Tetrahedron Vol. 40, No. 4, pp. 737 to 748, 1984
Printed in Great Britain.

0040-4020/84 13.00 + .00
Pergamon Press Ltd.

ALKALOIDS OF *TABERNAEMONTANA EGLANDULOSA*†

TERIS A. VAN BEEK,* ROBERT VERPOORTE and ANDERS BAERHEIM SVENDSEN
Department of Pharmacognosy, Gorlaeus Laboratories, State University of Leiden, P.O. Box 9502, 2300 RA Leiden, The Netherlands

(*Received in the UK 24 March 1983*)

Abstract—From the leaves and twigs of *Tabernaemontana eglandulosa* 22 alkaloids, of which 12 are new, have been isolated. Besides tacamine (pseudo-vincamine) 7 other alkaloids of this new class of indole alkaloids were identified. A further three alkaloids of the cleavamine type, namely the known (+)20R-I 5, 20-dihydro-cleavamine and (−)20S-1 5,20-dihydro-cleavamine and one new alkaloid, which was tentatively identified as 14S, 20R-velbanamine, two new alkaloids of the pseudo-aspidospermidine type, namely 20R-1,2-dehydro-pseudo-aspidospermidine and 20S-hydroxy-1,2-dehydro-pseudo-aspidospermidine and an alkaloid of a novel iboga type were isolated. More common alkaloids isolated were coronaridinc, 11-hydroxycoronaridinc, voaphylline, tubotaiwinc, ibogaminc, norfluorocurarine and probably a mixture of 20R- and 20S-pseudo-vincadifformine. Only quantitative differences in the alkaloid content of the leaves, twigs and stembark were observed.

Tabernaemontana eglandulosa Stapf (syn. Gabunia eglandulosa (Stapf)Stapf) is a small liane widely distributal in Central Africa and occurring in the forest usually as solitary plants. They flower at night.' In Zaire the root is used against snake bites.' Only the rootbark and the stembark of *T. eglandulosa* have been chemically investigated before. In these previous investigations the rootbark yielded the alkaloids perivine, vobasine, coronaridine, 3-hydroxy-coronaridine, 3-hydroxy-isovoacangine, isovoacanginc and voacamine[3] and the stembark the alkaloids conopharyngine, coronaridiae, 6-hydroxy-3-oxo-coronaridine, 3,6R-oxido-coronaridine and voacangine.[4,5] No report of an investigation of the leaves is known to the authors, therefore an examination of the alkaloidal composition of this part of the plant as well as a comparison with the alkaloids present in the twigs and the stembark were carried out. The results of the studies are set out below.

RESULTS AND DISCUSSION

The leaves were extracted with alcohol and the alkaloids separated by an acid-base extraction. The tertiary alkaloids were separated and purified by means of LC and prep TLC. As the alkaloidal composition of the twigs did not differ qualitatively from the leaves, the tertiary alkaloids from the twigs were worked up in a similar fashion and fractions were combined for greater yield. Table 1 lists the identified alkaloids from the leaves and the twigs, together with an indication of their relative abundance.

Of the 22 alkaloids isolated 12 were new, 8 of them belonging to the new tacaman class, the other 13 to the igbogan, plumeran, aspidospermatan and strychnan classes.[6] One alkaloid was of a new iboga type, the structure elucidation of which will be the subject of a separate paper.' The elucidation of the structures of the other new alkaloids is described in the following with the exception of the major alkaloid, tacamine 1, whose structure elucidation has already been published.* However for comparison its spectral data are also presented in this publication. In Fig. 1 the ^{1}H NMR spectrum of tacamine is given, since it was of utmost importance for the identification of the other seven related alkaloids.

16-*epi*-*Tacamine* 2. The second most abundant alkaloid in the leaves and twigs gave similar colour reactions as tacaminc, *viz* a greenish-black colour with ferric chloride and perchloric acid on heating and a yellow colour with ceric sulphate and sulfuric acid, which intensified upon heating. The alkaloid decomposed readily and quantitatively to tacamine, when left in solution or on a TLC-plate. These facts suggest a similar but less stable structure. The *W* and mass spectral data of the alkaloid were similar to those of tacamine, except for the presence of a fragment at *m/z* 59 (the base peak in the spectrum of the new alkaloid) which is absent in the spectrum of tacamine.

The easy loss of the fragment CH_3OCO^+ suggests that the alkaloid might be 16-epi-tacamine, which has the bulky carbomethoxy group in the less favoured axial position. This compound, being a carbinolaminc, can be readily converted by epimerization at C-l 6 to tacamine, which has the carbomethoxy group in equatorial position. The ^{1}H NMR and ^{13}C NMR data support the proposed structure. In the ^{1}H NMR spectrum the characteristic appearance (see Fig. 1) and thus the coupling constants of the aliphatic signals (see Table 2), have remained unchanged and therefore the relative stereochemistry must be the same as in tacamine. The chemical shifts of most protons however have changed (see Table 2). In analogy with the difference between vincamine and 16-epi-vincamine[9] two aromatic protons resonate at 7.4 ppm and two at 7.1 ppm, furthermore all protons except H-17α and H-1 7β are shifted upfield in 16-epi-tacamine if compared with tacamine. In Table 3 the ^{13}C NMR data are given. In column 2 the values for tacamine are given, in column 4 the predicted values for 16-epi-tacamine based on the extrapolation of the difference between vincamine and 16-epi-vincamine[9] (column 3) and in column 5 the actual values for 16-epi-tacamine. The good agreement between the predicted and the experimental values confirms that the alkaloid is 16-epi-tacamine. To determine the absolute stereochemistry a CD spectrum was recorded. From the minima at 272 and

†Part 4 in the series "Pharmacognostical studies of *Tabernaemontana* species". For Part 3 see Ref. 8.

Table 1. Alkaloids isolated from the leaves and twigs of *Tabernaemontana eglandulosa*

Alkaloid	Re l. abundance[a]	Hethods of identification
Tacamine 1	+++	UV,CD,MS, ^{1}H NMR, ^{13}C NMR
16-Epi-tacamine 2	+++	UV,CD,MS, ^{1}H NMR, ^{13}C NMR
16R-Descarbomethoxy-tacamine 3	++	UV,CD,MS, ^{1}H NMR
16S-Descarbomethoxy-tacamine 4	++	UV,CD,MS, ^{1}H NMR
Tacamonine 6	+++	UV,CD,MS, ^{1}H NMR
17-Hydroxy- tacamonine 7	t	UV,CD,MS, ^{1}H NMR
16,17-Anhydro-tacamine 8	++	UV,CD,MS, ^{1}H NMR
19S-Hydroxy-tacamine 5	++	UV,CD,MS, ^{1}H NMR
(+) 20R- 15,20-Dihydro-cleavamine 17	t t	UV,[α],CD,MS, ^{1}H NMR, ^{13}C NMR
(−)20S- 15,20-Dihydro-cleavamine 16	t	^{13}C NMR
14S,20R-Velbanamine 18	+	UV,MS,coTLC
(+)20R-1,2-Dehydro-pseudoaspido-spermidine 9	++t	UV,[α],CD,MS, ^{1}H NMR, ^{13}C NMR
20S-Hydroxy-1,2-dehydro-pseudo-aspidoapermidine 11	++	UV,CD,MS, ^{1}H NMR
20R-Pseudovincadifformine 15	+	UV,MS, ^{1}H NM?
20S-Pseudovincadifformine	+	UV,MS, ^{1}H NMR
Coronaridine 19	++	UV,CD,MS, ^{1}H NMR, coTLC
I I-Hydroxy-coronaridinc 20	++	UV,MS, ^{1}H NMR
Ibogamine 21		UV.MS, ^{1}H NMR, coTLC
Voaphylline 22	+	UV,CD,MS, ^{1}H NMR, coTLC
novel type of iboga alkaloid[7]	++	UV,CD,MS, ^{1}H NMR, coTLC
Tubotaivine 24	++	UV,MS, ^{1}H NMR, coTLC
Norf luorocurarine 23		UV,MS

[a] +++ = major component, t+ = minor component, + = trace component

283 nm it can be concluded that H-3 as in tacamine had the **β-configuration.**[10,11] The maximum at 240 nm indicates that the configuration at C-16 is opposite to that of **tacamine,**[10,11] as already concluded from ^{1}H NMR and ^{13}C NMR. The CD spectrum is the mirror image of the CD spectrum of **16-epi-vincamine.**[11] The absolute configuration is thus given by structure 2.

16R-*Descarbomethoxytacamine* 3. This minor alkaloid was shown to belong to the tacaman class by means of its ^{1}H NMR spectrum. The signals, readily recognized by their characteristic appearance, of H-3, **H-5α, H-5β, H-6α, H-6β,** H-l 4, H-l **5α, H15-β,** H-20, **H21α** and **H21β** were all present and indicated that the relative stereochemistry at C-3, C-14, N-4 and C-20 was the same as in tacamine. Compared with the ^{1}H NMR spectrum of **tacamine** the absence of the **COOCH$_3$** signal was noted and the **occurence** of a double doublet at 5.58 ppm with coupling constants of 9.5 and 5.4 Hz. These coupling constants were also present in the signals of H-l **7β** and **H-17α** respectively. This suggested that the alkaloid was **16-descarbomethoxytacamine.** This was confirmed by the *W* and mass spectral data.

