), 184 (20), 160 (25), 148 (11), 137 (11), 136 (50), 135 (18), 124 (24), 122 (24); ^1H NMR (100 MHz): δ 7.71 (*br s*, NH), 7.46–6.75 (*m*), 3.83 (*s*, OMe), 3.72 (*s*, OMe), 3.65 (*s*, CO_2Me), 0.91 (H-18).

Conopharyngine (**14**). TLC: R_f in system A 0.40, D 0.36; Fe^{3+}: green; UV λ_{max}^{MeOH} nm: 225, 302, 305, 312 (sh); MS (175°) *m/z* (rel. int.): 398 $[M]^+$ (100), 383 (12), 369 (3), 339 (9), 313 (9), 275 (10), 274 (13), 214 (11), 208 (19), 205 (25), 204 (14), 203 (20), 199 (17), 191 (22), 190 (50), 148 (27), 136 (97), 135 (35), 124 (28), 122 (45); ^1H NMR (100 MHz): δ 7.82 (*br s*, NH), 6.91 (*s*, H-9 or H-12), 6.77 (*s*, H-9 or H-12), 3.91 (*s*, OMe), 3.86 (*s*, OMe), 3.71 (*s*, CO_2Me), 3.54 (*s*, H-21), 0.89 (*t*, J = 7.1 Hz, H-18); ^{13}C NMR: δ 175.8 ($\underline{C}O_2Me$), 146.9 (C-11), 144.7 (C-10), 135.1 (C-2), 129.7 (C-13), 121.4 (C-8), 110.0 (C-7), 100.7 (C-9), 94.2 (C-12), 57.6 (C-21), 56.5 (10-OMe), 56.2 (11-OMe), 55.0 (C-16), 53.1 (C-5), 52.5 ($CO_2\underline{Me}$), 51.4 (C-3), 39.2 (C-20), 36.6 (C-17), 32.0 (C-15), 27.4 (C-14), 26.7 (C-19), 22.3 (C-6), 11.6 (C-18).

3R-*Hydroxyconopharyngine* (**15**) *and* 3S-*hydroxyconopharyngine* (**16**) *(mixture)*. TLC: R_f in system A 0.34, D 0.28; Fe^{3+}: purple; UV λ_{max}^{MeOH} nm: 223, 273 (sh), 285 (sh), 302, 306, 311 (sh); MS (190°) *m/z* (rel. int.): 414 $[M]^+$ (30), 412 (40), 398 (40), 397 (43), 396 (100), 395 (20), 381 (41), 367 (18), 355 (24), 353 (14), 338 (22), 337 (68), 313 (12), 310 (14), 309 (27), 300 (11), 298 (21), 297 (14), 288 (20), 219 (19), 208 (20), 190 (26), 151 (22), 136 (39), 135 (29), 122 (39); ^1H NMR (300 MHz): δ 7.65 (*br s*, NH), 6.92 (*s*, H_R-9 or H_R-12), 6.91 (*s*, H_S-9 or H_S-12), 6.80 (*s*, H_R-9 or H_R-12), 6.79 (*s*, H_S-9 or H_S-12), 4.43 (*br s*, $W_{1/2}$ = 6 Hz, H_S-3), 4.12 (*d*, J = 1.6 Hz, H_R-3), 3.92 (*s*, OMe), 3.89 (*s*, OMe), 3.69 (*s*, CO_2Me_S), 3.69 (*s*, CO_2Me_R), 0.92 (*t*, J = 7.5 Hz, H_R-18), 0.90 (*t*, J = 7.5 Hz, H_S-18).

