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ANTIMICROBIALLY ACTIVE ALKALOIDS FROM *TABERNAEMONTANA CHIPPII*[1]

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ABSTRACT.—From *Tabernaemontana chippii* root bark, forty-five alkaloids were isolated; thirty-four were fully characterized by means of their spectral data and/or co-tlc; eight alkaloids were new, four of them being 3-hydroxy derivatives of known dimeric voacamine type alkaloids. Most of the twenty-six known alkaloids belonged to the corynanthean, ibogan, or bisindole classes. The structures of eleven other alkaloids—all minor—were only partially elucidated, most of them being new alkaloids. All the dimeric alkaloids were shown to possess strong antimicrobial activity against gram-positive bacteria and moderate to weak activity against gram-negative bacteria.

In a recent antimicrobial screening of 19 different *Tabernaemontana* species (2), it was found that the ethanolic extracts of the root bark of *Tabernaemontana chippii* (Stapf) Pichon showed strong antimicrobial activity against both gram-positive and gram-negative bacteria and weak antifungal and antiyeast activity. This observation prompted a closer examination of the species in order to identify the compounds responsible for the antimicrobial activity. The results of this study are set out below.

T. chippii (syn. *Conopharyngia chippii* Stapf) is a small understory tree growing in light forest or bush and occurring in Ivory Coast, Ghana, and perhaps Liberia (3). No ethnomedicinal uses of the plant are known to the authors. In an earlier phytochemical investigation, voaphylline (**22**) (syn. conoflorine) was isolated from this species (4). Renner and Prins stated that the plant is suitable for the extraction of conopharyngine (5).

RESULTS AND DISCUSSIONS

GENERAL.—The ground root bark was extracted with EtOH and the crude ethanolic extract was separated into four fractions: an acidic EtOAc fraction (fats, steroids, triterpenes, etc.), a basic $CHCl_3$ fraction (crude tertiary alkaloids), a basic aqueous fraction (quaternary alkaloids and other water-soluble compounds) and an insoluble fraction. As only the crude tertiary alkaloid fraction showed antimicrobial activity, only this fraction was investigated further. The tertiary alkaloids were separated and purified by means of lc and preparative tlc. The antimicrobially active alkaloids were identified by using the biogram technique as a bioassay during the fractionation (6). *Bacillus subtilis* and *Escherichia coli* were used as test organisms. For phytochemical and chemotaxonomical interest, the nonantimicrobially active alkaloids were also separated and, as far as possible, identified. Table 1 lists the 34 fully characterized alkaloids together with an indication of their relative abundance and the methods used for their identification. Table 2 does the same for the partially identified alkaloids. Of the 34 fully identified alkaloids, eight were new and three have not been isolated previously

[1]Part 11 in the series: "Pharmacognostical Studies of *Tabernaemontana* species." For part 10 see van Beek *et al.* (1).

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from the genus. The structure elucidation of the new as well as the partially identified alkaloids is described in the following.

3*R*- AND 3*S*-HYDROXYCONODURAMINE (**36**).—This major antimicrobially active alkaloid showed the mass spectral behavior of a 3-hydroxy substituted iboga alkaloid with a small M^+ at *m/z* 720 and larger peaks at M^+-2, M^+-16, and M^+-18. The further fragmentation pattern was characteristic for a dimeric indole alkaloid of the voacamine type, suggesting that the alkaloid might be a 3-hydroxy derivative of one of the four known isomeric voacamine-type alkaloids. This was confirmed by the ^{1}H-nmr spectrum, which showed the alkaloid to be a mixture of 3*R*- and 3*S*-hydroxy-conoduramine (**36**). Extra evidence for the correctness of the proposed structure was gained from the ^{13}C-nmr spectra (see Table 3), which proved both the 3′-10 attachment between the two halves (C-12 at 92.8 ppm) and the presence of a carbinolamine function (95.9 ppm for the 3*R*-isomer and 86.1 ppm for the 3*S*-isomer). The absolute configura-

TABLE 1. Alkaloids Isolated from the Root Bark of *Tabernaemontana chippii*

Alkaloid	Relative abundance[a]	Methods of identification[b]
Isositsirikine (**1**)	+++	uv, ms, ^{1}H nmr, co-tlc
16-*Epi*-isositsirikine (**2**	+	co-tlc
Normacusine B (**3**)	++	ms, ^{1}H nmr, co-tlc
Pericyclivine (**4**)	++	uv, ms, ^{1}H nmr, co-tlc
16-*Epi*-affinine (**5**)	++	uv, ms, ^{1}H nmr, co-tlc
Anhydrovobasindiol (**7**)	++	uv, ms, ^{1}H nmr, co-tlc
Vobasine (**6**	++	uv, ms, ^{1}H nmr, co-tlc
Vobasinol (**8**)	++	uv, ms, ^{1}H nmr
Akuammiline (**10**)	++	uv, ms, ^{1}H nmr
Desacetylakuammiline (**11**	++	uv, ms, ^{1}H nmr
Picraline (**12**)	+++	uv, ms, ^{1}H nmr
Pleiocarpamine (**16**)	++	uv, ms, ^{1}H nmr
12-Hydroxyakuammicine (**17**)	++	uv, ms, ^{1}H nmr, [α]
Apparicine (**18**)	+++	uv, ms, co-tlc
Tubotaiwine (**21**)	+++	uv, ^{1}H nmr, co-tlc
Voaphylline (**22**)	+	uv, ms, co-tlc
Conopharyngine (**26**)	+++	uv, ms, ^{1}H nmr, cd, co-tlc
3 *R/S*-Hydroxyconopharyngine (**28**)	++++	uv, ms, ^{1}H nmr, ^{13}C nmr, cd, chcr, co-tlc
3-Oxoconopharyngine (**30**)	+	uv, ms, ^{1}H nmr
Conopharyngine-hydroxyindolenine (**45**)	+	uv, ms, ^{1}H nmr
3 *R/S*-Hydroxyconopharyngine-hydroxyindolenine (**46**)	+	uv, ms, ^{1}H nmr
Coronaridine (**31**)	+	uv, ms, cd, co-tlc
Ibogaline (**32**)	+	uv, ms, ^{1}H nmr, cd, co-tlc
Isovoacangine (**33**)	++	uv, ms, cd, co-tlc
3 *R/S*-Hydroxyisovoacangine (**34**)	++	uv, ms, ^{1}H nmr, ^{13}C nmr, cd, chcr
Conoduramine (**35**)	+++	uv, ms, ^{1}H nmr, cd, co-tlc
3 *R/S*-Hydroxyconoduramine (**36**)	+++	uv, ms, ^{1}H nmr, ^{13}C nmr, cd, chcr
Conodurine (**37**)	+++	uv, ms, ^{1}H nmr, ^{13}C nmr, cd, co-tlc
3 *R/S*-Hydroxyconodurine (**39**)	+++	uv, ms, ^{1}H nmr, ^{13}C nmr, cd, chcr
3 *R/S*-Hydroxy-16-decarbomethoxy-conodurine (**40**)	+	uv, ms ^{1}H nmr
3 *R/S*-Hydroxyvoacamine (**44**)	+	uv, ms, ^{1}H nmr, cd, chcr
Vobparicine (**19**)	++	uv, ms, ^{1}H nmr, ^{13}C nmr, cd, co-tlc
Vobparicine-N_4-oxide (**20**)	+	uv, ms, ^{1}H nmr, chcr
Monogagaine	++	uv, ms, ^{1}H nmr

[a]++++=main component; +++=major component; ++=minor component; +=trace component.

[b]chcr=chemical correlation.

TABLE 2. Partially Characterized Alkaloids Isolated from the Root Bark of *Tabernaemontana chippii*

Alkaloid (code)	Relative abundance[a]	Methods of identification[b]
Chippiine (**47** or **48**)	+	uv, ms, ^{1}H nmr, cd, chcr
TC-A (**23**)	+	uv, ms
TC-B (**25**)	+	uv, ms
TC-C (**24**)	++	uv, ms, ^{1}H nmr
TC-D (**9**)	++	uv, ms, ^{1}H nmr, ^{13}C nmr
Tacraline (**14**)	+	uv, ms, ^{1}H nmr
Desacetyltacraline (**15**)	+	uv, ms, ^{1}H nmr
TC-E (M^+ 416)	+	uv, ms, ^{1}H nmr[c]
TC-F (M^+ 600)	+	uv, ms, ^{1}H nmr[c]
TC-G (M^+ 588)	++	uv, ms, ^{1}H nmr[c]
TC-H (M^+ 606)	+	uv, ms, ^{1}H nmr[c]

[a]++=minor component; +=trace component.
[b]chcr=chemical correlation.
[c]Spectral data available upon request.

tion was determined by means of a chemical correlation with conoduramine (**35**). Standard $NaBH_4$ reduction of **36** gave only one product that was identical with conoduramine (**35**) (co-tlc, chromogenic reactions, uv, and cd).

3*R*- AND 3*S*-HYDROXYCONODURINE (**39**).—This major antimicrobially active alkaloid showed the same mass spectral behavior as the preceding alkaloid, suggesting it to be an isomer of **36**. This was confirmed by its ^{1}H-nmr and ^{13}C-nmr spectra, which showed the presence of a carbinolamine function and the 3′-12 attachment between the two monomeric halves. This alkaloid was also present as a mixture of the 3*R*- (C-3 at 93.7 ppm) and 3*S*-isomer (C-3 at 86.0 ppm). Many other signals of the iboga half were doubled as well in both the ^{1}H-nmr and ^{13}C-nmr spectra. The fact that the two isomers could not be separated on tlc in any solvent tested, while 19*R*- and 19*S*-hydroxy-iboga alkaloids, for example, can be easily separated on tlc (1), seems to suggest that the isomers are in equilibrium with each other (see Figure 1) and that this process is slow on the nmr time scale, making it possible to see both isomers, and fast on the tlc time scale, making it impossible to separate them. An alternative possibility is that the interconversion is catalyzed by the SiO_2 layer. The absolute configuration of 3*R*/*S*-hydroxyconodurine (**39**) was proven by a chemical correlation ($NaBH_4$ reduction) with conodurine (**37**) (co-tlc, chromogenic reactions, uv, and cd).

3*R*- AND 3*S*-HYDROXY-16-DECARBOMETHOXYCONODURINE (**40**).—This trace component showed again the typical mass spectral behavior of a 3-hydroxy substituted iboga alkaloid with a small M^+ at *m/z* 662 and larger peaks at *m/z* 660, 646, and 644. These data and the further fragmentation pattern suggested a 3-hydroxy substituted voacamine-type alkaloid without the 16-decarbomethoxy group (*m/z* 704−*m/z*

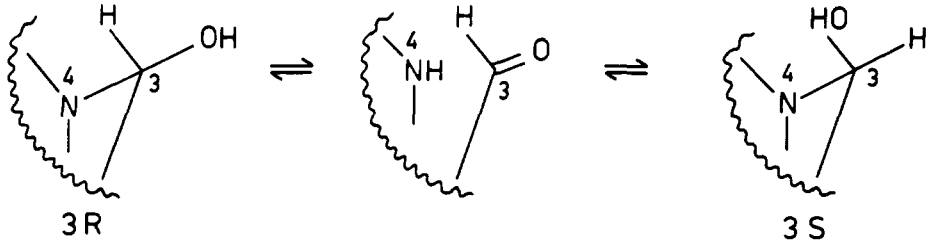
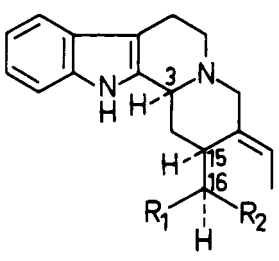
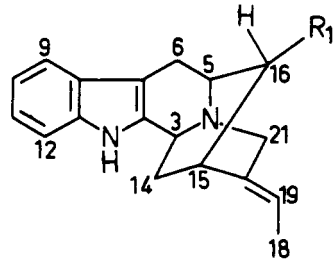


FIGURE 1

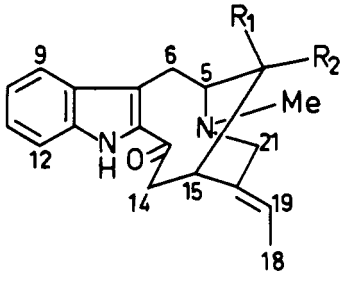
662=58 mass units loss). This was confirmed by the ^{1}H-nmr spectrum, which was almost superimposable with the spectrum of 3*R*/*S*-hydroxyconodurine (**39**) except for the absence of a peak for the carbomethoxy group at 3.65 ppm and some small changes in the aliphatic part. Thus, the alkaloid is 3*R*/*S*-hydroxy-16-decarbomethoxyconodurine (**40**). Reduction with $NaBH_4$ gave one product which showed a slightly higher Rf-value than **40** on tlc in solvent systems S1 and S4 and a much lower Rf value than **40** in S8 (See Experimental for solvent system composition). This is strong evidence for the absence of a 16-carbomethoxy group. In the base-containing solvent systems S1 and S4, the Rf is higher because the polar 3-hydroxy group is removed by the reduction. In the base-free S8, however, the Rf of the reduction product **38** is lower because by removal of the hydroxy group, the nitrogen at the 4-position becomes much more basic. This phenomenon of low Rf values in base-free solvents is not observed for alkaloids of the voacamine series (because the 16-carbomethoxy group also lowers the basicity) and is thus quite specific for alkaloids lacking the 16-carbomethoxy group. Another example of this behavior is tabernamine (**41**) (1). The absolute configuration could not be proven by a correlation with 16-decarbomethoxyconodurine (**38**) because this alkaloid was unavailable. However, by the conodurine-like cd spectrum and by reasoning based on



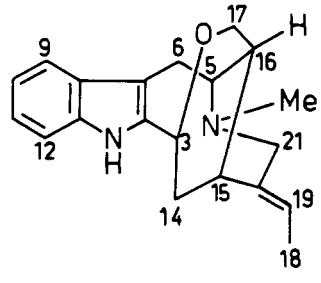
1 R_1=CH_2OH, R_2=CO_2Me
2 R_1=CO_2Me, R_2=CH_2OH



3 R_1=CH_2OH
4 R_1=CO_2Me



5 R_1=H, R_2=CH_2OH
6 R_1=CO_2Me, R_2=H



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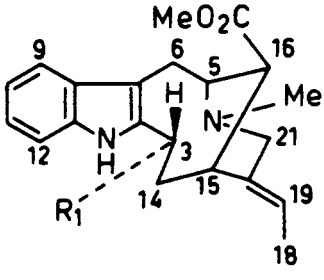
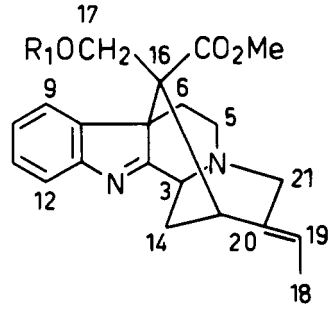