The UV was the same as for **tacamine** indicating an unchanged chromophore while the mass **spectrum** confirmed the **bruto** formula **$C_{19}H_{24}N_2O$** (**M$^+$** ***m/z*** 296). The stereochemistry at C-16 was determined in **three ways. Firstly** by the value of 9.5 Hz for one of the coupling constants of H-l 6. This value is only possible when H-16 is in an axial position, the angle with the axial H- 17 **being** 180". Secondly by the shift of H-15a. **Because** this proton is situated under the aromatic pyrrole ring (see threedimensional drawing of 3) it is strongly shielded and resonates at 0.37 ppm. This **behaviour** is exhibited by five of the eight tacamine-type alkaloids found. In the three cases, where a **16α-hydroxy** group is present, such as in tacamine itself, the shielding effect of the pyrrole nucleus is countered by a deshielding **effect** of the interaction between the electronegative oxygen of the **16α-OH** and the H-l **5α.** Resuming, H-l **5α** resonates between 0.37 ppm and **0.69 ppm,** if no **16α-OH** is present and between **l.** 13 ppm and 1.29 **ppm,** if a **16α-OH** is present. Thirdly a **difference** between the two **16-descarbomethoxy-tacamines** can be made by **means** of the CD spectrum. Toth ***et al.***[12] have published the CD spectra for the two possible descarbomethoxy-vincamines. As the 1 **6-descarbo**methoxy-tacamines are the mirror-images of the **16-descarbomethoxy-vincamines,** only the ethyl side chains being attached at different carbons, their CD spectra must also be their mirror images, as the attachment of the ethyl group will be of little importance in determining the CD spectrum. By comparing the CD data of the alkaloid with the data given by Toth ***et al.***[12] it can be concluded that the alkaloid must have an equatorial **16-OH (16α-H)** and a **3β-H** (see structure 3).

16S-*Descarbomethoxytacamine 4.* The UV and mass spectral data of this alkaloid were similar to those of **16R-descarbomethoxytacamine,** suggesting that this alkaloid might the **16S-isomer.** This was confirmed by its ^{1}H NMR data. The ^{1}H NMR was similar, only most of the protons had been shifted

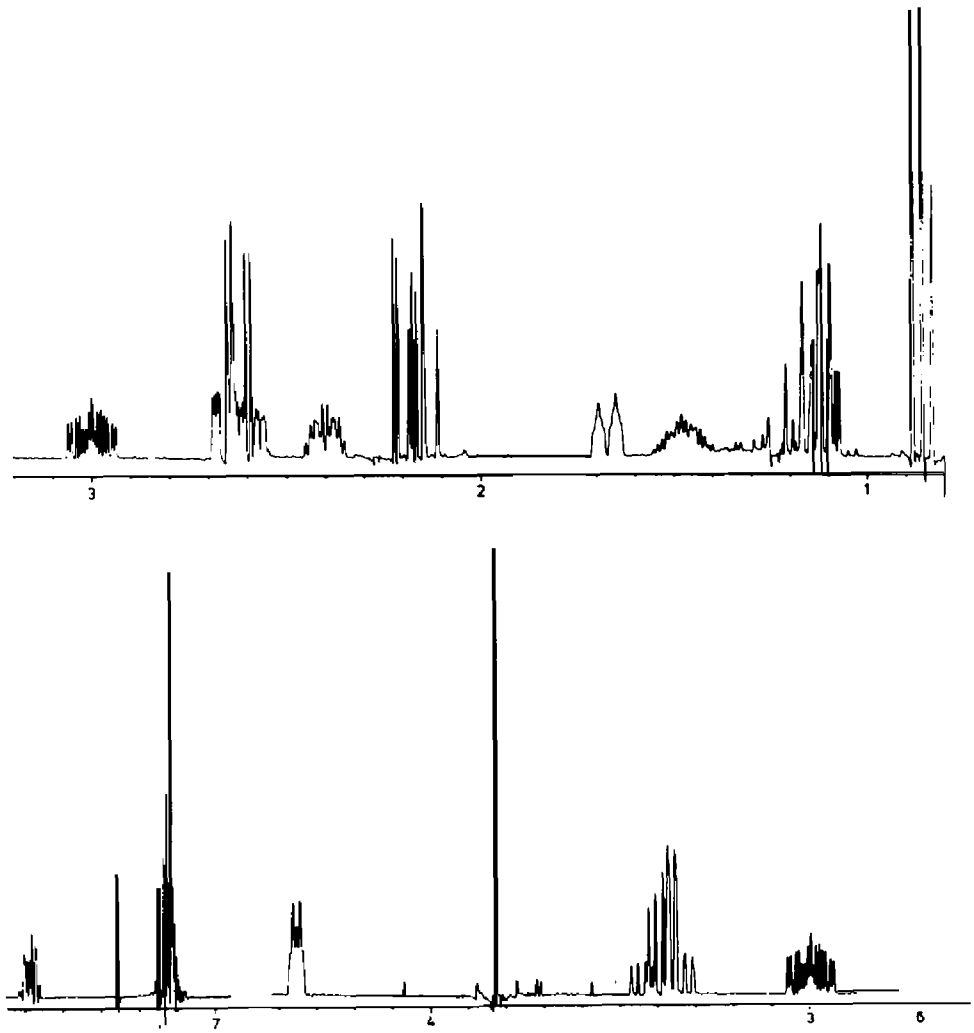
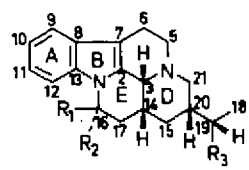


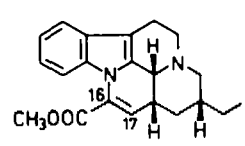
Fig. 1.

downfield 0.1–0.3 ppm and a double doublet at 6.06 ppm due to H-16 with coupling constants of 4.0 and 1.2 Hz with H-17β and H-17α respectively had appeared. Also H-15α was shifted to 1.13 ppm (tacamine: 1.14 ppm). All these data fitted the structure of 16S-descarbomethoxytacamine 4 well. Finally its CD spectrum was the mirror-image of the CD spectrum published by Toth[12] for 16-descarbomethoxyvincamine confirming the absolute and relative stereochemistry of the alkaloid 4.

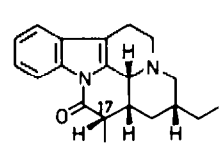
Tacamonine 6 This major component was again shown to belong to the tacamine type by its tacamine-



1 R_1=$COOCH_3$, R_2=OH, R_3=H
2 R_1=OH, R_2=$COOCH_3$, R_3=H
3 R_1=OH, R_2=R_3=H
4 R_1=R_3=H, R_2=OH
5 R_1=$COOCH_3$, R_2=R_3=OH



8



6 R_1=H
7 R_1=OH

Table 2. ^{1}H NMR data of the 8 tacaman type alkaloids

	Tacamine		16-epi-Tacamine		16R-Descarbomethoxy-tacamine		16S-Descarbomethoxy-tacamine	
	δ	J	δ	J	δ	J	δ	J
3	4.35 dddd	5.8, 2.3, 1.9, <0.5	4.11 m[a]		4.20 m[a]		4.35 m[a]	
5α	3.34 ddd	14.0, 6.5, 0.6	3.02 m[a]		3.14 m[a]		3.38 m[a]	
5β	3.43 ddd	14.0, 11.2, 5.5	3.07 m[a]		3.20 m[a]		3.40 m[a]	
6α	3.00 dddd	16.3, 11.2, 6.5, 2.3	2.90 m[a]		2.91 m[a]		3.02 dddd	15.9, 11.0, 6.7, 2.4
6β	2.59 dddd	16.3, 5.5, 1.9, 0.6	2.48 m[a]		2.50 m[a]		2.65 bd[a]	15.9
9	7.12 m		7.42 m		7.46 dd	6.9, 1.8	7.43 dd	7.4, 1.2
10	7.12 m		7.10 m		7.17 ddd	6.9, 6.9, 1.8	7.22 ddd	7.4, 7.4, 1.2
11	7.12 m		7.10 m		7.15 ddd	6.9, 6.9, 1.7	7.17 ddd	7.4, 7.4, 1.2
12	7.48 m		7.42 m		7.73 dd	6.9, 1.7	7.52 dd	7.4, 1.2
14	2.40 ddddd	12.7, 5.8, 4.2, 4.0, 3.1	2.08 m[a]		2.24 m[a]		2.39 m[a]	
15α	1.14 ddd	13.2, 12.7, 12.6	0.69 ddd	12.7, 12.7, 12.7	0.37 ddd	12.7, 12.7, 2.7	1.13 ddd	12.8, 12.5, 12.5
15β	1.67 ddddd	13.2, 4.0, 3.0, 1.6, <0.5	1.31 m[a]		1.53 bd[a]		1.77 bd	12.8[a]
16	-		-		5.58 dd	9.5, 5.4	6.06 dd	4.0, 1.2
17α	2.62 dd	14.3, 4.2	2.67 dd	14.9, 2.9	2.45 ddd	13.9, 5.4, 3.2	2.26 ddd	15.5, 3.5, 1.2
17β	2.19 dd	14.3, 3.1	2.22 dd	14.9, 4.2	1.89 ddd	13.9, 9.5, 3.6	2.40 ddd	15.5, 4.0, 4.0
18	0.86 t	7.3	0.77 t	7.3	0.81 t	7.2	0.88 t	7.3
19	~1.20 ddq	~14, 7.3, ~6.5	0.98 m		1.02 m		1.13 m	
19'	~1.20 ddq	~14, 7.3, ~6.5	0.98 m		1.02 m		1.13 m	
20	1.48 dddddd	12.6, 10.7, ~6.5, ~6.5, 3.5, 3.0	1.30 m[a]		1.40 m[a]		1.58 m[a]	
21α	2.15 dd	10.9, 10.7	2.06 dd	10.9, 10.9	1.92 dd	11.0, 11.0	2.26 dd	10.9, 10.9
21β	2.66 ddd	10.9, 3.5, 1.6	2.43 bd	10.9	2.50 m[a]		2.77 bd	10.8
CO_2Me	3.83 s		3.67 s		-		-	