19S-*Hydroxyconopharyngine* (**17**). TLC: R_f in system A 0.30, D 0.28; Fe^{3+}: green; UV λ_{max}^{MeOH} nm: 222, 280 (sh), 303, 310 (sh); MS *m/z* (rel. int.): 414 $[M]^+$ (100), 399 (16), 397 (30), 396 (77), 381 (7), 369 (11), 274 (16), 214 (7), 206 (6), 205 (10), 204 (8), 203 (10), 190 (23), 152 (25), 122 (13); ^1H NMR (300 MHz): δ 7.65 (*br s*, NH), 6.89 (*s*, H-9 or H-12), 6.79 (*s*, H-9 or H-12), 4.16 (*qd*, J = 6.4 and 1.5 Hz, H-19), 3.92 (*s*, OMe), 3.89 (*s*, OMe), 3.83 (*s*, H-21), 3.74 (*s*, CO_2Me), 3.44 (*m*, H-5a), 3.20–2.96 (*m*, H-3a, H-5b, 2 × H-6), 2.83 (*br d*, J = 9.5 Hz, H-3b), 2.57 (*br d*, J = 14 Hz, H-17a), 2.02 (*m*, H-14), 2.05–1.85 (*m*, H-17b, H-20), 1.55 (*br dd*, J = 14 and 10 Hz, H-15a), 1.45 (*br dd*, J = 10 and 7 Hz, H-15b), 1.10 (*d*, J = 6.4 Hz, H-18).

11-*Demethylconoduramine* (**18**). TLC: R_f in system A 0.31, D 0.23; Fe^{3+}: grey-purple; colour with Fast-blue salt B [23]: intense purple colour; UV λ_{max}^{MeOH} nm: 224, 286, 294, 303; $\lambda_{max}^{MeOH+NaOH}$ nm: 285, 294, 323; MS (140°) *m/z* (rel. int.): 704 [M

$+15-1]^+$ (37), 690 $[M]^+$ (100), 675 (15), 659 (25), 645 (12), 631 (25), 510 (30), 509 (17), 508 (17), 497 (30), 496 (30), 495 (52), 479 (17), 437 (12), 395 (12), 379 (28), 368 (17), 354 (25), 338 (43), 324 (37), 308 (74), 293 (42), 279 (42), 269 (34), 264 (34), 249 (42), 225 (27), 208 (35), 194 (44), 182 (80), 180 (80), 167 (40), 152 (52), 148 (34), 136 (80), 122 (100), 108 (47); ^{1}H NMR (300 MHz): δ 7.83 (*br s*, NH), 7.53 (*m*, H-9'), 7.45 (*br s*, N'H), 7.10–7.02 (*m*, H-10', H-11', H-12'), 6.85 (*s*, H-9), 6.75 (*br s*, H-12), 5.38 (*br q*, J = 7.0 Hz, H-19'), 5.1 (*br s*, H-3'), 4.12 (*m*, H-5'), 3.81 (*m*), 3.66 (*s*, CO_2Me), 3.48 (*s*, H-21), 3.38–3.27 (*m*), 3.18–2.97 (*m*), 2.91–2.81 (*m*), 2.78 (*dd*, J = 3.5 and 2.5 Hz, H-16'), 2.72 (*br d*), 2.66 (s, NMe'), 2.47 (*s*, CO_2Me'), 2.0 (*m*), 1.82 (*br s*, H-14), 1.66 (*br d*, J = 7.0 Hz, H-18'), 1.55 (*m*, H-19a), 1.42 (*m*, H-19b), 1.30 (*m*, H-20), 1.11 (H-15), 0.87 (*t*, J = 7.5 Hz, H-18).