8 R_1=OH
9 R_1=$C_2H_6NO_2$



10 R_1=COMe
11 R_1=H

TABLE 3. ^{13}C-nmr Data[a] of Conopharyngine (**26**) 3*R*-Hydroxyconopharyngine (**28**) 3*R*- and 3*S*-Hydroxyisovoacangine (**34**) Conodurine (**37**) 3*R*- and 3*S*-Hydroxyconodurine (**39**) Conoduramine (**35**) and 3*R*- and 3*S*-Hydroxyconoduramine (**36**)

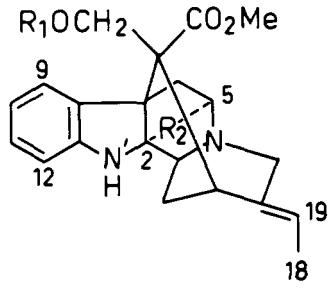
Carbon atom	cono-pharyngine[b]	3*R*-hydroxy-cono-pharyngine	hydroxy-isovoacangine		cono-durine	hydroxy-conodurine		conodur-amine[b]	hydroxy-conoduramine	
			3*R*	3*S*		3*R*	3*S*		3*R*	3*S*
2	135.1	135.0	136.1[c]	136.1[c]	135.7	135.9	135.9	134.6	134.7	134.7
3	51.4	93.9	93.8	85.9	53.0	93.7	86.0	53.0	95.9	86.1
5	53.1	52.1	52.0	51.2	51.4	51.8	51.1	51.4	52.5	51.2
6	22.3	22.1	21.9	21.9	21.9	21.7	21.6	22.1	21.9	21.9
7	110.0	109.8	110.0	110.0	109.8	109.8	109.8	110.1	109.8	109.8
8	121.4	120.9	122.6	122.6	124.3	123.9	123.9	122.5	121.9	121.9
9	100.7	100.6	118.9	118.9	117.2	117.0	117.0	117.4	117.8	117.8
10	144.7[c]	144.8	109.1	109.1	105.0	105.0	105.0	127.0	127.4	127.4
11	146.9[c]	146.9	n.o.	n.o.	151.9	151.9	151.9	155.8	153.3	153.3
12	94.2	94.3	94.2	94.2	114.0	114.3	114.3	92.7	92.8	92.8
13	129.7	129.7	134.6[c]	134.6[c]	135.0	135.1	135.1	138.0	138.1	138.1
14	27.4	30.8	30.8	34.5	27.0	30.5	34.2	27.3	30.0	34.5
15	32.0	25.2	25.1	24.8	31.7[c]	24.9	24.6	32.0[c]	25.0	24.8
16	55.0	54.4	54.4	54.4	54.4	54.0	54.0	54.9	54.2	54.2
17	36.6	35.5	35.5	35.5	33.5[c]	33.7[c]	33.7[c]	36.6[c]	36.6[c]	36.8[c]
18	11.6	11.7	11.7	11.7	11.6	11.6	11.6	11.6	11.7	11.7
19	26.7	26.6	26.6	26.8	26.5	26.5	26.8	26.7	26.6	26.9
20	39.2	38.0	38.0	37.8	38.9	38.0	37.5	39.1	37.8	37.8
21	57.6	56.0	56.0	56.3	57.3	55.7	55.8	57.4	54.0	55.4
10-Ome	56.5	56.5								
11-Me	56.2	56.2	55.7	55.7	56.8	56.8	56.8	55.9	56.0	56.0
CO_2Me	52.5	52.6	52.6	52.6	52.2	52.4	52.4	52.4	52.5	52.5
CO_2Me	175.8	175.1	175.1	175.1	174.9	174.2	174.0	175.7	175.0	175.0
2′					135.9	136.9	136.9	135.2	135.0	135.0
3′					34.8[c]	33.7[c]	33.7[c]	36.8[c]	35.4[c]	35.4[c]
5′					59.8	59.6	59.6	59.9	59.8	59.8
6′					19.7	19.4	19.4	19.5	19.4	19.4
7′					109.1	108.7	108.7	109.8	110.4	110.4

8′					129.3	129.3	129.3	129.8	129.9	129.9
9′					117.8	117.9	117.9	117.9	117.4	117.4
10′					119.6	119.1	119.1	118.7	118.3	118.3
11′					122.2	122.2	122.2	121.4	121.3	121.3
12′					109.8	109.8	109.8	109.8	109.8	109.8
13′					135.7	135.8	135.8	135.8	135.8	135.8
14′					35.0[c]	35.2[c]	35.2[c]	36.5[c]	36.9[c]	36.9[c]
15′					33.1[c]	33.4[c]	33.4[c]	33.5[c]	33.6[c]	33.6[c]
16′					46.6	47.1	47.2	46.8	47.2	47.2
18′					12.5	12.4	12.4	12.3	12.3	12.3
19′					120.7	119.5	119.5	119.1	118.7	118.7
20′					136.9	137.3	137.3	137.3	137.8	137.8
21′					52.4	52.4	52.4	52.4	52.5	52.5
NMe′					41.9	42.3	42.3	42.2	42.4	42.4
CO_2Me'					50.2	50.1	50.1	49.9	49.8	49.8
CO_2Me'					170.8	171.6	171.6	171.2	171.6	171.6

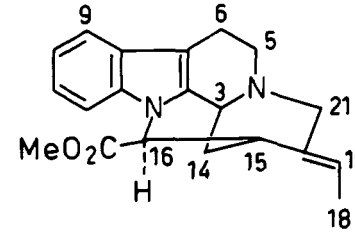
[a]The assignments were based on published ^{13}C-nmr data of similar alkaloids, normal chemical shift theory and in the case of **28** and **39** also on the signal multiplicity in the off-resonance ^{13}C nmr. The differentiation between the 3*R* and 3*S* isomers was possible after a careful comparison of the ^{13}C nmr spectra of **28** (90%R) and **34** (80%R).

[b]These data were taken from van Beek, *et al.* (7).

[c]Values may be interchanged within each column.



12 R_1=COMe, R_2=O
13 R_1=H, R_2=O
14 R_1=COMe, R_2=NH
15 R_1=H, R_2=NH



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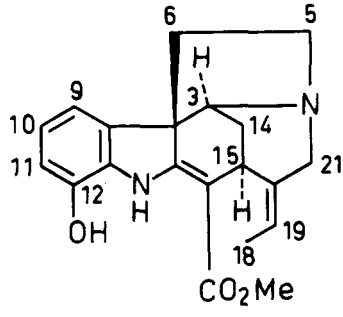

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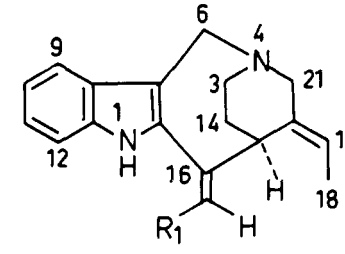
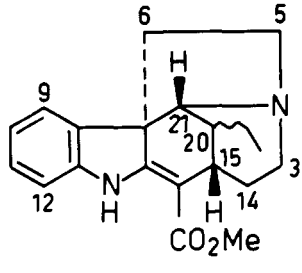


18 R_1=H
19 R_1=3′-vobasinyl
20 R_1=3′-vobasinyl, N_4→O



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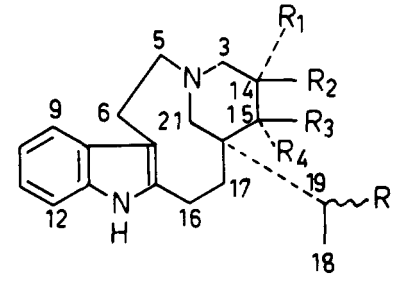
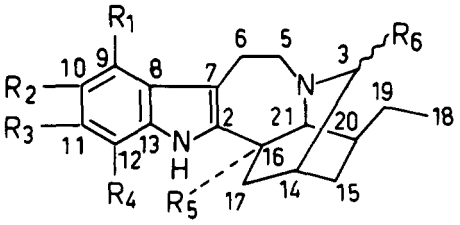


22 R_1=R_4=R_5=H, R_2+R_3=O
23 R_1=R_4=R_5=H, R_2=R_3=OH
24 R_1=R_3=OH, R_2=R_4=R_5=H
25 R_1=R_3=R_5=OH, R_2=R_4=H

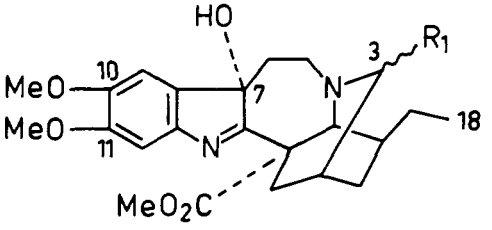
biosynthesis, it seems likely that the stereochemistry of the iboga part will be the same as in all the other iboga alkaloids isolated from this species (coronaridine type) and that the absolute configuration is thus given by **40**.

3*R*- AND 3*S*-HYDROXYVOACAMINE (**44**).—By means of its similar mass spectral data but different Rf value and color with the $FeCl_3$-perchloric acid spray reagent, this trace component was shown to be yet another isomer of alkaloids **36** and **39**. The only two remaining possibilities were 3*R*/*S*-hydroxyvoacamine (**44**) and 3*R*/*S*-hydroxyvoacamidine (**42**). Its ^{1}H-nmr spectrum showed two broad singlets at 6.93 ppm and 6.75 ppm, indicating it to be the former of these two possibilities. This was proven by a correlation ($NaBH_4$ reduction) with voacamine (**43**) (co-tlc, chromogenic reactions, uv, and cd); thus, the absolute configuration is as indicated in structure **44**.

3*R*/*S*-HYDROXYCONOPHARNYGINE-HYDROXYINDOLENINE (**46**).—This alkaloid was always present in very small amounts in fractions containing 3*R*/*S*-hydroxyconopharyngine (**28**) even after repurification and is therefore thought to be a decom-



26 $R_1=R_4=R_6=H$, $R_2=R_3=OMe$, $R_5=CO_2Me$
27 $R_1=R_4=H$, $R_2=R_3=OMe$, $R_5=CO_2Me$, $R_6=OEt$
28 $R_1=R_4=H$, $R_2=R_3=OMe$, $R_5=CO_2Me$, $R_6=OH$
29 $R_1=R_4=H$, $R_2=R_3=OMe$, $R_5=CO_2Me$, $R_6=OMe$
30 $R_1=R_4=H$, $R_2=R_3=OMe$, $R_5=CO_2Me$, $R_6=O$
31 $R_1=R_2=R_3=R_4=R_6=H$, $R_5=CO_2Me$
32 $R_1=R_4=R_5=R_6=H$, $R_2=R_3=OMe$
33 $R_1=R_2=R_4=R_6=H$, $R_3=OMe$, $R_5=CO_2Me$
34 $R_1=R_2=R_4=H$, $R_3=OMe$, $R_5=CO_2Me$, $R_6=OH$
35 $R_1=R_4=R_6=H$, $R_2=3'$-vobasinyl, $R_3=OMe$, $R_5=CO_2Me$
36 $R_1=R_4=H$, $R_2=3'$-vobasinyl, $R_3=OMe$, $R_5=CO_2Me$, $R_6=OH$
37 $R_1=R_2=R_6=H$, $R_3=OMe$, $R_4=3'$-vobasinyl, $R_5=CO_2Me$
38 $R_1=R_2=R_5=R_6=H$, $R_3=OMe$, $R_4=3'$-vobasinyl
39 $R_1=R_2=H$, $R_3=OMe$, $R_4=3'$-vobasinyl, $R_5=CO_2Me$, $R_6=OH$
40 $R_1=R_2=R_5=H$, $R_3=OMe$, $R_4=3'$-vobasinyl, $R_6=OH$
41 $R_1=R_2=R_4=R_5=R_6=H$, $R_3=3'$-vobasinyl
42 $R_1=3'$-vobasinyl, $R_2=OMe$, $R_3=R_4=H$, $R_5=CO_2Me$, $R_6=OH$
43 $R_1=R_4=R_6=H$, $R_2=OMe$, $R_3=3'$-vobasinyl, $R_5=CO_2Me$
44 $R_1=R_4=H$, $R_2=OMe$, $R_3=3'$vobasinyl, $R_5=CO_2Me$, $R_6=OH$



45 $R_1=H$
46 $R_1=OH$

position product of **28**. Its uv spectrum and unusual yellow color with the $FeCl_3$-perchloric acid spray reagent (1) suggested that this probable artifact might be 3*R*/*S*-hydroxyconopharyngine-hydroxyindolenine (**46**). It could be formed in a similar fashion as conopharyngine-hydroxyindolenine (**45**) is formed from conopharyngine (**26**) (8). Its ms showed no peak at the expected M^+ (*m/z* 430) but instead a small peak at *m/z* 428 (M^+-2) and a large peak at *m/z* 414 (M^+-16). The further fragmentation pattern was similar to the one of conopharyngine-hydroxyindolenine (**45**). In the ^{1}H-nmr spectrum, no NH was observed, and some signals for the H 3_S and H 3_R at 4.47 and 4.00 ppm could be observed.

MASS SPECTRAL BEHAVIOR OF 3-HYDROXY-IBOGA ALKALOIDS.—The troublesome mass spectral behavior of 3-hydroxy substituted iboga alkaloids under electron-impact conditions was first observed by Agwada *et al.* (9). They explained the fact that no clear single molecular ion could be observed by the occurrence of thermal processes within the mass spectrometer. Two molecules can react with each other (Figure 2), giving rise to an $M^{+\cdot}$, $[M-2]^+$ and an $[M-16]^+$. Also, loss of the hydroxy group and/or

H_2O is observed ([M-17]+, $[M-18]^+$). The ratio between these peaks varies strongly with the experimental conditions (probe/source temperature, eV, apparatus, etc.)

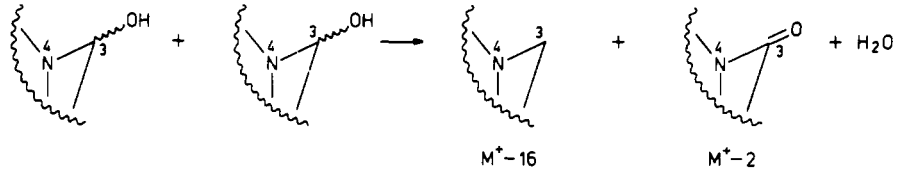
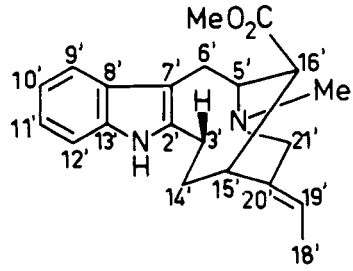


FIGURE 2.