[a] The coupling constants have not been actually determined, because this is only possible by series of decoupling experiments. It is however, evident from the similar, characteristic appearance of the signal from this proton to the corresponding proton in tacamine, that the individual coupling constants will not differ more than a few tenths of a hertz from those determined for tacamine.

	Tacamonine		17-Hydroxy-tacamonine		16,17-Anhydro-tacamine		19S-Hydroxy-tacamine	
	δ	J	δ	J	δ	J	δ	J
3	4.33 m[a]		4.65 m[a]		4.49 m[a]		4.40 m[a]	
5α	3.30 m[a]		3.37 m[a]		3.35 m[a]		3.37 m[a]	
5β	3.38 m[a]		3.37 m[a]		3.35 m[a]		3.46 m[a]	
6α	2.89 m[a]		2.93 m[a]		3.01 dddd	16.3,8.8,8.5,2.8	3.02 m[a]	
6β	2.48 bddd	17.0, 7.1, 3.1	2.52 bd	16	2.81 m[a]		2.63 m[a]	
9	7.43 m		7.46 m		7.24 m		7.13 m	
10	7.32 ddd	7.3, 7.3, 1.8	7.33 m		7.18 ddd	7.0, 7.0, 1.9	7.13 m	
11	7.28 ddd	7.3, 7.3, 1.8	7.33 m		7.13 ddd	7.0, 7.0, 1.9	7.13 m	
12	8.38 bdd	7.3, 1.8	8.34 bdd	7.0, 2.0	7.47 m		7.49 m	
14	2.45 m[a]		2.62 m		2.58 dddd	12.6,7.2,3.0,2.0	2.46 m[a]	
15α	0.56 ddd	12.6, 12.6, 12.6	0.40 ddd	12.4, 12.4, 12.4	0.52 ddd	13.0, 12.6, 12.6	1.29 ddd	13.0, 13.0, 13.0
15β	1.66 bd[a]	12.6	1.66 m		1.73 bd	13.0	1.62 bd	13.0
17α	2.99 dd	17.0, 4.9	-		6.39 d	7.2	2.65 dd	14.3, 4.0
17β	2.66 dd	17.0, 2.3	4.36 d	2.6	-		2.21 dd	14.3, 3.1
18	0.85 t	7.4	0.85 t	7.3	0.85 t	7.4	1.16 d	6.3
19	1.10 m		1.12 m		1.10 m		3.44 dd	6.6, 6.3
19'	1.10 m		1.12 m		1.10 m		-	
20	1.52 m[a]		not observable		1.54 m[a]		~1.7 m	
21α	2.03 dd	11.0, 10.9	2.06 dd	10.9, 10.9	2.23 dd	11.1, 11.1	2.31 dd	11.0, 11.0
21β	2.64 ddd	10.9, 3.7, 1.7	2.62 bd	10.0	2.71 ddd	11.1, 3.5, 1.9	2.99 bd	11.0
CO_2Me	-		-		3.94 s		3.84 s	

Table 3. ^{13}C NMR data of tacamine and 16-epi-tacamine

Carbon	tacamine	δ 16-epi-vincamine minus δ vincamine[a]	16-epi-tacamine predicted	16-epi-tacamine found
2	130.9	+0.2	131.1	130.9
3	54.2	+0.2	54.4	54.0
5	50.7	-0.1	50.6	50.4
6	17.0	-0.2	16.8	16.7
7	106.1	+0.2	106.3	106.2
8	128.8	-0.5	128.3	128.1
9	118.4	-0.3	118.1	118.0
10	121.6	-0.2	121.4	121.4
11	120.2	-0.1	120.1	120.0
12	110.4	+2.0	112.4	112.4
13	134.4	+1.5	135.9	135.9
14	32.1	+1.2	33.3	33.2
15	31.1	-1.0	30.1	29.6
16	81.8	+1.0	82.8	82.8
17	40.2	+2.4	42.6	42.6
18	11.5	-0.1	-[b]	11.2
19	26.9	0.0	-[b]	26.8
20	38.3	-0.1	-[b]	37.3
21	50.5	+0.1	50.6	50.2
CO	174.3	-2.1	172.2	172.3
OMe	54.2	-1.1	53.1	53.0

[a] The data used are those published by Bombarelli et al.[9]; except for carbon 21 in which case the data given by Kalaus et al.[28] were used.

[b] No prediction is possible due to the different site of attachment of the ethyl group.

like ^1H NMR. However its UV spectrum showed maxima at 242, 265, 294 and 303 nm indicating a different type of chromophore. As eburnamonine of the vincamine type has a similar UV spectrum, structure **6** was proposed for this alkaloid. This was confirmed by its ^1H NMR and mass spectral data. Its mass spectrum showed a M^+ at m/z 294 and in the ^1H NMR spectrum no methoxy signal or H-16 signal was observed. Except for H-12 (0.9 ppm deshielded), H-17α and H-17β (0.4 ppm deshielded) and H-15α (0.6 ppm shielded) its spectrum was similar to that of tacamine. Its CD spectrum is both qualitatively and quantitatively different from the mirror-image of the spectrum previously described for e-burnamonine.[12] A reason for this could be the relatively larger influence of the asymmetric C-20 (e-burnamonine)/C-14 (tacamonine) because C-16 is no longer an asymmetric center. From the minimum at 270 nm in the CD spectrum of tacamonine **6** and by biosynthetical reasoning it is thought that tacamonine has, as the other tacamines, a 3β-H. Recently tacamonine (syn. pseudovincamone) was synthesized by Massiot *et al.*[13] Their MS and 400 MHz ^1H NMR data are in excellent agreement with the data presented here. Their explanation of the extraordinary shielding of H-15α however is not followed here, *vide supra.*

17-*Hydroxy-tacamonine* **7**. This trace component has a similar UV spectrum as tacamonine **6**, suggesting the same kind of chromophore. This was confirmed by its colour reaction with ceric sulphate, which was also similar to tacamonine. Its R_f-value was smaller in solvents B and D suggesting the introduction of some polar group. Its ^1H NMR spectrum resembled the one of tacamonine, except that no H-17α and H-17β could be observed at their usual positions and instead a doublet at 4.36 ppm had appeared. The only explanation is the introduction of 17-OH group in tacamonine. This was proven by its mass spectrum showing a M^+ m/z 310, 16 mass units more than tacamonine. It is assumed that the OH group is in the equatorial position because of the small coupling constant between H-17 and H-14 and because the heavier group is then in the preferred position. Insufficient material precluded further substantiation of the configuration at C-17. The CD spectrum of 17-hydroxy-tacamonine is similar to that of tacamonine from 200–240 nm but is different from 240–350 nm. This is due to the introduction of an extra asymmetrical centre (C-17) near the chromophore, invalidating a comparison.

For biosynthetical reasons it is assumed that the alkaloid has a 3β-H. The absolute configuration is thus given by structure **7**.