Conoduramine (**19**). TLC: R_f in system A 0.40, D 0.34; Fe^{3+}: gold; UV λ_{max}^{MeOH} nm: 228, 285, 294, 303 (sh); MS (120°) *m/z* (rel. int.): 718 $[M+15-1]^+$ (23), 704 $[M]^+$ (68), 689 (5), 673 (18), 659 (7), 645 (18), 524 (18), 509 (39), 451 (11), 395 (14), 352 (21), 335 (11), 323 (11), 308 (18), 279 (9), 250 (9), 210 (18), 208 (18), 194 (41), 182 (55), 180 (77), 148 (21), 136 (55), 124 (27), 122 (100), 108 (27); ^{1}H NMR (300 MHz): δ 7.65 (*br s*, NH, N'H), 7.54 (*m*, H-9'), 7.11–7.02 (*m*, H-10', H-11', H-12'), 6.91 (*br s*, H-9), 6.79 (*br s*, H-12), 5.33 (*br q*, J = 7.0 Hz, H-19'), 5.13 (*br d*, J = 11 Hz, H-3'), 4.08 (*m*, H-5'), 3.96 (*br s*, OMe), 3.78 (*m*), 3.66 (*s*, CO_2Me), 3.56–3.48 (*m*), 3.44 (*br s*, H-21), 3.30–3.17 (*m*), 3.12–2.82 (*m*), 2.73 (*m*), 2.63 (*s*, NMe'), 2.51 (*br d*, J = 13 Hz, H-17), 2.44 (*s*, CO_2Me'), 2.0–1.8 (*m*), 1.66 (*d*, J = 7.0 Hz, H-18'), 1.50 (*m*, H-19a), 1.38 (*m*, H-19b), 1.09 (*m*, H-15), 0.85 (*t*, J = 7.0 Hz, H-18); ^{13}C NMR: δ 175.7 ($\underline{C}O_2Me$), 171.2 ($\underline{C}O_2Me'$), 155.8 (C-11), 138.0 (C-13), 137.3 (C-20'), 135.8 (C-13'), 135.2 (C-2'), 134.6 (C-2), 129.8 (C-8'), 127.0 (C-10), 122.5 (C-8), 121.4 (C-11'), 119.1 (C-19'), 118.7 (C-10'), 117.9 (C-9'), 117.4 (C-9), 110.1 (C-7), 109.8 (C-7'), 109.8 (C-12'), 92.7 (C-12), 59.9 (C-5'), 57.4 (C-21), 55.9 (OMe), 54.9 (C-16), 53.0 (C-3), 52.4 (CO_2Me), 52.4 (C-21'), 51.4 (C-5), 49.9 (CO_2Me'), 46.8 (C-16'), 42.2 (NMe'), 39.1 (C-20), 36.8 (C-3'), 36.6 (C-17), 36.5 (C-14'), 33.5 (C-15'), 32.0 (C-15), 27.3 (C-14), 26.7 (C-19), 22.1 (C-6), 19.5 (C-6'), 12.3 (C-18'), 11.6 (C-18).

Conodurine (**20**). TLC: R_f in system A 0.45, D 0.53; Fe^{3+}: dark-green; UV λ_{max}^{MeOH} nm: 224, 285, 293; MS (275°) *m/z* (rel. int.): 704 $[M]^+$ (9), 673 (0.5), 524 (5), 511 (5), 509 (2), 281 (4), 279 (4), 194 (6), 182 (12), 181 (50), 180 (35), 136 (12), 135 (11), 124 (16), 123 (16), 122 (100); ^{1}H NMR (100 MHz): δ 7.69 (*br s*, NH), 7.65 (*m*, H-9'), 7.56 (*br s*, NH'), 7.25 (*d*, J = 8.4 Hz, H-9), 7.06 (*m*, H-10', H-11', H-12'), 6.82 (*d*, J = 8.4 Hz, H-10), 5.30 (*m*, H-3', H-19'), 3.97 (*s*, OMe), 3.70 (*s*, CO_2Me), 2.64 (*s*, NMe'), 2.51 (*s*, CO_2Me'), 1.66 (*dd*, J = 7.0 and 1.4 Hz, H-18'), 0.81 (*t*, J = 7.1 Hz, H-18).

Voacamine (**21**). TLC: R_f in system A 0.45, D 0.52; Fe^{3+}: blue-green; UV λ_{max}^{MeOH} nm: 222, 285, 293; MS (350°) *m/z* (rel. int.): 718 $[M+15-1]^+$ (1), 704 $[M]^+$ (2.5), 645 (2), 524 (2.5), 512 (2), 511 (3), 510 (2), 509 (2), 503 (2), 455 (2), 453 (3.5), 194 (32), 182 (28), 181 (100), 180 (54), 136 (63), 122 (82).