In order to obtain a clear $M^{+\cdot}$, many fdms and a few fabms were recorded for almost all the 3-hydroxy-iboga alkaloids isolated from the species investigated here. Usually even thermally labile or non-volatile compounds exhibit only one peak ($M^{+\cdot}$ or $M^+ + H$) with no further fragmentation in a fdms. However, the above mentioned process happens seemingly so easily that none of the isolated 3-hydroxy-iboga alkaloids ever showed the anticipated $M^{+\cdot}$. Usually a similar pattern as observed in the ei spectra was observed in the fdms, i.e., peaks at the $M^{+\cdot}$, $[M-2]^+$, $[M-16]^+$, $[M-17]^+$, and $[M-18]^+$. The ratios between the peaks varied strongly with the emitter current. Contrary to the eims, no other peaks at lower masses were observed in the fdms. Also, the solvent chosen to dissolve the alkaloids and to bring them on the tungsten wire fd emitter played an important role. Using 3*R*/*S*-hydroxyconopharyngine (**28**) as a model compound, the influence of the solvent on the fdms was studied in some detail (Table 4). When CCl_4 was used, no single molecular ion, but the earlier mentioned pattern, was observed at lower emitter currents. Even a peak at *m/z* 810 was observed suggesting the formation of a dimeric ion by reaction between two molecules **28** and the subsequent loss of H_2O. At higher emitter currents, the $[M-18]^+$ became the predominant peak. When $CHCl_3$ stabilized with 1% EtOH was used, the same pattern with an additional peak at *m/z* 442 was observed. This indicated, at least to some extent, the formation of the ethoxy derivative **27**. This was checked by using pure MeOH as the solvent. Under these conditions, one peak at *m/z* 428 with no other peaks at all was observed. When deuterated MeOH was used, the peak shifted to *m/z* 431 and *m/z* 432, clearly proving the quantitative formation of the methoxy derivative **29**. Because of these results, it was therefore hoped that if H_2O was chosen as solvent, maybe a single peak at the expected molecular weight (*m/z* 414) might be observed. As a tertiary alkaloid the model compound **28** is not soluble in H_2O, and therefore 0.001 N HCl in H_2O was used. However, under these conditions a single stable peak at *m/z* 397 $[(M+H)-H_2O]^+$ was observed. The dimeric 3-hydroxy-iboga alkaloids showed a similar behavior as **28**.

VOBPARICINE (**19**).—The structure elucidation of this antimicrobially active minor component was described elsewhere (10).



3'-vobasinyl

TABLE 4. Influence of the Solvent on the fdms of 3 *R/S*-Hydroxyconopharyngine (**28**)

solvent	emitter current (mA)	peaks observed at *m/z* (rel. int.)
CCl_4	9	810(−), 414(50), 412(100), 397(50), 396(90)
	12	810(−), 792(−), 414(60), 412(40), 398(40), 397(80), 396(100)
	20	793(−), (792(−), 414(50), 412(30), 398(25), 397(55), 396(100)
$CHCl_3$+1% C_2H_5OH	5	442(100), 414(20), 413(20), 412(40), 398(10), 397(10), 396(10)
	10	442(40), 414(40), 413(40), 412(100), 397(45), 396(85)
	18	442(5), 414(20), 413(10), 412(20), 398(20), 397(50), 396(100)
CH_3OH	10	428(100)
	12	428(100)
CD_3OD	10	431(35), 432(100)
0.001 N HCl in H_2O	8	398(40), 397(100)
	10	414(1), 398(25), 397(100)
	12	398(10), 397(100)

VOBPARICINE-N_4-OXIDE (**20**).—This trace alkaloid possessed a ^{1}H-nmr spectrum that showed a great deal of similarity to the one of vobparicine (**19**). The only major observable differences were the 0.7 ppm downfield shifts of the two H6 protons and the two H21 protons of the apparicine half and the clear separation of the signals for the pair of H19 protons and the pair of H18 methyl groups, which both had almost identical shifts in vobparicine. Based on these changes, it was concluded that the alkaloid might be the mono-N_4-oxide of vobparicine (**19**). This would correspond with the much higher polarity of the alkaloid on tlc in several solvents. The ms was almost identical with the one of vobparicine except for the presence of small peaks at *m/z* 616 (M^+) and *m/z* 630 (M^++14). Due to the easy loss of oxygen, the major peaks, as in the ms of vobparicine itself, were observed at *m/z* 600 (M^+-16) and *m/z* 614 (M^+-16+14).

The loss of oxygen and the intramolecular transmethyleneation, a phenomenon also observed with leurosine-N_4-oxide (11), occur seemingly so easily that even in the fdms no single molecular ion could be observed. Instead, three peaks were observed, two minor ones at *m/z* 617 (M^++H, 25%) and *m/z* 615 (M^+-O+CH_2+H, 25%) and the base peak at *m/z* 601 (M^+-O+H, 100%). To obtain more proof, two chemical correlations with vobparicine were attempted, one of which was successful. Treatment of an ethanolic solution of vobparicine (**19**) with 3% H_2O_2 for 30 min gave, apart from a small portion of unreacted vobparicine, three products. The major product which showed the highest Rf value gave the same Rf values and showed the same chromogenic reactions as the alkaloid isolated. The two other products, which were more polar, were probably the mono-$N_{4'}$-oxide and the di-N_4,$N_{4'}$-oxide. The attempted reduction of the isolated product to vobparicine with an aqueous H_2SO_3 solution was not successful. The starting material was recovered unchanged. Whether or not vobparicine-N_4-oxide (**20**) is an artifact is not clear. The facts suggest that it may be a genuine product because, following column chromatography, **20** was isolated from a different fraction than vobparicine and also fractions containing vobparicine never showed any accompanying spot of the N_4-oxide. However, we do not exclude the possibility that it was formed somewhere earlier in the isolation process.

MONOGAGAINE.—This minor alkaloid, originally coded 6F, was named after the site of collection. It was independently isolated from the stem bark of the Sri Lankian

Tabernaemontana dichotoma (12). The structure elucidation of this complex new dimeric indole alkaloid (M^+ at *m/z* 600) will be the subject of a separate publication, which will appear in due course.

PARTIALLY CHARACTERIZED ALKALOIDS.—*Chippiine* (**47** or **48**): This alkaloid, which was isolated in an amount of 1-2 mg, was rather unstable and decomposed slowly into another, somewhat less polar alkaloid. This decomposition product could be isolated and was identified by means of co-tlc, chromogenic reactions, ms, uv, and cd as 3-hydroxyconopharyngine (**28**). This suggested that the alkaloid chippiine is either an unstable precursor of 3-hydroxyconopharyngine (**28**) or a biosynthetically more evolved alkaloid which is, however, not stable and can revert to its precursor **28**. Its uv spectrum was, as expected, typical for a 10,11-dimethoxyindole. Its ^{1}H-nmr spectrum showed a great deal of resemblance to the one of 3*R*-hydroxyconopharyngine (**28**) with two singlets at 6.98 and 6.95 ppm for the H9 and H12 protons, three singlets at 3.95, 3.93, and 3.73 ppm for the two methoxy- and the carbomethoxy groups and a triplet (J=7 Hz) at 0.92 ppm for the methyl group of the side chain. Major differences were the absence of a signal for the indolic NH and the H21, the presence of a doublet (J=2.1 Hz, 1H) at 5.38 ppm and a multiplet at 0.37 ppm. Also, the integral showed a total number of 27 protons which is two less than the number of observable protons in the ^{1}H-nmr spectrum of **28**. Because of these differences between the ^{1}H-nmr spectra and also by biogenetic speculation (vide infra), structure **47** was proposed for the new alkaloid.

To verify this structure, a detailed high resolution ^{1}H-nmr study of **47** was performed. The results are presented in Table 5. With the aid of a Dreiding model, these data could be compared with that expected for structure **47**. The results completely confirmed the proposed structure and its conformation. Important evidence for the indicated stereochemistry at C20 was the large coupling constant of 13.8 Hz between H15β and H20. The 0.51 ppm deshielding of H20 might be due to the introduction of the N4-C21 double bond. The unusual shift of 0.37 ppm of H15β could be explained

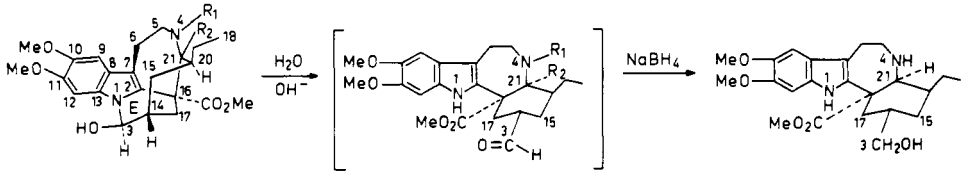
TABLE 5. ^{1}H-nmr Data of Chippiine (**47** or **48**)

Hydrogen Number	δ	J(Hz)	
3	5.38	d	2.1
5α	3.25	m	
5β	2.90	m	
6α	2.70	m	
6β	2.70	m	
9	6.98	s	
12	6.95	s	
14	2.54	ddddd	0.8; 2.1; 5.4; 7.1; 10.6
15α	1.67	ddd	3.2; 10.6; 14.2
15β	0.37	ddd	7.1; 13.8; 14.2
17α	2.34	dd	0.8; 13.5
17β	2.05	dd	5.4; 13.5
18	0.92	t	7.0
19	1.03	dqd	~6.5; 7.0; ~13.5
19′	1.43	dqd	~6.5; 7.0; ~13.5
20	1.84	dddd	3.2; ~6.5; ~6.5; 13.8
10-MeO	3.95	s	
11-MeO	3.93	s	
CO_2Me	3.73	s	
3-OH	not observable		

by its position above the pyrrole ring where it experiences a strong shielding effect. The stereochemistry at C3, which could not be determined unambiguously from the coupling constant between H3 and H14, was determined by means of the chemical shift of H3. In the eburnamines (13,14) and the 16-descarbomethoxytacamines (15) which have the E-ring in an almost identical conformation as **47**, the corresponding protons resonate both at 5.58 ppm, when the hydroxy group possesses a pseudo-equatorial position, and at 6.07 and 6.06 ppm, respectively, when the hydroxy group is in the pseudo-axial position. The observed chemical shift of 5.38 ppm for H3 indicated that the hydroxy group possessed the pseudo-equatorial position.

The absolute configuration of chippiine was proven by determination (cd) of the absolute configuration of its decomposition product, 3-hydroxyconopharyngine (**28**). Standard $NaBH_4$ reduction of **47** gave one somewhat more polar product, the ms of which showed a base and parent peak at *m/z* 416. The most likely structure for this product is **49**. Its formation could be explained by the breaking of the N1-C3 bond with the simultaneous formation of an N1H group and an aldehyde group at C3 in this alkaline medium. Next, both the N4-C21 imine double bond and the aldehyde group at C3 are reduced giving **49** as the end-product (Figure 3). Finally, an ms of chippiine was recorded. Surprisingly, the M^+ was observed not at the expected *m/z* 412 corresponding with $C_{23}H_{28}N_2O_5$ but at *m/z* 414 suggesting a brutoformula of $C_{23}H_{30}N_2O_5$, identical with the one of 3-hydroxyconopharyngine (**28**). Apart from the M^+, the rest of the ms was almost superimposable with the ms of 3-hydroxyconopharyngine (**28**) with large peaks at *m/z* 399, 398 (100%), 397, 396, 337, 313, 274, 190, 136, 124, and 122. As these data are in contradiction with the ^{1}H-nmr spectrum, which indicated a brutoformula of $C_{23}H_{28}N_2O_5$ (27 visible protons and one non-observable OH), it was thought that the unstable alkaloid decomposed in the mass-spectrometer to 3-hydroxyconopharyngine, although this would require the breaking of the N1-C3 bond, reduction of the N4-C21 double bond, and formation of the C3-N4 bond in the ion source. To test this hypothesis, a series of fdms were recorded starting with an emitter current of 0 mA and gradually increasing the current until the whole sample was desorbed. At all currents, almost exclusively a peak at *m/z* 414 was observed. Only very low intensity peaks at *m/z* 412, 398, 397, and 396, which are typical for the fdms of **28**, were observed suggesting that the real M^+ for chippiine was indeed at *m/z* 414 and was not caused by its decomposition to **28**.

This M^+ could only be explained by structure **48**, i.e., the N4-C21 dihydro derivative of the earlier proposed structure for chippiine. As mentioned before, this is, however, in strong disagreement with the ^{1}H-nmr spectrum. Although the absence of a signal for the N4H is unusual, the absolute certain absence of any possible signal for the H21, which according to the Dreiding model should appear as a small doublet, is unexplainable. As a last experiment to distinguish between the two possible structures, the reduction was repeated with $NaBD_4$ in D_2O. After the usual workup procedure with non-deuterated solvents, one would expect an M^+ at *m/z* 417 if **48** were the correct structure (1 H replaced by D at C3) and an M^+ at *m/z* 418 if **47** were the correct



47 R_1+R_2=bond
48 $R_1=R_2=H$

49

FIGURE 3

structure (2 H replaced by D at C3 and C21, respectively). The ms showed clearly the M^+ at *m/z* 417 with all the higher fragments shifted one mass unit as well. This strongly supported structure **48**, which seemed also biogenetically more probable. It also explained better the easy decomposition of chippiine to **28**. Why no signal in the ^1H nmr for the H21 could be observed remains a mystery. The best way to solve the problem of which of the two structures is correct, is probably to synthesize one or both alkaloids and to record their mass- and ^1H-nmr spectra or preferably their ^{13}C-nmr spectra. The biosynthetic precursor of chippiine is almost certainly 3-hydroxyconopharyngine (**28**). After the breaking of the N4-C3 bond, an intermediate would be formed which could lead to **48**. For **47** the additional oxidation of the N4H-C21H function to the corresponding imine is necessary.

Artifact formation: To minimize the possibility that chippiine is an artifact formed from **28** during the isolation procedure, the three most critical steps in the isolation procedure (refluxing with EtOH under 0.2 atm, partition between 2% HOAc and EtOAc, and partition between H_2O/NH_3 and $CHCl_3$/iPrOH) were repeated with some 3*R*-hydroxyconopharyngine (**28**). As no chippiine could be detected on tlc after these operations, the possibility that chippiine is an artifact is greatly reduced. Even preliminary attempts to convert **28** to chippiine under more drastic circumstances were not successful.

We propose the name chippiine for this alkaloid whether structure **47** or **48** is correct.

TC-A (**23**), TC-B (**25**), AND TC-C (**24**).—These three trace alkaloids all showed a similar chromogenic response and possessed similar Rf values and uv spectra suggesting that they were closely related. This was confirmed by their ms. The ms of TC-A and TC-C were identical indicating that they were isomers. TC-B had its M^+ 16 mu higher but otherwise the ms was similar to the other two. The ms of TC-A and TC-C (M^+ at *m/z* 314) were identical to the one published for synthetically prepared 14*S*,15*S* voaphyllinediol (**24**) (16) isolated earlier from *Voacanga africana* leaves (17,18). Probably TC-A and TC-C are 14*R*,15*S* voaphyllinediol (**23**) and 14*S*,15*S*-voaphyllinediol (**24**), although it could not be determined which is which. The ^1H-nmr spectrum of TC-C confirmed the basic structure of a voaphyllinediol, but the interpretation was not straightforward and did not allow a differentiation between the two isomers. TC-B (M^+ at *m/z* 330) is probably a hydroxy substituted voaphyllinediol (**25**), the most likely position for such a substitution being C19 (the most frequently encountered position for hydroxylation in the plumeran class). The fragments in the ms at *m/z* 313 (M^+-17) and *m/z* 295 (M^+-17-18) support this suggestion.