16,17-*Anhydrotacamine* **8**. The UV spectrum of this alkaloid showed maxima at 228, 273 and 312 nm, which is characteristic for 16,17-anhydro-vincamine (apovincamine). It was therefore thought that the alkaloid might be 16,17-anhydro-tacamine. This was confirmed by both its mass spectral and ^1H NMR data. The mass spectrum showed M^+ m/z 336, which is 18 mass units ($-H_2O$) less than tacamine and the ^1H NMR was similar to the one of tacamine except for the absence of the signals of H-17α and H-17β at their

usual positions, the shielding of H-15α by 0.6 ppm, a change in the characteristic appearance of H-14 and the appearance of a doublet (J = 7.2 Hz) at 6.39 ppm. This is all in accordance with structure **8** for 16,17-anhydro-tacamine. To determine the absolute configuration 16,17-anhydro-tacamine was prepared semisynthetically from tacamine according to a procedure given by Mokrý *et al.*[14] and its CD spectrum compared with the CD-spectrum of the alkaloid isolated. The spectra were identical indicating that structure **8** is correct.

To minimize the possibility that 16,17-anhydro-tacamine might be an artefact formed from tacamine during the isolation procedure the two most critical steps in the isolation procedure (refluxing with 96% ethanol, extraction with 2% HOAc) were repeated with some pure tacamine. As no spots apart from the starting material could be detected on TLC after these operations, the possibility that 16,17-anhydro-tacamine might be an artefact is greatly reduced.

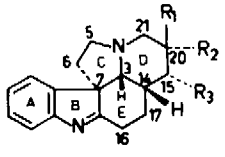
19S-*Hydroxy-tacamine* **5**. From its ^{1}H NMR spectrum it was evident that this alkaloid belonged to the tacamine-type. However the methyl group had changed from a triplet at 0.86 ppm into a doublet at 1.16 ppm. As the alkaloid was more polar than tacamine it was assumed to be a 19-hydroxy-tacamine. This was confirmed by its UV spectrum, identical to the one of tacamine, and its mass spectrum, M^+ at *m/z* 370, i.e. 354 + 16. No H-19 could be observed in the ^{1}H NMR spectrum. By a series of homonuclear decoupling experiments it became clear that this signal was obscured by H-5α and H-5β. The configuration at C-19 is thought to be S, because H-15α, H-21α and H-21β are deshielded respectively by 0.15 ppm, 0.16 ppm and 0.33 ppm, while H-15β is not deshielded at all. This indicates that the hydroxyl group is nearest to H-21β, which automatically means that the configuration must be S, as the relatively large methyl group is the least sterically hindered when it is as far away as possible from H-15α, H-15β, H-21α and H-21β. From the CD-spectrum it can be concluded that the absolute configuration at C-3 is the same as in tacamine. Thus 19S-hydroxy-tacamine has the absolute configuration as presented in structure **5**.

20R-1,2-*Dehydropseudoaspidospermidine* **9**. This major alkaloid exhibited an UV-spectrum typical of an indolenine (λ_{max} 221, 265 and 330 nm). Its mass-spectrum showed a M^+ *m/z* 280 and the base peak at *m/z* 137. This mass-spectral behaviour is characteristic for 1,2-dehydropseudoaspidospermidine isolated earlier from *Tabernaemontana eusepala* (syn. *Pandaca eusepala*[1]) and *T. mocquerysii* (syn. *Pandaca boiteaui*[1]).[15,16] From the positive $[\alpha]_D^{20}$ it was concluded that the configuration at C7 is the same as in the previously isolated (+) 20S-1,2-dehydropseudoaspidospermidine **10**.[15,16]

By careful comparison of the ^{1}H NMR spectra of this alkaloid and of 20S-hydroxy-1,2-dehydropseudoaspidospermidine **11** (vide infra), it was possible to determine the shifts and coupling constants of most of the protons, except for H-14, H-15, H-19 and H-20. From the similar shift coupling constants of H-3, H-5α and β, H-6α and β, H-16α and β, and, H-17α and β as in 20S-hydroxy-1,2-dehydropseudoaspidospermidine **11** it can be concluded that the conformation of all the rings is the same. Only the stereochemistry of C-20 remains to be determined. This is possible by measuring the values of the coupling-constants of H-21α and H-21β with H-20. However these signals coincide with H-16α and H-17α respectively, making them unintelligible. The signals of H-16α and H-17α, which do not coincide with other signals in the ^{1}H NMR spectrum of 20S - hydroxy - 1,2 - dehydro - pseudoaspidospermidine **11**, were therefore selected from this spectrum and after careful adjustment of the shift and the peak area, substracted by means of a computer from the mixed signals of H-16α and H-21α, and H-17α and H-21β in the spectrum of 20R-1,2-dehydro-pseudoaspidospermidine **9**. This is possible because the signals of H-16α and H-17α, due to the same conformation, are of exactly similar shape and spectral width in the two alkaloids. What remains after substraction is the pattern of H-21α and H-21β only. H-21α is clearly revealed as a broad doublet (J 21α- 21β: 11 Hz and $W_{\frac{1}{2}}$ of each peak 4.5 Hz) and H-21β as a doublet of doublets (J 21β − 21α: 11.0 Hz and J 21β − 20: 3.9 Hz).

From the coupling constants it can be concluded that the ethyl group is in the α-position (axial) and H-20 in the β-position (equatorial). In the opposite 20S configuration H-21α would have appeared as a ddd with J = *ca* 11, 3.5 and 1.6 Hz and H-21β as a dd with J = *ca* 11 and 11 Hz as for instance in tacamine which also has the D-ring in a chair conformation. In the present 20R-configuration the coupling constants are comparable with those found for H-21α and H-21β in the enantiomer of 20-epi-tacamonine.[13] Thus the absolute configuration of (+) 20R-1,2-dehydropseudoaspidospermidine is presented by structure **9**.

20S - *Hydroxy* - 1,2 - *dehydropseudoaspido-spermidine* **11**. This minor alkaloid had a similar UV spectrum and gave the same orange colour with Ce^{4+} as the alkaloid described above **9**. Its mass spectrum was also similar, except that almost all peaks were

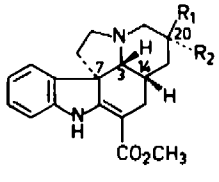


9 $R_1 = R_3 = H$, $R_2 = C_2H_5$

10 $R_1 = C_2H_5$, $R_2 = R_3 = H$

11 $R_1 = OH$, $R_2 = C_2H_5$, $R_3 = H$

12 $R_1 = H$, $R_2 = C_2H_5$, $R_3 = OH$



13 $R_1 = C_2H_5$, $R_2 = OH$

14 $R_1 = OH$, $R_2 = C_2H_5$

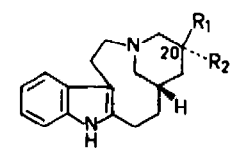
15 $R_1 = H$, $R_2 = C_2H_5$

Table 4. ^{1}H NMR data of 20S-hydroxy-1,2-dehydro-pseudoaspidospermidine

H nr	δ	coupling constants (Hz)
3	2.82	J3-14: 2.9; J3-17β < 1
5α	3.18	J5α-5β: 8.4; J5α-6α: 6.7; J5α-6β ≈2
5β	2.88	J5α-5β: 8.4; J5β-6α: 11.4; J5β-6β: 4.8
6α	2.31	J5α-6α: 6.7; J5β-6α: 11.4; J6α-6β: 12.1
6β	1.82	J5α-6β ≈2; J5β-6β: 4.8; J6α-6β: 12.1
9	7.35	J9-10: 7.7; J9-11: 1.4; J9-12 <0.5
10	7.30	J9-10: 7.7; J10-11: 7.7; J10-12: 1.1
11	7.17	J9-11: 1.4; J10-11: 7.7; J11-12: 7.7
12	7.51	J9-12 <0.5; J10-12: 1.1; J11-12: 7.7
14	1.53	J3-14: 2.9; J14-15α: 1.9; J14-15β: 5.6; J14-17α: 12.9; J14-17β: 5.6
15α	1.83	J14-15α:1.9; J15α-15β: 13.6; J15α- 21α: 1.8
15β	1.41	J14-15β: 5.6; J15α-15β: 13.6; J15β-19 ≈0.5
16α	2.97	J16α-16β: 15.4; J16α-17α:11.0; J16α-17β: 3.7
16β	2.78	J16α-16β: 15.4; J16β-17α: 6.5; J16β-17β: 9.9
17α	2.52	J14-17α: 12.9; J16α-17α: 11.0; J16β-17α: 6.5; J17α-17β: 12.2
17β	1.75	J3-17β <1; J14-17β: 5.6; J16α- 17β:3.7; J16β-17β: 9.9; J17α-17β: 12.2
18	0.97	J18-19: 7.5; J18-19': 7.5
19	1.77	J15β-19 ≈0.5; J18-19: 7.5; J19-19': 14.8
19'	1.96	J18-19': 7.5; J19-19': 14.8
21α	3.12	J15α-21α: 1.8; J21α-21β: 10.5
21β	2.36	J21α-21β: 10.5
OH	not observed	