Conopharyngine-hydroxyindolenine (**22**). TLC: R_f in system A 0.34; Fe^{3+}: yellow; UV λ_{max}^{MeOH} nm: 230, 288 (sh), 294, 308 (sh), 325 (sh); MS *m/z* (rel. int.): 414 $[M]^+$ (100), 399 (20), 398 (18), 397 (32), 385 (6), 367 (5), 355 (7), 290 (5), 220 (6), 207 (6), 206 (6), 192 (7), 191 (6), 190 (7), 136 (11), 122 (12). This alkaloid may be an artefact formed during the isolation procedure.

Lochnericine (**23**). TLC: R_f in system A 0.55, D 0.49; Fe^{3+}: blue; UV λ_{max}^{MeOH} nm: 225, 295, 325; MS (175°) *m/z* (rel. int.): 352 $[M]^+$ (59), 323 (12), 322 (15), 309 (7), 295 (9), 293 (9), 214 (28), 168 (25), 167 (30), 149 (36), 138 (100), 108 (72). Reduction of 3*R*/*S*-hydroxyconopharyngine (**15** and **16**) with $NaBH_4$ according to ref. [22] yielded conopharyngine (**14**), identified by TLC with systems A and D. After 2 days of treatment with CH_2N_2, with or without BF_3, 11-demethylconoduramine (**18**) was recovered unchanged according to TLC with systems A and D.

Biograms. This was carried out in the same manner as described earlier [21].

Determination of antimicrobial activity by means of the agar diffusion technique. A suspension ($\sim 10^8$ bacteria/ml) of *B. subtilis*, *S. aureus*, *E. coli* or *Ps. aeruginosa* was spread on TSB–agar plates [21], after which the plates were dried in sterile air. In all test plates, four holes, 1.0 cm in diameter, were made and filled with 0.1 ml test soln. Fifteen different concns were tested in duplicate. Inhibition zones were measured after 20 hr at 37°. The alkaloids were dissolved in a citrate–Pi buffer pH 4 (see below), which was also used as a negative control. The MIC values were calcd by plotting the diameter of inhibition minus 1.0 against log C. The results are shown in Table 3. Prior to the actual testing, several solvents were tested with voacamine as ref. Voacamine base was suspended in distilled H_2O, 20% DMSO, 20% MeOH, Pi–citrate buffer, pH 5 and pH 4.5, or dissolved in Pi buffer, pH 4, 3.5 and 3. Also, a soln of voacamine–HCl in distilled H_2O was tested. The soln in citrate–Pi buffer, pH 4, gave the largest and most reproducible inhibiting zones while the buffer itself did not inhibit the bacteria in their growth. Therefore this solvent was chosen as the testing solvent.

Steroids and triterpenes. α-Amyrenyl acetate: UV: no absorption above 200 nm: MS (155°) *m/z* (rel. int.): 468 $[M]^+$ (10), 219 (19), 218 (100), 203 (10), 189 (11); ^{1}H NMR (100 MHz): δ 5.12 (*t*, J = 3.8 Hz, H-12), 4.50 (*dd*, J = 9.0 and 6.6 Hz, H-3), 2.05 (*s*, CO_2Me), 1.06 (*s*, Me), 1.01 (*s*, Me), 0.98 (*s*, Me), 0.87 (*s*, Me), 0.80 (*s*, Me); ^{13}C NMR: δ 171.0, 139.3, 124.3, 80.9, 58.9, 55.2, 47.6, 42.1, 41.5, 40.0, 39.7, 39.6, 38.4, 37.8, 37.6, 33.8, 32.8, 31.2, 28.7, 28.1, 28.0, 26.5, 23.5, 23.4, 23.3, 21.3, 21.3, 18.2, 17.5, 16.8, 16.8, 15.7; mp 222–224°.