TC-D (**9**).—The 300 MHz ^1H-nmr spectrum of this minor alkaloid was similar to the one of vobasinol (**8**). The only major differences were the 0.65 ppm deshielding of the NH, the 0.75 ppm shielding of the H3, and the presence of approximately six extra protons in the 3.0-2.5 ppm region. This suggested the presence of a small extra fragment at C3 of the vobasinyl part. As the number of couplings of H3 was still the same as in vobasinol (**8**) (J= 13.1 and 3.3 Hz) the first atom of the additional group attached to C3 must be a hetero atom, most likely an oxygen. The uv spectrum was identical to the one of vobasinol. The ms showed an M^+ at *m/z* 413. The rest of the spectrum was characteristic for a vobasinyl-part with major fragments at *m/z* 338, 337 (base peak), 336, 277, 180, and 122. The peaks at *m/z* 413 and *m/z* 337 were peakmatched and corresponded to an elemental composition of $C_{23}H_{31}N_3O_4$ and $C_{21}H_{25}N_2O_2$, respectively. Thus, the side chain must have the following composition: $C_2H_6NO_2$, which means that it is fully saturated. In interpreting the ^1H-nmr spectrum at 500 MHz, the spectrum was essentially first order, and it was possible to assign all the protons and to determine all the coupling constants. Apart from H21b, H14a, H16, and the NMe of the

vobasinyl-part, the six additional protons of the side chain could be observed in the 3.0-2.5 ppm region. Four were present as three double doublets with couplings of approximately 12.5, 7, and 6 Hz, and two were spread out from 2.95-2.65 ppm as an extremely broad singlet. The latter signal disappeared after addition of D_2O. These data could only be explained by the presence of an R_1-CH_2-CH_2-R_2 group and two exchangeable protons attached to either an oxygen or a nitrogen. The -CH_2CH_2-group must have a restricted rotation since all four protons have slightly different chemical shifts (2.92, 2.83, 2.72, and 2.53 ppm). As the number of possible structures, which fitted the above spectral data was still quite large, a ^{13}C nmr was recorded in the hope that it would be possible to differentiate between the various isomers (for instance -OCH_2CH_2NHOH). This was, however, not the case. Although all the signals for the vobasinyl-part with the exception of C3 were present at their expected positions (19,20), the shifts of the three signals (42.1, 41.7, and 32.9 ppm), which should belong to C3 and the R_1-CH_2CH_2-R_2 group of the side chain, were 20-30 ppm more upfield than expected for any of the likely structures, i.e., C3 in vobasinol itself is at 66.8 ppm and CH_2 in $HON(C_2H_5)_2$ at 54.1 ppm (21).

The ms data, which indicates a fully saturated side chain with two oxygens and one nitrogen, and the ^{13}C-nmr data, which suggests an oxygen free side chain, are contradictory, making it impossible to propose a reasonable structure. The question whether or not TC-D (**9**) is an artifact may possibly be answered when the correct structure is deduced. The addition of such a small molecular weight fragment to a *Tabernaemontana* alkaloid is not very common and is observed only in 3-(2′-oxopropyl) coronaridine and similar artifacts.

TACRALINE (**14**) AND DESACETYLTACRALINE (**15**).—These alkaloids showed uv spectra (maxima at 206, 236, and 294 nm) and chromogenic reactions (purple-orange upon prolonged heating) typical of dihydroindoles substituted at the 2-position by a nitrogen or an oxygen such as picraline (**12**, dichomine, and corymine (1,22,23). Tacraline showed its M^+ at *m/z* 409 and desacetyltacraline at *m/z* 367. The odd molecular weight suggested the introduction of an additional nitrogen atom in both alkaloids while the difference of 42 mass-units between the two alkaloids was indicative of the presence of an acetyl group in tacraline when compared with desacetyltacraline. This would also fit the relatively higher polarity of desacetyltacraline on tlc (free CH_2OH group versus CH_2OAc group). The eims-fragmentation pattern (Table 6) of both alkaloids was similar; both showed intense fragments at M^+-42 and M^+-43. These ions then, in turn, lost the CH_2OH (desacetyltacraline) or CH_2OAc (tacraline) group. In the *m/z* 100-200 region, both alkaloids showed many common fragments. This part of the ms also showed some similarity with the corresponding part in the ms of picraline (**12**). Due to the small amount isolated, the ^1H-nmr spectra were not of high quality, but nevertheless allowed some conclusions to be drawn. The indole NH and the four aromatic protons had shifts typical for a dihydroindole substituted at the 2-position by nitrogen or oxygen, confirming the earlier conclusions from tlc and uv. Furthermore, the presence of a carbomethoxy group and an ethylidene side chain in both alkaloids was evident, while in tacraline an acetyl group could be distinguished as well. All these data suggested a strong similarity between tacraline/desacetyltacraline (M^+ 409/367) and picraline (**12**)/desacetylpicraline (**13**) (M^+ 410/368), respectively. The only reasonable explanation for the loss of one mass unit from the new alkaloids in comparison with the two picraline alkaloids is the replacement of the oxygen bridge between C2 and C5 by an NH bridge. This would explain the chromogenic reactions, the uv spectra, and the picraline-like ^1H-nmr spectra. The strong fragments at M^+-42 and M^+-43 in the ms, which are not observed in the ms of the picraline-type alkaloids (24), must be caused by the replacement of the oxygen bridge by the nitrogen bridge, giving rise to a different

TABLE 6. Ms Data of Tacraline (**14**) and Desacetyltacraline (**15**)

tacraline			desacetyltacraline		
mass	M^+	Relative intensity	mass	M^+	Relative intensity
409	M^+	18	367	M^+	22
—	—	—	336	31	9
367	42	36	325	42	35
366	43	43	324	43	40
350	59	5	308	59	4
336	73	2	—	—	—
307	−43-59	10	—	—	—
294	−42-73	22	294	−42-31	29
293	−43-73	100	293	−43-31	100
261		9	261		18
247		12	247		12
243		8	243		9
194		13	194		16
158		23	158		48
157		16	157		31
130		8	130		21
108		32	108		63

fragmentation pattern. Most likely, the M^+-42 involves the loss of C6, C5, the additional third nitrogen atom, and the attached protons (M^+-C_2H_4N). The even stronger M^+-43 peak might be caused by the loss of the same atoms and an additional proton (M^+-C_2H_5N) picked up from one of the other carbons or the indole NH.

Although, due to lack of substance, no detailed ^{1}H-nmr or ^{13}C-nmr data is available to prove the correctness of these structures, at the moment the structures **14** and **15** explain the available spectral data satisfactorily. Whether these trace alkaloids are genuine alkaloids or artifacts formed, for instance, by reaction of akuammiline (**10**) and desacetylakuammiline (**11**), which were also isolated from the species, and NH_3, which was used during the isolation and separation procedure, remains to be determined. The fact that these alkaloids were detected in only one fraction, which did not contain akuammiline or desacetylakuammiline, indicates that in any case they were not formed during column chromatography. If they are artifacts, they must have been formed during the partition between $CHCl_3$/iPrOH and the basified aqueous layer.

Apart from the above mentioned, almost completely identified, alkaloids four alkaloids were isolated, the structures of which remain for the greater part uncharacterized (TC-E, TC-F, TC-G, and TC-H). The spectral data that were recorded (see Table 2) are available on request.

PHYTOCHEMISTRY AND CHEMOTAXONOMY.—The most abundantly occurring alkaloids in *T. chippii* belong either to the ibogan class (coronaridine-type only) or to the dimeric voacamine type. Within these groups, there is a strong tendency for the introduction of a great number of methoxy groups in the aromatic ring and the hydroxylation of the 3-position of the iboga part. Although 3-hydroxy-substituted iboga alkaloids have been encountered before in *Tabernaemontana* species [sometimes as their 2′-oxopropyl- or ethoxy-derivatives (25,26)], they have never before been isolated in such high concentrations as in this species. In this species, methoxy substitution at the 11-position seems to be much favored over substitution at the 10-position. Only one trace alkaloid (3*R*/*S*-hydroxyvoacamine, **44**) has a 10-methoxy group without having an 11-methoxy group at the same time. Alkaloids of these two groups lacking the carbo-

methoxy group at the 16-position of the iboga half also occur only as trace components (ibogaline, **32** and 3*R*/*S*-hydroxy-16-decarbomethoxyconodurine, **40**). Other alkaloids not belonging to the aforementioned groups and occurring in high concentrations include the common alkaloids tubotaiwine (**21**) and apparicine (**18**) (aspidospermatan class) and the less common isositsirikine (**1**) (corynanthean class), the biosynthetically least evolved alkaloid isolated from *T. chippii*. The species is further capable of synthesizing a whole range of other corynanthean class alkaloids. Two of these have not been identified before in the genus *Tabernaemontana* (vobasinol, **8** and picraline, **12**). 12-Hydroxyakuammicine (**17**) (syn. vinervine) belonging to the strychnan class is also new to the genus, although this alkaloid was recently isolated from a tissue culture of *Tabernaemontana divaricata* (27). The isolation of the minor alkaloid vobparicine (**19**) and its N_4-oxide **20** and the partially identified trace alkaloids TC-D, TC-F, TC-G, and TC-H indicate that vobasinol can combine with many other indole alkaloids apart from coronaridine type alkaloids to form dimeric indole alkaloids. The alkaloid chippiine (**47** or **48**), which is almost certainly derived from the biogenetically advanced iboga alkaloids, is the first example of a type of indole alkaloids possessing a N1-C3 bond.

Summarizing, one can conclude that the species investigated in this study is a typical example (28) of the genus *Tabernaemontana* with a high content of corynanthean class, ibogan class, and dimeric voacamine type alkaloids as well as the aspidospermatan alkaloids apparicine and tubotaiwine. In many respects, it is similar to the recently restudied Nigerian *Tabernaemontana pachysiphon* (7) although the *T. chippii* species investigated here is much richer in 3-hydroxy-substituted iboga alkaloids and is also capable of synthesizing a broader, more varied range of corynanthean class alkaloids.

ANTIMICROBIAL ACTIVITY.—Using the biogram technique with *B. subtilis* and *E. coli* as test organisms, the antibacterially active alkaloids could be identified. The most abundant compounds which also showed strong activity were dimeric alkaloids of the voacamine type [conodurine (**37**), 3-hydroxyconodurine (**39**), conoduramine (**35**), and 3-hydroxyconoduramine (**36**)]. The minimum inhibiting concentration (MIC value) of these alkaloids was determined against *B. subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *E. coli* (ATCC 8739), and *Pseudomonas aeruginosa* (ATCC 9027) by means of the agar diffusion test (Table 7). The MIC value against the gram-positive bacteria *B. subtilis* and *S. aureus* was approximately 10 and 50 μg/ml. The MIC value against the gram-negative bacteria *E. coli* and *P. aeruginosa* varied for the individual alkaloids from 70 to approximately 750 μg/ml. The dimeric alkaloid vobparicine (**19**) which does not belong to the voacamine type showed MIC values of 50 and 100 μg/ml against *B. subtilis* and *E. coli*, respectively. The dimeric alkaloids 3-hydroxyvoacamine (**44**), vobparicine-N_4-oxide (**20**), TC-F, TC-G, and TC-H were also active against *B. subtilis* and *E. coli* according to the biogram technique, but too little was available for the determination of their MIC values. 3-Hydroxyconopharyngine (**28**) and 3-hydroxyisovoacangine (**34**) were the only monomeric alkaloids to show activity in the biogram assay. They were approximately five times less active than the dimeric alkaloids in the agar diffusion test (Table 7).

ANTIYEAST AND ANTIFUNGAL ACTIVITY.—In the antimicrobial screening mentioned earlier, the ethanolic root bark extract of *T. chippii* showed weak antiyeast and antifungal activity (2). Of the initial four fractions obtained in the separation process of *T. chippii* (acidic EtOAc-, basic $CHCl_3$-, basic aqueous- and insoluble fraction), only the basic $CHCl_3$ fraction showed activity, indicating that probably tertiary alkaloids were responsible for the antiyeast/antifungal activity. Contrary to the antimicrobial testing above, here the agar diffusion test was used as a bioassay. The biogram technique could not be used because of its relatively low sensitivity. However, when the dif-

TABLE 7. Minimum Inhibitory Concentration (MIC) as Determined for Some Alkaloids Isolated from *Tabernaemontana chippii* and Streptomycin

Compound	solvent	MIC (μg/ml) against:			
		Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
conoduramine	buffer pH=4	15	35	110	~400
3-hydroxy-conoduramine	buffer pH=4	8	65	170	65
conodurine	buffer pH=4	4	50	70	~400
3-hydroxy-conodurine	buffer pH=4	14	45	~750	~150
3-hydroxycono-pharyngine	buffer pH=4	60	140	>1000	>1000
3-hydroxyiso-voacangine	buffer pH=4	50	~500	~500	~300
vobparicine	buffer pH=4	50	—	100	—
streptomycin	buffer pH=4	1	5	80	7
streptomycin	distilled H_2O	0.2	1	0.5	12

ferent fractions obtained after column chromatography were assayed for their antifungal activity, none of them showed any activity. The reason for this apparent loss of activity could be a possible decomposition of the active compounds or a synergistic effect of two or more compounds which were later separated from each other.

EXPERIMENTAL

PLANT MATERIAL.—The root bark of *T. chippii* was collected in November 1981, by Dr. A.J.M. Leeuwenberg in Ivory Coast near Monogaga. Voucher specimens (Lg 12291) are kept in the herbarium of the Laboratory for Plant Systematics, Wageningen, The Netherlands.

APPARATUS.—Uv and cd spectra were recorded in MeOH. Ir spectra were recorded in KBr. Optical rotations were recorded in $CHCl_3$. All ^{1}H-nmr spectra were recorded on a Bruker WM300 at 300 MHz, except for the spectra of vobparicine and TC-D, which were recorded on a Bruker WM500 at 500 MHz. All ^{13}C-nmr spectra were recorded on a JEOL PS-100 at 25.2 MHz, except for the spectra of vobparicine and TC-D, which were recorded on a Bruker WM300 at 75.2 MHz. All nmr spectra were recorded in $CDCl_3$ unless otherwise stated. Eims (70 eV) were recorded on an AEI MS 20, AEI MS 902, a LKB 2091, a Varian MAT 711, or a JEOL D300 spectrometer. Fdms were obtained with a Varian MAT 711 double focusing mass spectrometer with a combined EI/FI/FD source. 10 μm tungsten wire fd emitters containing carbon microneedles with an average length of 30 μm were used. The samples were dissolved in a particular solvent and then loaded onto the emitters with the dipping technique. An emitter current of 0-25 mA was used to desorb the samples. Fabms were recorded on a VG Analytical ZAB-HF mass spectrometer.