shifted 16 mass units, suggesting the introduction of an oxygen in the molecule. The only known alkaloid of this type is capuronidine **12** isolated from *Tabernaemontana capuronii* (syn. *Capuronetta elegans*[1]).[17,18] However the 300 MHz ^{1}H NMR spectrum was different from the 240 MHz ^{1}H NMR spectrum given for capuronidine.[18] For instance, no signal at 3.6 ppm for H-15 was observed. This indicated that the hydroxy group was attached to another carbon, the most likely carbon being C-20 as several indole alkaloids with a C-20 hydroxy group are known (pandoline **13**, velbanamine). To prove this structure a detailed high resolution ^{1}H NMR study of the alkaloid was performed. By means of homonuclear decoupling experiments it was possible to assign all the protons and to determine all the coupling constants (Table 4). From the coupling constants, especially those between H-3 and H-14 (J = 2.9 Hz) and, H-15α and H-21α (J = 1.8 Hz, W-path), it could be concluded that the alkaloid has a conformation of the C- and D-rings identical to the conformation of these rings in pandoline[13]/20-epipandoline **14**.[19] For the E-ring a boat-conformation automatically follows, which was confirmed by the large coupling constants between H-16α and H-17α (J = 11.0 Hz) and, H-16β and H-17β (J ≈ 9.9 Hz). The configuration at C-20 can be determined by the shift of H-19 and H-19'. In pandoline **13** H-19 and H-19' resonate at 1.47 and 1.54 ppm respectively and in 20-epi-pandoline **14** at 1.75 and 1.85 ppm.[20] In the present alkaloid H-19 and H-19' were found at 1.77 and 1.96 ppm indicating that the configuration at C-20 was the same as in 20-epi-pandoline **14**, namely 20S. The position of H-3 was determined as β, because the alkaloid had a CD spectrum similar to that of 20R-1,2-dehydro-pseudoaspidospermidine **9**, whose absolute configuration is known (vide supra). Thus the absolute configuration of 20S-hydroxy-1,2-dehydro-pseudoaspidospermidine is presented by structure **11**. The C-20 isomer of this alkaloid has been prepared from pandoline **13** by Le Men *et al.*[21] during the structure elucidation of pandoline.

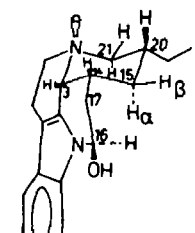
14S,20R-*Velbanamine* **18**. This alkaloid, which was isolated in trace amounts, gave a similar purple colour with Ce^{4+} as the dihydrocleavamines **16** and **17** and also showed the UV spectrum of a normal



16 $R_1 = C_2H_5$, $R_2 = H$

17 $R_1 = H$, $R_2 = C_2H_5$

18 $R_1 = C_2H_5$, $R_2 = OH$



3

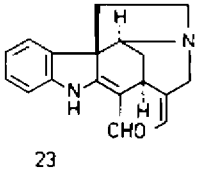
indole. Its mass spectrum was similar to that of (+)20R-15,20-dihydro-cleavamine except for the fact that many peaks were shifted 16 mass units, indicating the presence of a hydroxy group. The hydroxy group must be situated at the 20-position

because of the presence of a peak at m/z 267 and the absence of a peak at m/z 280 (M^+-H_2O). This last peak is characteristic for capuronine (15-hydroxy-dihydro-cleavamine).[18] The stereochemistry of C-14 is thought to be S because all ibogan alkaloids and tacaman alkaloids isolated from this plant or the genus *Tabernaemontana* in general have the same configuration at C-14. If the configuration at C-14 is assumed to be S, then the configuration at C-20 is R, because coTLC in systems B and D showed that the alkaloid isolated had similar R_f values and gave similar colour reactions as an authentic sample of (−) velbanamine. The C-20 epimer, (+)-isovelbanamine gave different R_f values and different colours with the spray reagents. Although the velbanamines have been known for a long time, because they are part of the oncolytic agents vinblastine and vincristine (14R configuration) they have not yet been isolated as such. 14S, 20S- and 14S, 20R-velbanamine were recently synthesized by Takano *et al.*[22] The other alkaloids (see Table 1) isolated are all known alkaloids and all of them except for norfluorocurarine **23** have been isolated earlier from one or more *Tabernaemontana* species. (−)20S-15,20-Dihydro-cleavamine **16** was identified only by the presence of some characteristic minor signals in the ^{13}C NMR of (+)20R-15,20-dihydro-cleavamine **17**. Due to the minimal amount of (−)20S-15,20-dihydro-cleavamine **16** present, no separation of the two isomers was attempted. The pseudovincadifformine isolated is probably a mixture of the two 20-epimers, although this could not be concluded with absolute certainty because the amount isolated was too small for a separation of the two possible epimers.

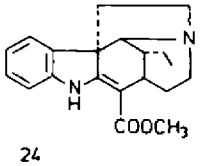
Biosynthesis. It seems likely that the tacaman class alkaloids are derived from pseudovincadifformine **15** in the same manner as the eburnan class of alkaloids are derived from vincadifformine.[23,8] The exact biosynthetic pathways are not yet known; most recently the biosynthesis of vincamine was summarized by Kutney.[24]

Chemotaxonomy. The *T. eglandulosa* species investigated in this study contains more alkaloids from the latter stages of the indole alkaloid biosynthesis than any other *T.* species investigated so far. It contains mainly alkaloids of the new tacaman class and the ibogan class.[6] From the ibogan class it contains three of the currently known six types, viz the pseudoaspidospermidine type (C-14/C-17 bond and C-3/C-7 bond), the cleavamine type (C-14/C-17 bond only) and the common ibogamine type (C-14/C-17 bond and C-16/C-21 bond). No ibogan alkaloids of the pandine, ibophyllidine and iboxyphylline type were isolated. Besides the alkaloids of these classes traces of voaphylline **22**, which belongs to the plumeran class,[6] tubotaiwine **24**, which belongs to the aspidospermatan class and norfluorocurarine **23**, which belongs to the strychnan class were also isolated indicating that the plant is still capable of synthesizing alkaloids of quite different and biosynthetical earlier classes. No alkaloids of the non-rearranged corynanthean class or of the dimeric corynanthean-ibogan type were isolated, although these alkaloids occur frequently in *T.* species.[25] Why alkaloids of the tacaman class have not been found before, while now eight in relatively high concentrations have been isolated from an already investigated species is unclear. Of the 11 alkaloids previously isolated from the species only one, namely coronaridine **19**, the most common *T.* alkaloid has been isolated in small amounts from the species investigated in this study. This is not due to the plant part, as tacamine, 16-epi-tacamine and tacamonine were also detected in the stembark of the species investigated by means of TLC. Three possibilities remain therefore; firstly the *T. eglandulosa* grown in the greenhouse originated from Cameroun and may be of a different chemical race than the species growing in Nigeria and Tanzania, where the material used for earlier studies was collected; secondly during the 20 years of cultivation in The Netherlands some changes in the biosynthesis and metabolism of secondary plant products due to light changes, temperature and soil may have taken place; thirdly the plantmaterial of the earlier studies may have been wrongly identified. We think that a combination of the first two possibilities is most likely. The best way to check this, would be to collect some new material of *T. eglandulosa* from Cameroun. We hope to report about such an investigation shortly.

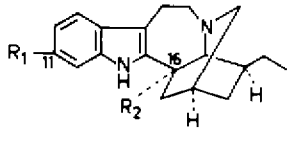
Pharmacology. The main reason for Massiot *et al.* to synthesize the four different isomers of tacamonine **6**,[13] was to compare their hypotensive effect with the



23



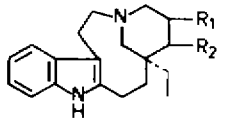
24



19 R_1 = H, R_2 = $COOCH_3$

20 R_1 = OH, R_2 = $COOCH_3$

21 R_1 = R_2 = H



22 R_1 + R_2 = β-epoxide

effect of the known eburnamonines. Similarly, it would be interesting to compare the hypotensive effect of the other seven tacamines with those of the corresponding vincamines. A necessary condition for these studies will be the synthesis of these alkaloids, as several of them occur only as minor components.

EXPERIMENTAL

Plant materials of *Tabernaemontana eglandulosa* Stapf were collected between 1977 and 1980 from a species cultivated in the greenhouse of the Agricultural University of Wageningen, The Netherlands. Voucher specimens (Lg 11028) are kept in the herbarium at Wageningen. The seeds were originally collected by F. J. Breteler in April 1961 in Cameroun, 6 km east of Bertoua, along the road to Batouri and Bétaré Oya (Br. 1297). ^{1}H NMR spectra were recorded in $CDCl_3$ at 300 MHz. The ^{13}C NMR spectra were recorded in $CDCl_3$ at 25.2 MHz in the Fourier transform mode.

High-resolution MS were determined at 70 eV using a direct inlet system. CD spectra were recorded in MeOH. The following TLC systems were used in combination with silicagel plates: A: Toluene—100% EtOH sat with NH_3 (49:1); B: Toluene—100% EtOH sat with NH_3 (19:1); C: Toluene—100% EtOH sat with NH_3 (4:1); D: Cyclohexane—$CHCl_3$–Et_2NH (6:3:1); E: EtOAc—iso-PrOH-conc. NH_4OH (17:2:1); F: EtOAc. Prior to development the plates were left standing in an atmosphere of NH_3 for 20 min in the case of solvents A–C.