Lupeyl acetate. UV: no absorption above 200 nm; MS *m/z* (rel. int.): 468 $[M]^+$ (100), 218 (64), 204 (48), 190 (45), 189 (97), 136 (43), 135 (52), 109 (58), 95 (53); mp 210°.

Sitosterol, stigmasterol and campesterol (mixture). MS (220°) *m/z* (rel. int.): 414 $[M]^+$ (100), 413 (24), 412 $[M]^+$ (63), 400 $[M]^+$ (72), 399 (30), 397 (31), 396 (20), 382 (18), 273 (28), 271 (27), 255 (46), 231 (23), 213 (24).

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REFERENCES

1. van Beek, T. A., Verpoorte, R. and Baerheim Svendsen, A., *Planta Med.* (in press).
2. van Beek, T. A., Verpoorte, R., Baerheim Svendsen, A., Leeuwenberg, A. J. M. and Bisset, N. G. (1984) *J. Ethnopharmacol.* **10**, 1.
3. Kingston, D. G. I., Li, B. T. and Ionescu, F. (1977) *J. Pharm. Sci.* **66**, 1135.
4. Patel, M. P. and Poisson, J. (1966) *Bull. Soc. Chim. Fr.* 427.
5. Renner, U. and Kernweisz, P. (1963) *Experientia* **19**, 244.
6. Crooks, P. A. and Robinson, B. (1970) *J. Pharm. Pharmacol.* **22**, 799.
7. Kingston, D. G. I., Ionescu, F. and Li, B. T. (1977) *Lloydia* **40**, 215.
8. Crooks, P. A. and Robinson, B. (1973) *J. Pharm. Pharmacol.* **25**, 820.
9. Thomas, J. and Starmer, G. A. (1963) *J. Pharm. Pharmacol.* **15**, 487.

10. Patel, M. B., Miet, C. and Poisson, J. (1967) *Ann. Pharm. Fr.* **25**, 379.
11. Crooks, P. A. and Robinson, B. (1970) *J. Pharm. Pharmacol.* **22**, 471.
12. Crooks, P. A., Robinson, B. and Smith, G. F. (1968) *J. Chem. Soc. Chem. Commun.* 1210.
13. Lucas, R. A., Dickel, D. F., Dziemian, R. L., Ceglowski, M. J., Hensle, B. L. and MacPhillamy, H. B. (1960) *J. Am. Chem. Soc.* **82**, 5688.
14. Bally, P. R. O. (1938) *Feddes Repert.* **102**, 58.
15. Kokwaro, J. O. (1976) *Medicinal Plants of East Africa*, p. 28, East African Literature Bureau, Kampala, Nairobi, Dar es Salaam.
16. Annotation from: J. B. Gillett 17972, Rondo Forest Reserve, Tanzania.
17. Agwada, V. C., Morita, Y., Renner, U., Hesse, M. and Schmid, H. (1975) *Helv. Chim. Acta* **58**, 1001.
18. Achenbach, H., Raffelsberger, B. and Brillinger, G.-U. (1980) *Phytochemistry* **19**, 2185.
19. Hesse, M. (1974) *Progress in Mass Spectrometry*, Vol. 1, p. 229. Verlag Chemie, Weinheim.
20. Bombardelli, E., Bonati, A., Gabetta, B., Martinelli, E. M., Mustich, G. and Danieli, B. (1976) *J. Chem. Soc. Perkin Trans.* 1, 1433.
21. Verpoorte, R., Ruigrok, C. L. M. and Baerheim Svendsen, A. (1982) *Planta Med.* **46**, 149.
22. van Beek, T. A., Verpoorte, R. and Baerheim Svendsen, A. (1983) *Planta Med.* **47**, 83.
23. Stahl, E. (1967) *Dünnschicht Chromatographie*, Vol. 2, p. 829, Springer, Berlin.