THIN LAYER CHROMATOGRAPHY.—For the separation of the alkaloids, the following tlc solvent systems were used: S1, toluene-100% EtOH saturated with NH_3 (19:1)[2]; S2, toluene-100% EtOH saturated with NH_3 (9:1)[3]; S3, $(iPr)_2O$-100% EtOH saturated with NH_3 (9:1)[2]; S4, cyclohexane-$CHCl_3$-Et_2NH (6:3:1); S5, $CHCl_3$-cyclohexane-Et_2NH (10:8:3); S6, *n*-hexane-MeCOEt-Et_2NH (8:1:1); S7, ClH_2CCH_2Cl-EtOAc-PrOH (6:3:1); S8, $CHCl_3$-MeOH (9:1); S9, $CHCl_3$-MeOH-NH_4OH (263:35:2); S10, $CHCl_3$-MeOH-NH_4OH (45:14:1); S11, EtOAc-iPrOH-NH_4OH (17:2:1). After development, the 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.

PREPARATION OF ALKALOID FRACTIONS.—A sample of 263 g ground root bark was extracted for 15 h with 96% EtOH in a Soxhlet apparatus working under a pressure of 0.2 atm. After filtration, the EtOH was evaporated in vacuo to dryness. The extracts were partitioned between EtOAc and 2% HOAc. The aqueous layer was collected and brought to pH~10 with NH_4OH and extracted with $CHCl_3$-iPrOH (9:1). The $CHCl_3$-iPrOH layer was collected, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The

[3]Prior to development, the plates were left in an atmosphere of NH_3 for 20 min.

yield was 2.25 g (0.85%) of crude tertiary alkaloids. The extract was separated on a Si gel column using toluene-100% EtOH saturated with NH_3 as the mobile phase. The percentage EtOH saturated with NH_3 was increased from 5% (begin) to 25% (end) during the separation. The collected fractions were further separated and purified by means of preparative tlc (0.25 or 0.50 mm) with solvent systems S1-S11. The alkaloids were identified by means of their spectral data, color reactions, co-tlc and chemical correlations (Table 1).

CHARACTERIZATION OF ALKALOIDS.—*Isositsirikine* (**1**): Rf-values and chromogenic reactions see (1); uv, ms, and ^{1}H-nmr data see (29).

16-Epi-isositsirikine (**2**): Rf-values and chromogenic reactions see (1). This trace alkaloid was not actually isolated but only detected in a fraction by means of co-tlc with two different solvents. This was possible because of a sensitive and selective detection method (strong blue fluorescence under 254 nm after spraying with $CeSO_4/H_2SO_4$ (1).

Normacusine-B (**3**): Rf-values and chromogenic reactions see (1); uv and ms data see (7); ^{1}H-nmr δ 8.03 (bs, NH), 7.45 (dd, J=7.1 and 1.2 Hz, H12), 7.30 (dd, J=7.1 and 1.2 Hz, H9), 7.13 (ddd, J=7.1, 7.1 and 1.2 Hz, H11), 7.08 (ddd, 7.1, 7.1 and 1.2 Hz, H10), 5.33 (bq, J=6.8 Hz, H19), 4.08 (dd, J=10.0 and 2.3 Hz, H3), 3.54 (dd, J ca. 10 and 6 Hz, H17a), 3.52-3.45 (m, H17b, H21a and H21b), 3.03 (ddd, J=15.4, 5.2 and ~1 Hz, H6a), 2.77 (bs, H15), 2.76 (bdd, J ca. 7.3 and 5.2 Hz, H5), 2.62 (dd, J=15.4 and 0.7 Hz, H6b), 1.97 (ddd, J=12.4, 10.0 and 2.0 Hz, H14a), 1.80 (dddd, J ca. 8, 7.5, 6 and 1.5 Hz, H16), 1.68 (ddd, J=12.4, 4.1 and 2.3 Hz, H14b), 1.61 (ddd, J=6.8, 1.9 and 1.8 Hz, H18).

Pericyclivine (**4**): Rf-values and chromogenic reactions see (1); uv and ms data see (7); ^{1}H nmr δ 7.81 (bs, NH), 7.42 (bd, J=7.5 Hz, H12), 7.29 (bd, J=7.5 Hz, H9), 7.11 (ddd, J=7.5, 7.5 and 1.3 Hz, H11), 7.04 (ddd, J=7.5, 7.5 and 1.3 Hz, H10), 5.27 (bq, J=6.9 Hz, H19), 4.25 (bd, J=10.0 Hz, H3), 3.70 (ddd, J=11.2, 4.8 and 1.8 Hz, H5), 3.64 (m, H21a and H21b), 3.25 (dd, J=15.8 and 1.8 Hz, H6a), 3.06 (s, CO_2Me), 2.97 (bs, H15), 2.92 (bdd, J=15.8 and 4.8 Hz, H6b), 2.83 (bdd, J=11.2 and 2.8 Hz, H16), 2.59 (ddd, J=12.9, 4.2 and 2.0 Hz, H14a), 1.78 (bdd, J=12.9 and 10.0 Hz, H14b), 1.62 (ddd, J=6.9, 1.7 and 1.7 Hz, H18).

16-Epi-affinine (**5**): Rf-values and chromogenic reactions see (1); uv, ms, and ^{1}H-nmr data see (7).

Anhydrovobasindiol (Taberpsychine) (**7**): Rf-values and chromogenic reactions see (1); uv and ms data see (7); ^{1}H nmr δ 7.95 (bs, NH), 7.63 (bd, J=7.7 Hz, H9 or H12), 7.33 (bd, J= .7 Hz, H9 or H12), 7.21 (ddd, J=7.7, 7.7 and 1.3 Hz, H10 or H11), 7.14 (ddd, J=7.7, 7.7 and 1.3 Hz, H10 or H11), 5.40 (bq, J=6.9 Hz, H19), 5.16 (dd, J=10.0 and 1.0 Hz, H3), 3.88 (dd, J=11.6 and 10.1 Hz, 1H), 3.62 (bd, J ca. 14.8 Hz, 1H), 3.34-3.14 (m, 5H), 2.98 (d, J=14.0 Hz, 1H), 2.58 (s, NMe), 2.58-2.47 (m, 2H), 2.02 (ddd, J=14.0, 10.3 and 1.0 Hz, H14b), 1.69 (dd, J=6.9 and 2.1 Hz, H18).

Vobasine (**6**): Rf-values and chromogenic reactions see (1); uv and ms data see (7); ^{1}H nmr δ 8.91 (bs, NH), 7.73 (dd, J=8.3 and 0.9 Hz, H9), 7.39-7.33 (m, H11 and H12), 7.17 (ddd, J=8.3, 5.0 and 3.1 Hz, H10), 5.50 (dq, J=7.0 and 1.0 Hz, H19), 4.03 (ddd, J=10.2, 8.6 and 3.5 Hz, H15), 3.89 (bs, J=14.6 Hz, H21a), 3.78 (ddd, J=11.6, 7.6 and 3.3 Hz, H5), 3.55 (dd, J=15.2 and 10.2 Hz, H14a), 3.45 (dd, J=15.2 and 8.6 Hz, H14b), 3.32 (dd, J=13.5 and 11.6 Hz, H6a), 3.04 (bd, J=14.6 Hz, H21b), 2.85 (dd, J=3.5 and 3.3 Hz, H16), 2.74 (dd, J=13.5 and 7.6 Hz, H6b), 2.654 (s, NMe or CO_2Me), 2.646 (s, NMe or CO_2Me), 1.72 (dd, J=7.0 and 1.9 Hz, H18).

Vobasinol (**8**): Rf-values and chromogenic reactions see (1); uv λ max 226, 280(sh), 285, and 292(sh) nm; ms (150°) *m/z* (rel. int.) 354 (M^+, 17), 338(9), 337(10), 336(38), 321(15), 277(25), 194(20), 182(73), 181(28), 180(97), 167(19), 166(18), 156(16), 130(28), 122(100), 120(63); ^{1}H nmr δ 8.21 (NH), 7.53 (d, J=7.8 Hz, H9), 7.26 (d, J=7.8 Hz, H12), 7.18 (dd, J=7.8 and 7.8 Hz, H10), 7.10 (dd, J=7.8 and 7.8 Hz, H11), 5.49 (q, J=6.8 Hz, H19), 5.19 (dd, J=12.1 and 4.2 Hz, H3), 4.09 (m, ΣJ=22 Hz, H5), 3.85 (bd, J=14 Hz, H21a), 3.67 (m, ΣJ=22 Hz, H15), 3.38 (dd, J=14.6 and 10.8 Hz, H6a), 3.27 (dd, J=14.6 and 8.4 Hz, H6b), 3.15 (bd, J=14 Hz, H21b), 2.79 (bs, H16), 2.67 (s, NMe), 2.59 (ddd, J=14.2, 12.7 and 12.1 Hz, H14a), 2.45 (s, CO_2Me), 2.16 (ddd, J=14.2, 6.9 and 4.2 Hz, H14b), 1.72 (dd, J=6.8 and 1.4 Hz, H18).

Akuammiline (**10**): Rf-values and chromogenic reactions see (1); uv λ max 215, 220(sh), 264, and 286 nm (sh); ms (100°) *m/z* (rel. int.) 394 (M^+, 98), 393(23), 366(9), 363(7), 336(11), 335(40), 322(28), 321(100), 306(8), 292(11), 275(16), 262(12), 261(11), 249(17), 232(17), 194(10), 168(15), 121(17); ^{1}H nmr δ 7.65 (d, J=7.4 Hz, H9 or H12), 7.59 (d, J=7.6 Hz, H9 or H12), 7.30 (t, J=7.4 and 7.6 Hz, H10 or H11), 7.16 (t, J=7.4 and 7.6 Hz, H10 or H11), 5.49 (q, J=7.1 Hz, H19), 4.63 (d, J=4.8 Hz, H3), 4.11 (bd, J=17.0 Hz, H21a), 3.76 (s, CO_2Me), 3.65-3.48 (m, H5a and H15), 3.61 (d, J ca. 11Hz, H17a), 3.51 (d, J ca. 11 Hz, H17b), 3.15 (d, J=17.0 Hz, H21b), 2.67 (m, H6a), 2.59 (ddd, J ca. 14, 14 and 4.0 Hz, H6b), 2.48 (ddd, J=14.7, 4.8 and 2.3 Hz, H14a), 2.13 (bdd, J=15.1 and 4.0 Hz, H5b), 1.91 (bdd, J=14.7 and 2.3 Hz, H14b), 1.65 (dd, J=7.1 and 2.3 Hz, H18), 1.58 (s, O_2CMe).

Desacetylakuammiline (**11**): Rf values and chromogenic reactions see (1); uv λ max 219(sh), 265, and 285 nm(sh); ms (125°) *m/z* (rel. int.) 352 (M^+, 76), 351(17), 322(28), 321(100), 293(21), 289(11), 262(14), 261(14), 249(17), 232(20), 194(11), 180(17), 168(20), 167(25), 121(15); ^{1}H nmr δ 7.65 (bd, J=7.8 Hz, H9 or H12), 7.54 (dd, J=7.8 and 1.2 Hz, H9 or H12), 7.35 (ddd, J=7.8, 7.8 and 1.2 Hz, H10 or H11), 7.18 (ddd, J=7.8, 7.8 and 1.2 Hz, H10 or H11), 5.47 (bq, J=7.0 Hz, H19), 4.56 (d, J=5.0 Hz, H3), 4.11 (bd, J=17.3 Hz, H21a), 3.85 (s, CO_2Me), 3.77-3.63 (m, H5a, H15 and OH), 3.14 (d, J=17.3, H21b), 2.98 (bd, J ca. 13 Hz, H17a), 2.89 (bdd, J ca. 13 and 9 Hz, H17b), 2.67 (m, H6a), 2.60 (ddd, J ca. 14.2, 14.2 and 4.0 Hz, H6b), 2.45 (ddd, J=15.2, 5.0 and 2.8 Hz, H14a), 2.06 (ddd, J=15.2, 4.0 and 1.5 Hz, H5b), 1.92 (bdd, J=15.2 and 2.7 Hz, H14b), 1.64 (dd, J=7.0 and 2.5 Hz, H18).

Picraline (**12**): Rf values and chromogenic reactions see (1); uv λ max 231 and 285 nm; ms *m/z* (rel. int.) 410 (M^+, 32), 392(14), 351(25), 338(22), 337(93), 333(51), 247(32), 240(29), 239(100), 194(27), 182(25), 181(23), 180(20), 168(30), 157(67), 156(31), 130(26), 108(28), 106(29); ^{1}H nmr δ 7.42 (bd, J=7.5 Hz, H12), 7.05 (ddd, J=7.5, 7.5 and 1.1 Hz, H10), 6.82 (ddd, J=7.5, 7.5 and 0.6 Hz, H11), 6.73 (bd, J=7.5 Hz, H9), 5.39 (bq, J=7.1 Hz, H19), 4.82 (bs, NH), 4.77 (d, J=2.6 Hz, H5), 4.56 (d, J=11.0 Hz, H17a), 3.88 (d, J=11.0 Hz, H17b), 3.81 (dq, J=18.0 and 1.9 Hz, H21a), 3.69 (s, CO_2Me), 3.63 (bs, H15), 3.30 (bs, H3), 3.22 (d, J=14.0 Hz, H6a), 3.13 (bd, J=18.0 Hz, H21b), 2.45 (dd, J=14.0 and 2.6 Hz, H6b), 2.03 (ddd, J ca. 13, 3 and 3 Hz, H14a), 1.98 (ddd, J ca. 13, 3 and 3 Hz, H14b), 1.59 (dd, J=7.1 and 2.0 Hz, H18), 1.52 (s, O_2CMe).

Pleiocarpamine (**16**): Rf-values and chromogenic reactions see (1); uv λ max 231, 284, and 294 nm (sh); ms (100°) *m/z* (rel. int.) 322 (M^+, 100), 307(9), 293(6), 278(4), 276(4), 264(22), 263(100), 249(8), 234(28), 232(27), 218(15), 194(11), 180(56), 168(13), 167(13), 155(15), 131(19), 120(18), 108(19); ^{1}H nmr δ 7.56 (m, H9), 7.12 (m, H10 and H11), 6.98 (m, H12), 5.36 (dq, J=6.9 and 1.4 Hz, H19), 5.24 (d, J=4.1 Hz, H16), 3.98 (bs, H3), 3.59 (s, CO_2Me), 3.55 (m, 1H), 3.48 (ddd, J=13.2, 9.8 and 2.2 Hz, 1H), 3.19 (ddd, J=15.6, 8.5 and 2.4 Hz, 1H), ~2.77 (m, 1H), 2.72 (d, J=12.8 Hz, H21a), 2.53 (ddd, J=14, 3.5 and 2.5 Hz, 1H), 2.47-2.37 (m, 2H), 1.83 (bd, J=13 Hz, H21b), 1.51 (dd, J=6.9 and 2.1 Hz, H18).