After development the plates were sprayed with 1% $Ce(SO_4)_2$ in 10% H_2SO_4 or with 0.2 M $FeCl_3$ in 35% $HClO_4$, followed by heating with hot air. The isolation procedure was the same for leaves and twigs; 1500 g twigs and 675 g leaves were extracted for 15 h with 96% EtOH in a Soxhlet apparatus working under 0.25 atm. After filtration the EtOH was evaporated to dryness *in vacuo*. The extracts were partitioned between EtOAc and 2% HOAc. The aq layer was collected and brought to pH = 8 with NH_4OH and extracted with EtOAc. The EtOAc layer was collected, dried over dry Na_2SO_4 and evaporated *in vacuo*. Yield for the leaves 230 mg (0.034%) and for the twigs 530 mg (0.035%). The extracts were first separated on a Si gel column using system B as the mobile phase. The fractions were further separated and purified by means of prep. TLC (0.50 mm) with systems A–F. The alkaloids were identified by means of their spectral data, colour reactions and if possible by TLC comparison with authentic samples.

Tacamine **1**. TLC: R_f in system B 0.31, C 0.58, D 0.35, E 0.53; Ce^{4+}: yellow, intensifying upon heating; UV λ_{max}^{MeOH} nm: 227, 277, 282, 290 (sh); CD $\lambda(\Delta\epsilon)$: 212(0.0), 227(+14.8), 234(0.0), 238(−6.3), 250(−3.9), 272(−5.0); MS(50°) *m/z* (rel. int.) 355(31), 354(M^+, 100), 353(89), 339(22), 336(3), 295(23), 294(10), 293(37), 292(35), 267(4), 265(3), 252(62), 238(5), 237(5), 223(42), 196(13); ^{1}H NMR: see Table 2 and Fig. 1; ^{13}C NMR: see Table 3.

16-*Epi-tacamine* **2**. TLC: R_f in system B 0.13, C 0.46, D 0.08; Ce^{4+}: yellow, intensifying upon heating; UV λ_{max}^{MeOH} nm: 225, 274, 280, 289 (sh); CD $\lambda(\Delta\epsilon)$: 212(+2.6), 217(0), 222(+4.1), 232(+2.6), 240(+8.3), 257(0), 272(−2.4), 280(−2.0), 283(−2.2), 321(0); MS *m/z* (rel. int.) 354(M^+,49), 353(45), 336(4), 295(6), 293(14), 292(15), 267(3), 253(10), 252(23), 238(15), 237(15), 223(14), 197(3), 196(5), 59(100); ^{1}H NMR: see Table 2; ^{13}C NMR: see Table 3.

16R-*Descarbomethoxy-tacamine* **3**. TLC: R_f in system B 0.16; Ce^{4+}: yellow; UV λ_{max}^{MeOH} nm: 228, 277, 282, 290; CD $\lambda(\Delta\epsilon)$: 205(0), 229(−7.0), 240(+3.6), 248(0), 272(−3.8), 304(0), 315(+1.4); MS *m/z* (rel. int.) 296(M^+, 100), 295(90), 278(5), 277(10), 268(3), 267(3), 253(5), 252(8), 235(16), 234(62), 223(10), 221(6.5), 194(5), 193(10), 180(15), 170(6.5), 169(8), 168(14), 167(10.5); ^{1}H NMR: see Table 2.

16S-*Descarbomethoxy-tacamine* **4**. TLC: R_f in system B 0.22, E 0.50; Ce^{4+}: yellow UV λ_{max}^{MeOH} nm: 228, 277, 282, 290; CD $\lambda(\Delta\epsilon)$: 207(0), 228(+20.9), 238(0), 241(−1.8), 247(−1.3), 286(−3.2); MS(100°) *m/z* (rel. int.) 296(M^+, 64), 295(71), 278(26), 277(23), 268(1), 267(2), 253(5), 252(6), 235(25), 234(100), 223(11), 221(8), 207(5), 206(5), 194(10), 193(12), 180(48), 168(10), 167(11); ^{1}H NMR: see Table 2.

Tacamonine **6**. TLC: R_f in system B 0.37, D 0.32; Ce^{4+}: no immediate colour, after 15 min orange; UV λ_{max}^{MeOH} nm: 242, 265, 273 (sh), 294, 303; CD $\lambda(\Delta\epsilon)$: 205(−7.3), 217(0), 225(+5.2), 233(+3.8), 253(0), 256(−0.7), 270(−2.3), 284(0), 304(+1.8); MS *m/z* (rel. int.) 294(M^+,100), 293(88), 266(2), 265(2.5), 251(10), 250(22), 248(4), 247(7), 246(4.5), 223(5), 222(9), 210(5), 209(35), 196(7.5), 182(5), 181(6.5), 180(13), 169(8), 168(27), 167(25), 147(M^{2+},4); ^{1}H NMR: see Table 2; m.p. 180–181° (uncorrected).

17-*Hydroxy-tacamonine* **7** TLC: R_f in system B 0.18, D 0.14, E 0.43; Ce^{4+}: no immediate colour, after 15 min orange; UV λ_{max}^{MeOH} nm: 221; 225, 242, 267, 290, 302 (sh); CD $\lambda(\Delta\epsilon)$: 208(−6.1), 213(0), 222(+4.3), 255(+1.4), 263(+2.7), 284(0), 296(0), 321(+3.0); MS (150°) *m/z* (rel. int.) 310(M^+, 100), 309(100), 292(42), 291(47), 282(8), 281(10), 280(10), 268(18), 253(16), 252(9), 251(17), 250(16), 223(12), 209(35), 197(14), 196(17), 195(15), 180(26), 168(31), 167(40); ^{1}H NMR: see Table 2.

16,17-*Anhydro-tacamine* **8** TLC: R_f in system B 0.42, D 0.40; Ce^{4+}: yellow UV λ_{max}^{MeOH} nm: 228, 273, 312; CD $\lambda(\Delta\epsilon)$: 211(0), 230(−67.5), 265(−6.7), 279(−0.9), 293(−5.5), 306(0), 325(+6.2); MS *m/z* (rel. int.) 337(15), 336(M^+,67), 335(39), 293(35), 292(100), 278(14), 277(17), 276(20), 252(12), 251(8), 239(10.5), 238(59), 234(21), 219(4), 194(4), 193(6), 192(4), 191(3.5), 180(26), 179(13), 178(7.5), 168(8), 167(5); ^{1}H NMR: see Table 2.

19S-*Hydroxy-tacamine* **5**. TLC: R_f in C 0.25, F 0.05; UV λ_{max}^{MeOH} nm: 227, 277, 280, 291; CD $\lambda(\Delta\epsilon)$: 214(0), 225(+8.6), 232(0), 238(−5.9), 248(−3.4), 270(−4.2); MS (125°) *m/z* (rel. int.) 370(M^+,100), 369(84), 355(26), 352(3), 311(13), 310(16), 309(55), 308(26), 293(16), 269(19), 268(61), 267(23), 225(19), 223(16); ^{1}H NMR: see Table 2.

(+)20R-15,20-*Dihydro-cleavamine* **17**. TLC: R_f in system B 0.73, D 0.49, F 0.55; Ce^{4+}: purple, Fe^{3+}: no immediate colour, greyish-black upon heating; UV λ_{max}^{MeOH} nm: 228, 283, 290; $[\alpha]_D^{20}$ + 133° ($CHCl_3$; c = 0.078); CD $\lambda(\Delta\epsilon)$: 214(+4.2), 221(0), 231(−11.5), 240(0), 270(+2.1), 300(+0.6), 320(+2.0); MS(100°) *m/z* (rel. int.)283(13), 282(58), 281(20), 280(19), 254(8), 238(1.5), 237(2), 236(2.5), 196(5), 195(6), 182(6), 180(6), 170(2.5), 169(3), 168(5), 167(4), 158(5), 157(9), 156(7), 152(8), 144(11), 143(9), 141(13), 139(23), 138(100), 137(23), 125(11), 124(71), 122(7.3); ^{1}H NMR: δ 7.83(bs,NH), 7.44 (d, J = 6.8 Hz, H-12), 7.28 (m, H-9), 7.13–7.04 (m, H-10 and H-11), 3.65(bdd, J = 12 and 12 Hz), 1.30(m,H-19), 0.88(t,J = 7.3 Hz, H-18). ^{13}C NMR: δ 11.6 (C-18), 21.4(C-16), 26.0(C-6), 28.7(C-19), 31.2(C-15), 32.8(C-20), 33.8(C-17), 35.0(C-14), 51.4(C-3), 52-3(C-5), 58.7(C-21), 109.9(C-7 and C-12), 117.7(C-9), 118.8(C-10), 120.6(C-11), 128.5(C-8), 135.5(C-13), 138.6(C-2). The values presented here are identical with the data given by Wenkert *et al.*[26] for 15,20α-dihydro-cleavaine.