12-Hydroxyakuammicine (**17**): Rf-values and chromogenic reactions see (1); uv λ max 200, 219(sh), 285(sh), 290, and 333 nm, upon basification with NaOH the max at 333 nm shifted to 355 nm; ms (250°) *m/z* (rel. int.) 338 (M^+, 75), 263(18), 122(32), 121(100), 107(28), 106(22), 93(18), 92(22); ^{1}H nmr δ 9.05 (bs, NH), 6.83 (dd, J=6.5 and 1.8 Hz, H11), 6.75-6.67 (m, H9 and H10), 5.52 (q, J=6.8 Hz, H19), 4.33 (bs, W½=7 Hz, H3 or H15), 4.12 (bd, J=15.0 Hz, H21a), 4.02 (bs, W½=8 Hz, H3 or H15), 3.81 (s, CO_2Me), 3.60 (m, H5a), 3.17 (d, J=15.0 Hz, H21b), 3.13 (m, H5b or H6a), 2.58 (m, H5b or H6a), 2.45 (bd, J=14 Hz, H14a), 1.97 (dd, J=13.0 and 6.0 Hz, H6b), 1.64 (d, J=6.8 Hz, H18), 1.31 (bd, J=14 Hz, H14b); $[\alpha]^{20}D$=−655° (c=0.057).

Apparicine (**18**): Rf values and chromogenic reactions see (1); uv and ms data see (7).

Tubotaiwine (**21**): Rf values and chromogenic reactions see (1); uv, ms, and ^{1}H-nmr data see (7).

Voaphylline (**22**): Rf-values and chromogenic reactions see (1); uv and ms data see (15).

Conopharyngine (**26**): Rf-values and chromogenic reactions see (1); uv and ms data see (7); ir (cm^{-1}) 3375, 2960, 2937, 2865, 1731, 1634, 1559, 1491, 1470, 1372, 1330; ^{1}H nmr δ 7.75 (bs, NH), 6.90 (s, H9 or H12), 6.78 (s, H9 or H12), 3.91 (s, OMe), 3.86 (s, OMe), 3.71 (s, CO_2Me), 3.55 (bs, H21), 3.40 (m, ΣJ ca. 28 Hz, H5a), 3.25-3.10 (m, H6a and H6b), 2.96 (m, ΣJ ca. 28 Hz, H5b), 2.92 (bd, J=8.5 Hz, H3a), 2.82 (bd, J=8.5 Hz, H3b), 2.55 (ddd, J=14.5, 2.9 and 2.9 Hz, H17a), 1.90 (ddd, J=14.5, 3.2 and 2.8 Hz, H17b), 1.87 (m, H14), 1.87 (bdd, J ca. 11 and 11 Hz, H15a), 1.56 (ddq, J ca. 13.5, 7.5 and 6.5 Hz, H19a), 1.47 (ddq, J ca. 13.5, 7.5 and 6.5 Hz, H19b), 1.32 (m, ΣJ ca. 31 Hz, H20), 1.14 (m, ΣJ ca. 22 Hz, H15b), 0.90 (t, J=7.5 Hz, H18); cd λ (Δε) 204(0.0), 210(+7.6), 226(+1.1), 243 (+6.8), 275(0.0), 281(−0.7), 290(−0.6), 305(−1.6), 318 (0.0).

3 R/S-hydroxyconopharyngine (**28**) (mixture 3*R*:3*S*=9:1): Rf-values and chromogenic reactions see (1); uv and eims[4] data see (7); ir (cm^{-1}) 3380, 2960, 2944, 2880, 1734, 1638, 1551, 1492, 1465, 1378, 1334; fdms see Table 4; ^{1}H nmr δ 7.79 (bs, NH), 6.93 (s, $H9_R$ or $H12_R$), 6.92 (s, $H9_S$ or $H12_S$), 6.80 (s, $H9_R$ or $H12_R$), 6.79 (s, $H9_S$ or $H12_S$), 4.43 (bs, $H3_S$), 4.12 (d, J=2.0 Hz, $H3_R$), 3.92 (s, OMe_R), 3.88 (s, OMe_R), 3.78 (bs, $H21_R$), 3.69 (s, CO_2Me_R), 3.52 (ddd, J=13.5, 5.6 and 5.6 Hz, $H5a_R$), 3.20 (m, $H5b_R$ or $H6a_R$), 3.03 (m, $H5b_R$ or $H6a_R$ and $H6b_R$), 2.73 (dd, J=14.0 and 2.3 Hz, $H17a_R$), 2.69 (dd, J=14 and 2 Hz, $H17a_S$), 2.28 (bs, J=14 Hz, $H17b_S$), 1.99 (bs, W½=9 Hz, $H14_R$), 1.88 (ddd, J=14.0, 3.3 and 1.7 Hz, $H17b_R$), 1.53 (m, $H19a_R$), 1.60-1.43 (m, $H15a_R$, $H15b_R$, and $H19b_R$), 1.33 (m, $H20_R$), 0.92 (t, J=7.4 Hz, $H18_S$), 0.90 (t, J=7.4 Hz, $H18_R$); ^{13}C nmr see Table 3; cd λ (Δε) 214 (0.0), 227(−8.4), 255(−3.3), 280(−7.8), 293(−5.5), 300(−4.7), 318(0.0).

[4]The relative intensities of the different peaks can vary significantly depending on temperature, solvent used, type of apparatus, and operator.

Standard $NaBH_4$ reduction gave one product, which was in all respects (co-tlc, chromogenic reactions, uv, and cd) identical with conopharyngine (**26**).

3-Oxoconopharyngine (**30**)-tlc: Rf in S2 0.36, S8 0.59; Fe^{3+}: purple, orange upon heating; uv λ max 220, 285(sh), 296(sh), 300, 305(sh), and 311 nm(sh); ms (200°) *m/z* (rel. int.) 412 (M^+, 100), 353(9), 289(10), 288(11), 276(7), 275(9), 257(15), 255(12), 230(7), 214(8), 206(8), 203(8), 180(6), 138(7), 136(9), 124(18); fdms (12 mA) *m/z* 412; ^1H nmr δ 7.76 (bs, NH), 6.89 (s, H9 or H12), 6.77 (s, H9 or H12), 4.50 (m, H5a), 4.48 (s, H21), 3.91 (s, OMe), 3.88 (s, OMe), 3.73 (s, CO_2Me), 3.65 (m, 1H), 3.15 (m, 2H), 2.62 (bs, H14), 2.60 (dd, J=13.0 and 1.0 Hz, H17a), 2.29 (ddd, J=13.0, 4.0 and 2.6 Hz, H17b), 1.99 (ddd, J=13.5, 10.2 and 3.2 Hz, H15a), 1.74 (m, H20), 1.6-1.3 (m, H15b, H19a, H19b), 0.98 (t, J=7.4 Hz, H18).

Conopharyngine-hydroxyindolenine (**45**): Rf-values and chromogenic reactions see (1); uv and ms data see (7); ^1H nmr δ 7.10 (s, H9 or H12), 6.92 (s, H9 or H12), 3.92 (s, OMe), 3.89 (s, OMe), 3.73 (s, CO_2Me), 0.90 (bt, J ca. 7.5 Hz, H18). This alkaloid may be an artifact formed during the isolation procedure.

3 R/S-hydroxyconopharyngine-hydroxyindolenine (**46**) (mixture 3*R*:3*S*= 1:1): Rf-values and chromogenic reactions see (1); uv λ max 232, 298, 306(sh), and 328 nm (sh); ms (150°) *m/z* (rel. int.) 428(1), 414(100), 399(25), 398(13), 397(47), 385(8), 368(32), 367(18), 355(14), 353(11), 352(12), 290(9), 220(21), 206(19), 192(29), 190(24), 136(48), 122(43); ^1H nmr δ 7.10 (s, $H9_{RS}$ or $H12_{RS}$), 6.91 (s, $H9_{RS}$ or $H12_{RS}$), 4.47 (bd, J=7 Hz, $H3_S$), 4.00 (bs, $H3_R$), 3.91 (s, OMe_{RS}), 3.89 (s, OMe_{RS}), 3.71 (s, CO_2Me_{RS}), 0.92-0.85 (m, $H18_{RS}$). This alkaloid is probably an artifact formed during the isolation procedure.

Coronaridine (**31**): Rf-values and chromogenic reactions see (1); uv λ max 225, 285, and 293 nm; ms *m/z* (rel. int.) 338 (M^+, 100), 323(17), 309(4), 279(5), 253(7), 215(5), 214(14), 208(9), 195(4), 182(3), 169(M^{++}, 6), 168(5), 165(3), 154(7), 148(7), 137(8), 136(49), 135(14), 130(7), 124(37), 122(18); cd λ (Δε) 215(0.0), 241(+7.5), 282(0.0), 300 (−0.8), 307(0.0).

Ibogaline (**32**): Rf-values and chromogenic reactions see (1); uv and ms data see (7); ^1H nmr δ 7.53 (bs, NH), 6.99 (s, H9 or H12), 6.81 (s, H9 or H12), 3.93 (s, OMe), 3.90 (s, OMe), 0.95 (t, J=7.0 Hz, H18); cd λ (Δε) 208(−11), 243(−0.1), 284(−2.3), 330(0.0).

Isovoacangine (**33**): Rf-values and chromogenic reactions see (1); uv and ms data see (7); cd λ (Δε) 202(0.0), 210(+10), 217(+8.4), 241(+13), 278(+4.0), 285(+4.4), 300(0.0), 305(−0.7), 314(0.0), 324(+0.6), 343(0.0).

3 R/S-hydroxyisovoacangine (**34**) (mixture, 3*R*:3*S*=4:1): Rf-values and chromogenic reactions see (1); uv λ max 227, 280(sh), and 298 nm; ms *m/z* (rel. int.) 384 (M^+, 2), 382(7), 368(76), 367(51), 366(100), 353(7), 351(7), 337(6), 307(10), 283(6), 268(9), 252(9), 244(9), 184(9), 173(17), 160(11), 136(41), 124(16), 122(21); ^1H nmr δ 7.75 (bs, NH), 7.36 (m, $H12_{RS}$), 6.80-6.74 (m, $H9_{RS}$ and $H10_{RS}$), 4.42 (bs, W½=6 Hz, $H3_S$), 4.02 (d, J=1.9 Hz, $H3_R$), 3.83 (s, OMe_{RS}), 3.79 (bs, $H21_{RS}$), 3.692 (s, CO_2Me_S), 3.686 (s, CO_2Me_R), 3.53 (ddd, J=13.0, 5.3 and 5.3 Hz, $H5a_R$), 3.43 (m, $H5a_S$), 3.30 (m, $H5b_S$), ca. 3.15 (m, $H5b_R$), 3.05 (m, $H6a_{RS}$ and $H6b_{RS}$), 2.74 (dd, J=14.2 and 2.2 Hz, $H17a_R$), 2.68 (dd, J=14.2 and 1.8 Hz, $H17a_S$), 2.03 (m, W½ ca. 10 Hz, $H14_R$), 1.91 (dd, J=14.2 and 3.5 Hz, $H17b_R$), 1.70-1.45 (m, $H15a_{RS}$, $H15b_{RS}$, $H19a_{RS}$, and $H19b_{RS}$), 1.34 (m, $H20_R$), 0.92 (t, J=7.3 Hz, $H18_S$), 0.90 (t, J=7.3 Hz, $H18_R$); cd λ (Δε) 212(−5.8), 220(−7.3), 240(−0.8), 245(−1.2), 248(−0.7), 277 (−6.2), 290(−5.1), 300(−5.1), 343(0.0). Standard $NaBH_4$ reduction gave one product, which was in all respects (co-tlc, chromogenic reactions, uv, and cd) identical with isovoacangine (**33**).

Conoduramine (**35**): Rf-values and chromogenic reactions see (1); uv and eims data see (7); fdms (18 mA) *m/z* 704; ^1H nmr δ 7.67 (bs, NH or NH′), 7.65 (bs, NH or NH′), 7.54 (m, H9′), 7.04 (m, H10′, H11′ and H12′), 6.92 (bs, H9), 6.79 (bs, H12), 5.33 (q, J=6.8 Hz, H19′), 5.14 (bd, J ca. 12 Hz, H3′), 4.05 (ddd, J ca. 10.5, 8 and 3 Hz, H5′), 3.96 (bs, OMe), 3.76 (m), 3.74 (s, CO_2Me), 3.60-3.42 (m), 3.43 (bs, H21), 3.30-3.18 (m), 2.94 (d, J=14.0 Hz, H21b′), 2.98-2.82 (m), 2.78-2.67 (m), 2.73 (dd, J=2.8 and 2.8 Hz, H16′), 2.62 (s, NMe), 2.44 (s, CO_2Me'), 2.62-2.43 (m), 1.96 (m, H14b′), 1.83 (m, H14 and H17b), 1.69 (m, H15a), 1.66 (d, J=6.8 Hz, H18′), 1.49 (m, H19a), 1.42 (m, H19b), 1.37 (m, H20), 1.09 (m, H15b), 0.85 (t, J=7.4 Hz, H18); cd λ (Δε) 204(0.0), 224(−78), 233(0.0), 239(+59), 262(0.0), 273(−3.4), 287(−0.8), 290(−1.1), 295(−0.4), 304(−9.8), 323(0.0).