(−)20S-15,20-Dihydro-cleavamine **16**. ^{13}C NMR: δ 24.1(C-6), 27.5(C-19), 32.9(C-20), 37.6(C-15), 53.2(C-5), 61.2(C-21), 118.6(C-10). The signals of the other carbons were either obscured by the much larger signals of the 20R isomer or were lost in the noise. However the signals, which were observed are identical with the data given by Wenkert *et al.*[26] for 15,20β-dihydro cleavamine.

14S, 20R-*Velbanamine* **18**. TLC: R_f in system B 0.48, D 0.26; Ce^{4+}: purple UV λ_{max}^{MeOH} nm: 287; MS(200°) *m/z* (rel. int.) 229(4), 298(M^+, 18), 297(4), 296(10), 295(4), 267(3), 241(2), 198(3), 197(3), 196(4), 195(3), 182(6), 180(4), 170(3), 169(4), 168(8), 167(5), 157(8), 156(11), 155(12), 154(100%), 153(8), 144(11), 143(13), 130(8), 124(6).

(+)20R-1,2-*Dehydro-Ψ-aspidospermidine* **9**. TLC: R_f in system B 0.55, D 0.51; Ce^{4+}: orange with white center, after 15 min gold; UV λ_{max}^{MeOH} nm: 221, 265, 270, 328, 345, $\lambda_{max}^{MeOH+NaOH}$ nm: 221, 255, 292 (sh), $\lambda_{max}^{MeOH+HCl}$ nm: 220, 273,

290 (sh), 330; $[\alpha]_D^{20}$ + 209° ($CHCl_3$; c = 0.44); CD $\lambda(\Delta\varepsilon)$: 202(0), 223(−11.9), 234(−4.2), 246(−5.4), 263(0), 271(+2.1), 284(0), 300(−1.7), 305(−1.3); MS(30°) *m/z* (rel. int.) 281(20), 280(M^+, 75), 279(17), 265(2), 252(5), 251(13), 238(4), 237(11), 236(12), 197(8), 196(15), 195(31), 194(11), 193(16), 183(7), 182(18), 181(9), 180(26), 169(7), 168(12), 167(11), 158(21), 157(20), 138(21), 137(100), 136(19), 124(22); ^{1}H NMR; δ 7.50 (bd, J = 7.7 Hz, H-12), 7.35(bd, J = 7.7 Hz, H-9), 7.28(ddd, J = 7.7, 7.7 and 1.3 Hz, H-10), 7.16(ddd, J = 7.7, 7.7 and 0.9 Hz, H-11), 3.19 (ddd, J = 8.8, 6.7 and 2 Hz, H-5α), 3.00 (bd, J = 11.0, 1.8 and 1.8 Hz, H-21α), 2.97(ddd, J = 15.5, 11.1 and 3.9 Hz, H-16α), 2.78(ddd, J = 11.4, 8.8 and 4.8 Hz, H-5β), 2.75(ddd, J = 15.5, 10.2 and 6.3 Hz. H-16β), 2.71(bd, J = 3.1 Hz, H-3), 2.55(dddd, J = 12.9, 12.2, 11.1 and 6.3 Hz, H-17α), 2.53(dd, J = 11.0 and 3.9 Hz, H-21β), 2.28(ddd, J = 12.2, 11.4 and 6.7 Hz, H-6α), 1.79(bdd, J = 12.2, 4.8 and 2 Hz, H-6β), 1.8–1.4(7H, m, H-14, H-15α, H-15β, H-17β, H-19, H-19′, H-20), 0.92(t, J = 7.1 Hz, H-18); ^{13}C NMR δ: 13.1(C-18), 25.5*(C-16), 27.3*(C-19), 29.1*(C-15), 32.0*(C-17), 32.4*(C-20), 34.9*(C-14), 35.5*(C-6), 54.8■(C-5), 55.5■(C-21), 62.7(C-7), 74.3(C-3), 119.7(C-12), 121.5(C-9), 125.1(C-10), 127.6(C-11), 146.0(C-8), 154.6(C-13), 190.6(C-2). Assignments of signals with * or ■ are not certain. These signals may be interchanged.

20S-Hydroxy-1,2-dehydro-Ψ-aspidospermidine **11**. TLC: R_f in system B 0.26, D 0.24; Ce^{4+}: orange; UV λ_{max}^{MeOH} nm: 220, 258, 270, 290 (sh), $\lambda_{max}^{MeOH+NaOH}$ nm: 220 (sh), 258, 270 (sh), 292 (sh), $\lambda_{max}^{MeOH+HCl}$ nm: 220, 273, 288 (sh), 320; CD $\lambda(\Delta\varepsilon)$: 215(0), 233(−5.1), 238(−0.1), 248(−0.9), 255(0), 271(+4.3), 304(0), 310(−0.3); MS *m/z* (rel. int.) 296(M^+, 100), 295(16), 279(10), 268(7.5), 267(21), 254(5.5), 253(8), 252(7.5), 240(6), 239(8), 224(5.5), 209(4.5), 198(21), 197(21.5), 196(22), 195(30), 185(22), 184(8), 183(20), 182(25), 181(10), 180(23.5), 169(12), 168(21.5), 167(19), 158(16), 157(23), 156(18), 154(36), 153(68), 130(19), 124(14): ^{1}H NMR: see Table 4.

20R-Pseudo-vincadifformine **15** *and 20S-Pseudo-vincadifformine (mixture)*. TLC: R_f in system B 0.71, D 0.54, F 0.61; Ce^{4+}: blue; UV λ_{max}^{MeOH} nm: 205, 219, 293, 326; MS(125°) *m/z* (rel. int.) 339(5), 338(M^+,21), 323(1), 307(1.5), 305(1.5), 293(6), 239(2), 238(2), 214(2), 205(2.5), 194(2), 193(2), 180(3), 168(3.5), 167(3.5), 167(5), 154(4), 125(12), 124(100), 122(7); ^{1}H NMR: δ 8.9(bs, NH), 7.3–6.8 (aromatic H), 3.77(s, $COOCH_3$), 3.65(s, $COOCH_3$), 0.95(t, J = 7.5 Hz. H-18), 0.93(t, J = 7.5 Hz. H-18). The spectral data are in accordance with the data given by Kuehne *et al.*[27].

Coronaridine **19**. TLC: R_f in system B 0.69, D 0.48, F 0.69; Ce^{4+}: pale blue with white center, quickly fading; UV λ_{max}^{MeOH} nm: 226, 280(sh), 285, 293, CD $\lambda(\Delta\varepsilon)$: 205(0), 211(+4.6), 216(+2.0), 232(+4.1), 236(+3.3), 245(+7.4), 272(0), 299(−2.3); MS(90°) *m/z* (rel. int.) 338(M^+, 100), 323(21), 309(5), 279(6), 253(11), 214(17), 208(18), 195(9), 169(M^{2+}, 14), 154(19), 148(10), 136(71), 130(14), 124(38), 122(31); ^{1}H NMR: δ 7.75(bs, NH), 7.47(bd, J = 7.3 Hz, H-9), 7.24(dd, J = 7.3 Hz and 1.0 Hz, H-12), 7.14(ddd, J = 7.3, 7.3 and 1.0 Hz, H-11), 7.07(ddd, J = 7.3, 7.3 and 1.0 Hz, H-10), 3.70(s, $COOCH_3$), 3.55(s, H-21), 0.89(t, J = 7.4 Hz, H-18).

11-Hydroxy-coronaridine **20**. TLC: R_f in system B 0.16, D 0.04; Ce^{4+}: black–purple; UV λ_{max}^{MeOH} nm: 225, 277, 298; MS(100°) *m/z* (rel. int.) 354(M^+, 100), 353(14), 325(3), 295(5), 269(6), 230(11), 208(22), 177(M^{2+}, 14), 170(8), 148(11), 147(17), 146(20), 136(63), 124(40), 122(34); ^{1}H NMR: δ 7.60(bs, NH), 7.28(d, J = 8.5 Hz, H-9), 6.69(d, J = 2.1 Hz, H-12), 6.63(dd, J = 8.5 and 2.1 Hz, H-10), 3.71(s, $COOCH_3$), 3.52(s, H-21), 0.89(t, J = 7.3 Hz, H-18).

Ibogamine **21**. TLC: R_f in system A 0.60, D 0.46, F 0.12; Ce^{4+}: blue, quickly changing in purple; UV λ_{max}^{MeOH} nm: 224, 282, 289; MS(175°) *m/z* (rel. int.) 281(21), 280(M^+, 85), 265(18), 251(6), 195(45), 182(8), 181(6), 180(12), 168(14), 156(15), 149(42), 140(16), 136(100), 135(70), 124(29), 122(39).