3 R/S-hydroxyconoduramine (**36**) (mixture 3*R*:3*S*=2:1): Rf-values and chromogenic reactions see (1); uv λ max 227, 280(sh), 286, 295, 302(sh), and 310 nm (sh); ms *m/z* (rel. int.) 720 (M^+, 1), 719(2), 718(5), 717(1), 716(2), 705(8), 704(16), 703(7), 702(11), 674(2), 673(4), 672(2), 671(2), 646(2), 645(2), 524(2), 523(5), 522(9), 511(7), 510(11), 509(22), 508(8), 507(11), 205(17), 194(23), 182(48), 181(51), 180(100), 136(22), 122(64), 121(25); fdms (16 mA) *m/z* (rel. int.) 720 (M^+, 30), 718(40), 706(30), 705(65), 704(100), 703(30), 702(30); ^1H nmr δ 7.65 (s, NH_R or NH_S), 7.64 (s, NH_R or NH_S), 7.62 (bs, NH'_{RS}), 7.54 (m, $H9'_{RS}$), 7.05 (m, $H10'_{RS}$, $H11'_{RS}$, and $H12'_{RS}$), 6.95 (bs, $H9_{RS}$), 6.78 (bs, $H12_{RS}$), 5.32 (q, J=6.8 Hz, $H19'_{RS}$), 5.12 (bs, $H3'_{RS}$), 4.37 (bs, W½=7 Hz, $H3_S$), 4.07 (bs, $H3_R$), 4.04 (ddd, J=10.3, 8.1 and 3.2 Hz, $H5'_{RS}$), 3.95 (bs, OMe_{RS}), 3.80-3.58 (m, $H21_{RS}$, $H15'_{RS}$, and $H21a'_{RS}$), 3.53 (dd, J=14.6 and 10.3 Hz, $H6a'_{RS}$), 3.44 (m), 3.24 (dd, J=14.6 and 8.1 Hz, $H6b'_{RS}$),

3.13 (m), 2.92 (d, J=13.9 Hz, H21b'$_{RS}$), 2.86 (m), 2.72 (dd, J=3.2 and 3.2 Hz, H16'$_{RS}$), 2.72-2.59 (m), 2.62 (s, NMe'$_{RS}$), 2.45 (s, CO_2Me'$_{RS}$), 1.94 (m, H14$_{RS}$ and H14b'$_{RS}$), 1.82 (bd, J ca. 14 Hz, H17b$_{RS}$), 1.65 (dd, J=6.8 and 1.5 Hz, H18'$_{RS}$), 1.68-1.25 (m, H15a$_{RS}$, H15b$_{RS}$, H19a$_{RS}$, H19b$_{RS}$, and H20$_{RS}$), 0.88 (t, J=7.8 Hz, H18$_S$), 0.86 (t, J=7.8 Hz, H18$_R$); ^{13}C nmr see Table 3; cd λ (Δε) 204(−14), 233(−90), 223(0.0), 238(+41), 248(0.0), 270(−16), 296(−7.2), 303(−12), 335(0.0). Standard $NaBH_4$ reduction gave one product, which was in all respects (co-tlc, chromogenic reactions, uv, and cd) identical with conoduramine (**35**).

Conodurine (**37**): Rf-values and chromogenic reactions see (1); uv and ms data see (7); ^{1}H nmr ($CDCl_3$+CD_3OD) δ 7.68 (d, J=7.3 Hz, H9'), 7.67 (bs, NH), 7.57 (bs, NH'), 7.25 (d, J=8.8 Hz, H9), 7.15-7.06 (m, H10', H11' and H12'), 6.84 (d, J=8.8 Hz, H12), 5.39 (q, J=6.9 Hz, H19'), 5.29 (dd, J=13.2 and 3.4 Hz, H3'), 4.17 (m, H5'), 4.00 (s, OMe), 3.85 (m, H15'), 3.70 (s, CO_2Me), 3.68 (m, 1 H), 3.52-3.46 (m, 2 H), 3.42 (bs, H21), 3.32-3.25 (m, 1 H), 3.08-2.90 (m, 4 H), 2.78 (dd, J=3.2 and 3.2 Hz, H16'), 2.70 (m, 1 H), 2.68 (s, NMe'), 2.65 (m, H14a'), 2.54 (s, CO_2Me'), 2.43 (bd, J=8.5 Hz, H3b), 1.95 (ddd, J=15.6, 7.0 and 3.7 Hz, H14b'), 1.74 (bd, J ca. 14 Hz, 1 H), 1.69 (dd, J=6.9 and 1.4 Hz, H18'), 1.53 (m, H15a), ~1.47 (m, H14 and H19a), 1.35 (m, H19b), 1.13 (m, ΣJ=31.6 Hz, H20), 0.97 (m, ΣJ ca. 22 Hz, H15b), 0.83 (t, J=7.6 Hz, H18), 0.67 (ddd, J=14.2, 4.0 and 1.9 Hz, H17b); ^{13}C nmr see Table 3; cd λ (Δε) 205(0.0), 222(−133), 230(0.0), 236(+102), 266(0.0), 278(−4.4), 291(0.0), 293(+3.6), 296(0.0), 301(−5.8), 320(0.0).

3 R/S-hydroxyconodurine (**39**) (mixture, 3R:3S=1:1: Rf-values and chromogenic reactions see (1); uv λ max 224, 284, 293, and 310 nm (sh); ms *m/z* (rel. int.) 718(4), 717(7), 705(8), 704(26), 703(52), 702(9), 701(11), 672(7), 523(23). 511(8), 510(23), 509(18), 508(41), 194(23), 182(68), 181(91), 180(100), 136(25), 122(73); fdms (12 mA) *m/z* (rel. int.) 720 (M^+, 25), 718(30), 706(30), 705(75), 704(100), 703(15), 702(15); ^{1}H nmr δ 7.73 (bs, NH'$_{RS}$), 7.68 (m, H9'$_{RS}$), 7.63 (bs, NH$_R$ or NH$_S$), 7.59 (bs, NH$_R$ or NH$_S$), 7.27 (d, J=9.0 Hz, H9$_R$ or H9$_S$), 7.26 (d, J=9.0 Hz, H9$_R$ or H9$_S$), 7.17-7.02 (m, H10'$_{RS}$, H11'$_{RS}$, and H12'$_{RS}$), 6.83 (d, J=9.0 Hz, H12$_R$ or H12$_S$), 6.82 (d, J=9.0 Hz, H12$_R$ or H12$_S$), 5.35-5.28 (m, H3'$_{RS}$ and H19'$_{RS}$), 4.12 (m, H5'$_{RS}$), 4.03 (bs, W½=5 Hz, H3$_R$), 3.98 (s, OMe$_{RS}$), 3.83 (m, H15'$_{RS}$), 3.68 (s, CO_2Me$_R$ or CO_2Me$_S$), 3.67 (s, CO_2Me$_R$ or CO_2Me$_S$), 3.70-3.55 (m), 3.47-2.87 (m), 2.76 (dd, J=3.2 and 3.2 Hz, H16'$_{RS}$), 2.75-2.50 (m), 2.64 (s, NMe'$_R$ or NMe'$_S$), 2.63 (s, NMe'$_R$ or NMe'$_S$), 2.53 (s, CO_2Me'$_R$ or CO_2Me'$_S$), 2.52 (s, CO_2Me'$_R$ or CO_2Me'$_S$), 1.97-1.81 (m), 1.67 (d, J=6.8 Hz, H18'$_{RS}$), 1.70-1.05 (m), 0.83 (t, J=7.5 Hz, H18$_S$), 0.81 (t, J=7.5 Hz, H18$_R$), 0.63 (m, H15b$_{RS}$); ^{13}C nmr see Table 3; cd λ (Δε) 204(+26), 209(0.0), 222(−122), 230(0.0), 237(+102), 280(+4.3), 294(+10), 305(0.0), 317(+1.4), 337(0.0). Standard $NaBH_4$ reduction gave one product, which was in all respects (co-tlc, chromogenic reactions, uv, and cd) identical with conodurine (**37**).

3 R/S-hydroxy-16-decarbomethoxyconodurine (**40**) (mixture, 3R:3S=3:2): tlc: Rf in S1 0.35, S4 0.34, S6 0.34, S7 0.05, S8 0.37; Fe^{3+}: blue,. blue-grey after heating; uv λ max 225, 284, and 292 nm (sh); fdms (20 mA) *m/z* (rel. int.) 662 (M^+, 4), 661(4), 660(8), 648(28), 647(38), 646(100%), 645(8), 644(13); eims *m/z* (rel. int.) 662 (M^+, <0.5), 660(2), 648(8), 647(26), 646(55), 645(6), 644(6), 631(2), 466(10), 464(5), 458(10), 453(14), 452(19), 451(27), 340(23), 255(12), 194(20), 182(86), 181(34), 180(100), 149(28), 136(63), 135(38), 122(56); ^{1}H nmr δ 7.76 (bs, NH'$_{RS}$), 7.62 (m, H9' and NH, R or S), 7.52 (m, H9' and NH, R or S), 7.24 (d, J=9 Hz, H9$_{RS}$), 7.18-7.02 (m, H10'$_{RS}$, H11'$_{RS}$, and H12'$_{RS}$), 6.83 (d, J=9 Hz, H12$_R$ or H12$_S$), 6.82 (d, J=9 Hz, H12$_R$ or H12$_S$), 5.37-5.31 (m, H3'$_{RS}$ and H19'$_{RS}$), 4.48 (bs, W½ ca. 6 Hz, H3$_S$), 4.11 (m, H3$_R$ and H5'$_{RS}$), 3.97 (s, OMe$_{RS}$), 3.87 (m, H15'$_{RS}$), 3.76-2.87 (m), 2.78 (dd, J=3 and 3 Hz, H16'$_{RS}$), 2.63 (s, NMe'$_{RS}$), 2.51 (s, CO_2Me'$_{RS}$), 2.65-2.20 (m), 2.05-1.95 (m), 1.68 (d, J=6.6 Hz, H18'$_R$), 1.63 (d, J=6.6 Hz, H18'$_S$), 1.65-1.20 (m), 0.95 (m), 0.85 (t, J=7 Hz, H18$_S$), 0.83 (t, J=7 Hz, H18$_R$); cd λ (Δε) 204(+12), 216(0.0), 223(−17), 229(0.0), 237(+22), 290(+5.0), 294(+6.3), 345(0.0). Standard $NaBH_4$ reduction gave one product which possessed the following chromogenic and spectral data: tlc: Rf in S1 0.38, S4 0.35, S8 0.26; Fe^{3+}: blue, blue-grey upon heating; uv λ max 225, 284, and 293 nm; cd λ (Δε) 205(+11), 214(0.0), 223(−17), 229(0.0), 236(+18), 290(+3.3), 294(+4.0), 340(0.0).

3 R/S-hydroxyvoacamine (**44**) (mixture 3R:3S ca. 1:1): Rf-values and chromogenic reactions see (1); uv λ max 224, 278(sh), 286, 294, and 310 nm (sh); ms *m/z* (rel. int.) 720 (M^+, 3), 719(4), 718(5), 704(17), 703(32), 702(7), 701(7), 523(11), 522(7), 521(9), 510(16), 509(15), 508(26), 205(56), 194(30), 182(100), 181(55), 180(77), 136(55), 122(72); fdms (15 mA) *m/z* (rel. int.) 720 (M^+, 45), 718(85), 706(35), 705(80), 704(100), 703(40), 702(20); cd λ (Δε) 204(−5.8), 220(−77), 234(0.0), 240(+21), 248(0.0), 278(−17), 290(−8.5), 295(−0.8), 303(−8.1), 348(0.0); ^{1}H nmr δ 7.71 (bs, NH$_{RS}$), 7.52 (bs, H9'$_{RS}$ and NH'$_{RS}$), 7.07 (bs, H10'$_{RS}$, H11'$_{RS}$, and H12'$_{RS}$), 6.93 (bs, H9$_{RS}$), 6.75 (bs, H12$_{RS}$), 5.41 (bq, H19'$_{RS}$), 5.15 (bd, H3'$_{RS}$), 4.33 (bs, W½=6 Hz, H3$_S$), 4.17 (m), 4.00 (bs, OMe$_{RS}$), 4.0-2.4 (m), 3.64 (bs, CO_2Me$_{RS}$), 2.68 (bs, NMe'$_{RS}$), 2.46 (s, CO_2Me'$_{RS}$), 2.0-1.2 (m), 1.68 (d, J=7.1 Hz, H19'$_{RS}$), 0.90 (t, J=7.4 Hz, H18$_S$), 0.88 (t, J=7.4 Hz, H18$_R$). Standard $NaBH_4$ reduction gave one product, which was in all respects (co-tlc, chromogenic reactions, uv, and cd) identical with voacamine

(**43**); cd λ (Δε) 208(0.0), 223(−59), 232(0.0), 239(+44), 273(0.0), 275(−1.1), 282(0.0), 286(+3.8), 295(+10), 302(0.0), 304(−2.3), 312(0.0).

Vobparicine (**19**): Rf-values and chromogenic reactions see (1): uv data see (10); ms *m/z* (rel. int.) 614 (M^++14, 16), 600.3464 (M^+, 13; calc. for $C_{39}H_{44}N_4O_2$ 600.3464), 599(7), 435(3), 434(4), 432(4), 421(3), 420(5), 419(3), 418(3), 405(3), 404(4), 349(5), 337(7), 336(9), 277(20), 263(14), 262(13), 261(14), 247(17), 233(11), 232(11), 220(7), 208(8), 194(22), 183(19), 182(56), 181(23), 180(65), 168(18), 167(17), 166(13), 165(14), 156(13), 144(23), 130(23), 124(33), 123(23), 122(100), 107(63), 106(53); ^{1}H nmr, ^{13}C nmr, and cd data see (10). Synthesis of vobparicine: 0.2 mg vobasinol (**8**) and 1 mg apparicine (**18**) were dissolved in 2 ml 1.5% methanolic HCl and refluxed for 2 h under N_2. After cooling, the solution was basified with an aqueous $NaHCO_3$ solution and extracted twice with $CHCl_3$. After drying with Na_2SO_4, the solution was evaporated in vacuo. The amorphous product was separated into two fractions by means of preparative tlc with S5. The fraction with the lower Rf was in all respects (co-tlc, chromogenic reactions, uv, and cd) identical with the isolated vobparacine (**19**).

Vobparicine-N_4-oxide (**20**)-tlc: Rf in S2 0.16, S8 0.08, S11 0.07; Fe^{3+}: grey-blue upon heating; fdms *m/z* (rel. int.) 617 (M^++H, 25), 615 (M^+−O+CH_2+H, 25), 601 (M^+−O+H, 100); ms *m/z* (rel. int.) 630 (M^++14, 0.5), 616 (M^+, 2), 615(3), 614(6), 601(4), 600(8), 599(4), 492(2), 434(2), 432(2), 421(2), 420(3), 419(2), 418(2), 337(13), 336(16), 321(6), 307(5), 277(26), 263(20), 249(12), 247(10), 235(8), 221(8), 206(11), 195(12), 194(13), 183(16), 182(32), 181(24), 180(48), 156(29), 144(13), 143(17), 130(17), 122(100); ^{1}H nmr δ 8.09 (d, *J*=7.7 Hz, 1 H), 7.97 (bs, NH), 7.81 (bs, NH), 7.55-7.06 (m, 7 H), 6.03 (d, *J*=10.0 Hz, H22), 5.58 (q, *J*=7.0 Hz, H19), 5.41 (q, *J*=7.0 Hz, H19′), 5.27 (d, *J* ca. 16 Hz, H6a), 5.01 (d, *J* ca. 16 Hz, H6b), 4.70 (d, *J* ca. 15 Hz, H21a), 4.53 (ddd, *J* ca. 12, 10 and 3 Hz, H3′), 4.42 (m, H3a and H15), 4.21 (d, *J* ca. 15 Hz, H21b), 4.03 (m, H5′), 3.80-3.60 (m, H3b, H15′ and H21a′), 3.40 (dd, *J* ca. 14 and 10 Hz, H6a′), 3.23 (dd, *J* ca. 14 and 8 Hz, H6b′), 3.01 (d, *J*=14.5 Hz, H21b′), 2.72 (dd, *J*=3 and 3 Hz, H16′), 2.61 (s, NMe′), ca. 2.60 (m, H14a and H14a′), 2.46 (s, CO_2Me′), 2.33 (m, H14b), 1.67 (d, *J*=7.0 Hz, H18 or H18′), 1.57 (d, *J*=7.0 Hz, H18 or H18′), H14b′ is not observed due to the presence of an impurity around 1.9 ppm; cd λ (Δε) 207(+42), 217(0.0) 227(−37), 235(0.0), 244(+28), 292(+3.5), 297(+4.6), 303(0.0), 313(−3.3), 342(0.0).

Vobparicine-N_4-oxide (0.2 mg) was dissolved in 2 ml 5% aqueous H_2SO_3 solution and was left standing for 30 min. After basification with NH_4OH and addition of 2 ml H_2O, it was extracted twice with 4 ml $CHCl_3$. After drying with Na_2SO_4 and evaporation in vacuo, the residue was examined on tlc. No spot other than that of the starting material could be observed.

Vobparicine (0.2 mg) was dissolved in 2 ml 3% H_2O_2 in EtOH-H_2O (9:1) and was left standing for 30 min. After addition of 3 ml H_2O, it was extracted twice with 4 ml $CHCl_3$. After drying with Na_2SO_4 and evaporation in vacuo, the residue was examined on tlc with solvent systems S2, S9 and S11. The major product had identical Rf-values and chromogenic reactions as vobparicine-N_4-oxide (**20**).

Monogagaine: tlc-Rf in S1 0.48, S3 0.31, S8 0.39; Fe^{3+}: gold upon heating, dark blue-grey upon prolonged heating; uv λ max 222, 282(sh), 288, and 297(sh) nm; ms (220°) *m/z* (rel. int.) 600 (M^+, 38), 492(32), 491(35), 490(74), 458(18), 295(18), 182(72), 181(26), 180(100), 133(42), 122(46); ^{1}H nmr δ 7.51 (m, 2 H), 7.37 (bs), 7.15-7.02 (m, 3 H), 6.97 (ddd, *J*=7.6, 7.6 and 0.8 Hz), 6.79 (ddd, *J*=7.6, 7.6 and 1.0 Hz), 6.54 (d, *J*=7.6 Hz), 5.72 (q, *J*=6.7 Hz, H19), 5.24 (q, *J*=7.0 Hz, H19′), 4.78 (d, *J*=17.8 Hz, H6a), 4.43 (d, *J*=17.8 Hz, H6b), 4.08 (m, 1 H), 3.87-2.78 (m), 3.47 (s, CO_2Me), 2.45 (s, NMe), 2.40 (m, 1 H), 2.17-1.94 (m), 1.87 (dd, *J*=6.7 and 1.2 Hz, H18), 1.80 (m), 1.63 (d, *J*=7.0 Hz, H18′).

Chippiine (**47** or **48**): tlc-Rf in S2 0.42, S8 0.26; Fe^{3+}: purple, somewhat intensifying upon heating; uv λ max 207, 228, 282, 301, 306, and 310 nm (sh); fdms (emitter current 0-20 mA) *m/z* (rel. int.) 414(100%), minor peaks at 412, 398, 397, and 396 (intensity varies with emitter current); eims *m/z* (rel. int.) 414(31), 412(7). 399(28), 398(100), 397(23), 396(50), 383(11), 381(10), 339(9), 338(8), 337(18), 313(15), 275(11), 274(17), 231(8), 230(8), 214(9), 208(10), 205(10), 204(9), 203(11), 199(15), 190(28), 156(10), 148(14), 136(71), 135(17), 124(26), 122(33); ^{13}C nmr see Table 5; cd λ (Δε) 210(−11), 225(−13), 255(−3.2), 280(−7.1), 362(0.0). Standard $NaBH_4$ reduction gave one amorphous product with the following characteristics: tlc: Rf in S2 0.33, S8 0.28; Fe^{3+}: purple, somewhat intensifying upon heating. Uv λ max 218(sh), 285(sh), 300, 305(sh), and 311 nm(sh); ms (200°) *m/z* (rel. int.) 416 (M^+, 100), 401(8), 387(3), 385(2), 357(47), 328(15), 302(3), 285(5), 275(6), 262(19), 218(15), 214(19), 204(28), 190(17), 154(10). When the reduction was carried out in D_2O/C_2H_5OD with $NaBD_4$, a product with identical chromogenic and spectral data, except for the ms data, was obtained: ms (325°) *m/z* (rel. int.) 417 (M^+, 100), 402(8), 388(4), 385(8), 358(30), 329(13), 302(7), 275(10), 262(18), 218(13), 204(21), 190(19), 155(13). Chippiine was always contaminated with another compound. The contamination increased with time. This decomposition product was isolated by means of preparative tlc with S2. The band with the higher Rf-value was in all respects (co-tlc, chromogenic reactions, uv, and cd) identical with 3-hydroxyconopharyngine (**28**). The nmr experiments of chippiine were carried out as soon as possible after this purification.

Investigation of possible artifact formation from 3-hydroxyconopharyngine (**28**). Two mg 3-hydroxyconopharyngine was dissolved in 5 ml 96% EtOH and refluxed for 15 h under a pressure of 0.2 atm. After cooling, the solvent was evaporated in vacuo and the residue examined on tlc with S7 and S8. No spots other than those of 3-hydroxyconopharyngine and 3-hydroxyconopharyngine-hydroxyindolenine were visible.

Two mg 3-hydroxyconopharyngine was dissolved in 2.5 ml 2% HOAc and 2.5 ml EtOAc and the solution was left standing in daylight and in contact with air for 2 h. After separation of the EtOAc layer, basification of the HOAc layer with NH_4OH, extraction with $CHCl_3$, drying with Na_2SO_4, and evaporation in vacuo, the residue was examined on tlc with S2 and S8. No spots other than those of 3-hydroxyconopharyngine and 3-hydroxyconopharyngine-hydroxyindoline were visible.

Two mg 3-hydroxyconopharyngine was dissolved in 2.5 ml $CHCl_3$-iPrOH (9:1) and 2.5 ml 2% NH_4OH in H_2O. The solution was left standing in daylight and in contact with air for 2 h. After separation of the aqueous layer, drying of the $CHCl_3$ layer, and evaporation in vacuo, the residue was examined on tlc with S7 and S8. No spots other than those of 3-hydroxyconopharyngine and 3-hydroxyconopharyngine-hydroxyindolenine were visible.

TC-A (**23** or **24**): tlc-Rf in S2 0.42, S8 0.17, S11 0.50; Fe^{3+}: orange upon heating, very slowly changing to purple; uv λ max 228, 284, and 290(sh) nm; ms (200°) *m/z* (rel. int.) 314 (M^+, 77), 313(16), 297(27), 296(18), 285(12), 267(15), 249(12), 211(45), 210(38), 170(39), 158(72), 157(100), 156(75), 144(45), 143(45), 142(34), 140(34), 130(27), 129(25), 128(24), 122(27).

TC-B (**25**): tlc-Rf in S2 0.42, S8 0.14, S11 0.50; Fe^{3+}: orange upon heating, very slowly changing to purple; uv λ max 226, 282, and 293 nm; ms (200°) *m/z* (rel. int.) 330 (M^+, 28), 314(25), 313(100), 297(8), 296(13), 295(32), 277(8), 269(8), 267(6), 265(8), 212(13), 211(11), 210(16), 184(17), 172(18), 170(26), 169(17), 168(18), 167(17), 159(19), 158(22), 157(20), 156(37), 146(27), 144(30), 143(23), 130(30), 122(20).

TC-C (**24** or **23**): Rf-values and chromogenic reactions see (1); uv λ max 228, 284, and 289 nm (sh); ms (200°) *m/z* (rel. int.) 314 (M^+, 90), 313(27), 297(13), 296(14), 295(15), 285(14), 267(16), 211(20), 210(19), 199(20), 170(42), 158(80), 157(100), 156(99), 144(48), 143(52), 142(32), 140(30), 138(44), 130(24); ^1H nmr δ 7.77 (bs, NH), 7.46 (dd, *J*=7.0 and 1.7 Hz, H9 or H12), 7.27 (dd, *J*=7.0 and 1.5 Hz, H9 or H12), 7.10 (ddd, *J*=7.0, 7.0 and 1.7 Hz, H10 or H11), 7.05 (ddd, *J*=7.0, 7.0 and 1.5 Hz, H10 or H11), 3.56 (bs, H14 and H15), 3.03 (bd, *J*=12.7 Hz, H3a), 2.99-2.86 (m, H5a, H5b, H16a and H21a), 2.76 (bdd, *J*=14.8 and 9.0 Hz, H16b), 2.55 (ddd, *J*=12.0, 4.0 and 4.0 Hz, H6a), 2.43(bd, *J* ca. 11 Hz, H21b), 2.33 (ddd, *J*=11.9, 8.9 and 6.0 Hz, H6b), 2.19 (bdd, *J*=15.0 and 9.0 Hz, H17a), 1.93 (d, *J*=12.8 Hz, H3b), ~1.85 (m, H17b), 1.32-1.18 (m, H19a and H19b), 0.87 (t, *J*=7.5 Hz, H18).

TC-D (**9**): tlc-Rf in S5 0.40, S9 0.12, Fe^{3+}: brown on prolonged heating; uv λ max 223, 284(sh), 288, and 296 nm (sh); fdms *m/z* 413 (M^+); eims (250°) *m/z* (rel. int.) 413.2299 (M^+, 61; calcd for $C_{23}H_{31}N_3O_4$ 413.2314), 338(69), 337.1893 (100; calcd for $C_{21}H_{25}N_2O_2$ 337.1916), 336.1834 (51; calcd for $C_{21}H_{24}N_2O_2$ 336.1815), 305(8), 277(20), 233(15), 222(20), 206(8), 182(12), 181(22), 180(68), 168(11), 156(22), 144(11), 122(70), 121(18), 120(21), 107(13); ^1H nmr (500 MHz) δ 8.87 (bs, NH) 7.49 (dd, *J*=7.3 and 1.5 Hz, H9), 7.22 (dd, *J*=7.3 and 1.5 Hz, H12), 7.13 (ddd, *J*=7.3, 7.3 and 1.5 Hz, H10), 7.06 (ddd, *J*=7.3, 7.3 and 1.5 Hz, H11), 5.41 (qd, *J*=7.0 and 1.6 Hz, H19), 4.54 (dd, *J*=13.1 and 3.3 Hz, H3), 3.96 (ddd, *J*=10.4, 8.0 and 3.2 Hz, H5), 3.73 (dq, *J*=14.0 and 1.9 Hz, H21a), 3.65 (dddd, *J*=12.3, 6.9, 3.3 and 1.6 Hz, H15), 3.28 (dd, *J*=14.9 and 10.4 Hz, H6a), 3.16 (dd, *J*=14.9 and 8.0 Hz, H6b), 2.97 (d, *J*=14.0 Hz, H21b), 2.92 (ddd, *J*=12.7, 7.5 and 5.5 Hz, H1a'), 2.83 (ddd, *J*=12.7, 6.4 and 5.8 Hz, H1b'), 2.81 (ddd, *J*=15.1, 13.1 and 12.3 Hz, H14a), 2.78 (bs, W½ ca. 50 Hz, 2H; disappears after addition of D_2O), 2.72 (ddd, *J*=12.7, 5.8 and 5.5 Hz, H2a'), 2.66 (dd, *J*=3.3 and 3.2 Hz, H16), 2.58 (s, NMe), 2.53 (ddd, *J*=12.7, 7.5 and 5.8 Hz, H2b'), 2.41 (s, CO_2Me), 2.20 (ddd, *J*=15.1, 6.9 and 3.3 Hz, H14b), 1.70 (dd, *J*=7.0 and 1.9 Hz, H18); ^{13}C nmr (75.5 MHz, ^{12}C-enriched $CDCl_3$, $CDCl_3$=77.0 ppm): δ 171.5 (*C*O_2Me), 136.3 (C19), 135.7(C13), 133.7(C2), 129.0(C8), 122.4(C11), 120.1(C10), 119.1(C19), 117.7(C9), 110.6(C7), 110.2(C12), 59.2(C5), 52.0(C21), 50.1(CO_2*Me*), 46.7(C16), 42.1(NMe), 42.1(C3?), 41.7(C1'?), 36.5(C14), 32.9(C2'?), 29.7(C15), 19.0(C6), 12.6(C18).

Tacraline (**14**): tlc-Rf in S9 0.31, S11 0.11; Fe^{3+}: orange-purple upon prolonged heating; Ce^{4+}: purple changing to orange; uv λ max 205, 235, and 293 nm; ms (110°) *m/z* (rel. int.) 409 (M^+, 18), 367(36), 366(43), 350(5), 336(2), 307(10), 294(22), 293(100), 261(9), 247(12), 243(8), 226(5), 194(13), 183(10), 169(6), 158(23), 157(16), 130(8), 108(32); ^1H nmr δ 7.48 (d, *J*=7.3 Hz, H9 or H12), 7.07 (dd, *J*=7.3 and 7.3 Hz, H10 or H11), 6.78 (dd, *J*=7.3 and 7.3 Hz, H10 or H11), 6.66 (d, *J*=7.3 Hz, H9 or H12), 5.54 (bs, W½=24 Hz, H19), 4.50 (bs, NH), 3.72 (s, CO_2Me), 3.7-3.2 (m), 2.3 (m), 2.0-1.7 (m), 1.75 (s, O_2CMe), 1.69 (d, *J* ca. 7 Hz, H18).

Desacetyltacraline (**15**): tlc-Rf in S9 0.20, S11 0.08; Fe^{3+}: purple-orange upon prolonged heating;

Ce^{4+}: orange; uv λ max 207, 236, and 295 nm; ms (200°) *m/z* (rel. int.) 367 (M^+, 22), 336(9), 325(35), 324(40), 308(4), 294(29), 293(100), 261(18), 247(12), 243(9), 235(19), 221(17), 202(17), 194(16), 185(16), 183(15), 171(25), 158(48), 157(31), 130(21), 108(63); ^1H nmr δ 7.37 (d, J=7.5 Hz, H9 or H12), 7.12 (dd, J=7.5 and 7.5 Hz, H10 or H11), 6.82 (dd, J=7.5 and 7.5 Hz, H10 or H11), 6.73 (d, J=7.3 Hz, H9 or H12), 5.49 (q, J=7 Hz, H19), 4.62 (bs, NH), 3.77 (s, CO_2Me), 3.8-3.3 (m), 2.25 (m), 2.0-1.7 (m), 1.66 (d, J=7 Hz, H18).

Standard $NaBH_4$ reduction was carried out as described by van Beek *et al.* except that instead of EtOAc, $CHCl_3$ was used.

Bioassay by means of the biogram technique. This was carried out in the same manner as described earlier (6).

Determination of antimicrobial activity by means of the agar diffusion technique. This was carried out in the same manner as described earlier (7).

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