Voaphylline (syn. conoflorine) **22**. TLC: R_f in system A 0.45, B 0.67, D 0.37; Ce^{4+}: purple UV λ_{max}^{MeOH} nm: 228, 284, 293; CD $\lambda(\Delta\varepsilon)$: 210(−10.2), 226(0), 233(+7.4), 241(0), 270(−3.8), 315(0); MS(125°) *m/z* (rel. int.) 297(17), 296(M^+, 67), 267(9), 249(14), 224(11), 140(100), 124(45), 210(16), 156(38), 144(29), 143(31), 140(100), 124(45), 122(43); ^{1}H NMR: δ 7.79(bs,NH), 7.45(bd, J = 7.5 Hz), 4.18(bdd, J = 13.5 and 13.5 Hz, H-16), 1.12(q, J = 7.6 Hz, H-19), 0.75(t, J = 7.6 Hz, H-18).

Norfluorocurarine **23**. TLC: R_f in system B 0.34, D 0.31; Ce^{4+}: blue, fading within 5 sec.; UV λ_{max}^{MeOH} nm: 242, 296, 363; MS(175°) *m/z* (rel. int.) 293(10), 292(M^+,44), 277(4), 263(12), 249(27), 247(12), 236(13), 234(10), 231(14), 222(15), 221(7), 220(11), 219(7), 208(17), 206(14), 194(21), 193(16), 180(22), 168(22), 167(35), 154(14), 121(100), 106(20).

Tubotaiwine **24**. TLC: R_f in system B 0.33, D 0.39, E 0.48; Ce^{4+}: dark blue, after 5 min pink, Fe^{3+}: blue upon heating, after 5 min yellow center; UV λ_{max}^{MeOH} nm: 228, 295, 327; MS(125°) *m/z* (rel. int.) 324(M^+, 49), 309(4), 293(8), 267(25), 265(19), 253(19), 229(77), 194(44), 182(49), 181(54), 180(100), 167(79); ^{1}H NMR: δ 8.85(bs, NH), 7.16(bd, J = 7.3 Hz), 7.12(ddd, J = 7.3, 7.3 and 1.1 Hz), 6.89(ddd, J = 7.3, 7.3 and 1.1 Hz), 6.82(bd, J = 7.3 Hz), 3.90(bs, H-21), 3.77(s, $COOCH_3$), 0.82(m, H-19), 0.70(t, J = 6.7 Hz, H-18).

Preparation of 16,17-*anhydrotacamine* **8** *from tacamine* **1**

10 mg tacamine was dissolved in 4 ml dry MeOH, saturated with HCl gas, and refluxed for 2 hr. After cooling the solvent was evaporated *in vac.* and the residue examined on TLC with solvent B. Apart from some unreacted tacamine the residue consisted mainly of 16,17-anhydro-tacamine, which was purified by means of prep TLC. The amorphous product thus obtained (7 mg) was homogeneous on TLC and had a similar R_f-value, colourreaction with Ce^{4+}, UV- and CD-spectrum as the 16,17-anhydro-tacamine isolated.

Investigation of possible artefact formation from tacamine

5 mg tacamine was dissolved in 5 ml 96% EtOH and refluxed for 15 hr under a pres. of 0.2 atm. After cooling the solvent was evaporated *in vac.* and the residue examined on TLC with solvent B. No spot other than that of tacamine was visible.

5 mg tacamine was dissolved in 5 ml 2% HOAc and the solution was left standing in daylight and in contact with air for 6 hr. After evaporation of the solvent *in vac.* the residue was examined on TLC with solvent B. No spot other than that of tacamine was visible.

Acknowledgements—In the first place we wish to thank Dr. A. J. M. Leeuwenberg for the identification and the kind gift of the plantmaterial. Further we would like to thank Prof. L. le Men-Olivier and Dr. G. Massiot for the copies of the ^{1}H NMR-, UV- and mass spectra of (+) - 2OR - 15,20 - dihydro-cleavamine, (−)20S - 15,20 - dihydro-cleavamine and (+)20S - 1,2 - dehydro-pseudoaspidospermidine and pseudovincadifformine, Prof. M. Hesse for his gift of conoflorine and coronaridine, Prof. D. G. I. Kingston for his gift of ibogamine and coronaridine, Prof. G. A. Cordell for this gift of coronaridine, Prof. S. Takano for his prompt gift of (−)velbanamine and (+)isovelbanamine, Mr. A. W. M. Lefeber for recording the ^{13}C NMR spectra, Mr. J. J. van Houte for recording the mass spectra, Dr. J. van Thuijl for his advice in interpreting the mass spectra and Drs. P. P. Lankhorst and Mr. C. Erkelens for performing some of the specialized ^{1}H NMR measurements. General financial support by the "Fonds Harold Quintus Bosz" and the "Van Leersum Fonds" for studies of Tabernaemontana species is gratefully acknowledged.

REFERENCES

[1]A. J. M. Leeuwenberg, personal communication (1983).
[2]G. Hulstaert, in Notes de botanique Mongo (Memoire

17/12/63) p. 57, (1966). Koninklijke Academie voor Overzeese Wetenschappen, Belgium.
[3]V. C. Agwada, Y. Morita, U. Renner, M. Hesse and H. Schmid, *Helv. Chim. Acta* **58**, 1001 (1975).
[4]M. B. Patel, C. Miet and J. Poisson, *Ann. Pharm. Franc.* **25**, 379 (1967).
[5]J. Le Men, P. Potier, L. Le Men-Olivier, J. M. Panas, B. Richard and C. Potron, *Bull. Soc. Chim. France*, 1369 (1974).
[6]M. V. Kisakürek and M. Hesse, in: *Indole and Biogenetically Related Alkaloids*, (Edited by J. D. Phillipson and M. H. Zenk), p. 15. Academic Press, New York (1980).
[7]P. Perera, T. A. van Beek and R. Verpoorte, Planta Medica, in press.
[8]T. A. van Beek, P. P. Lankhorst, R. Verpoorte and A. Baerheim Svendsen, *Tetrahedron Letters* 4827 (1982).
[9]E. Bombardelli, A. Bonati, B. Gabetta, E. M. Martinelli and G. Mustich, *Fitoterapia* **46**, 51 (1975).
[10]K. Blaha, K. Kavkova, Z. Koblicova and J. Trojanek, *Coll. Czech.* Chem. Commun. **33**, 3833 (1968).
[11]B. Danieli, B. Gabetta, G. Gottarelli and B. Samori, *Gazz. Chim. Ital.* **106**, 39 (1976).
[12]G. Toth, O. Clauder, K. Gesztes, S. S. Yemul and G. Snatzke, *J. Chem. Soc.* Perkin II 701 (1980).
[13]G. Massiot, F. S. Oliveira and J. Lévy, *Bull. Soc. Chim. France II* 185 (1982).
[14]J. Mokrý, I. Kompis, J. Suchý, P. Sefcovic and Z. Votický, *Chem. Zvesti* **17**, 41 (1963).
[15]F. Quirin, M. M. Debray, C. Sigaut, P. Thepenier, L. Le Men-Olivier and J. Le Men, *Phytochemistry* **14**, 812 (1975).
[16]M. Andriantsiferana, F. Picot, P. Boiteau and H. P. Husson, *Phytochemistry* **18**, 911, (1979).
[17]I. Chardon-Loriaux and H. P. Husson, *Tetrahedron Letters* 1845 (1975).
[18]I. Chardon-Loriaux, Thesis (1975), Université de Paris-Sud.
[19]J. Le Men, G. Hugel, M. Zeches, M. J. Hoizey, L. Le Men-Olivier and J. Lévy, *C. R. Acad. Sci.* **283C**, 759 (1976).
[20]C. Kan, H. P. Husson, S. K. Kan and M. Lounasmaa, *Planta Medica* **41**, 195 (1981).
[21]J. Le Men, G. Lukacs, L. Le Men-Olivier, J. Lévy and M. J. Hoizey, *Tetrahedron Letters* 483 (1974).
[22]S. Takano, M. Yonoga, K. Chiba and K. Ogasawara, *Ibid.* 3697 (1980).
[23]J. Le Men, C. Caron-Sigaut, G. Hugel, L. Le Men-Olivier and J. Lévy, *Helv. Chim. Acta* **61**, 566, (1978).
[24]J. P. Kutney, *Heterocycles* **4**, 169, (1976).
[25]T. A. van Beek, R. Verpoorte, A. Baerheim Svendsen, A. J. M. Leeuwenberg and N. G. Bisset, *J. Ethnopharmacology* in press (1984).
[26]E. Wenkert, E. W. Hagaman, N. Kunesch, N. Wang and B. Zsadon, *Helv. Chim. Acta* **59**, 2711 (1976).
[27]M. E. Kuehne, C. L. Kirkemo, T. H. Matsko and J. C. Bohnert, *J. Org. Chem.* **45**, 3259 (1980).
[28]G. Kalaus, Z. Gyulai, M. Kajtár-Peredy, P. Győry, L. Szabó and C. Szántay, *Acta Chim. Acad. Sci. Hung.* **105**, 221 (1980).