Monogagine, a Novel Dimeric Indole Alkaloid from *Tabernaemontana chippii* and *Tabernaemontana dichotoma* [1]

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Monogagine, Novel Dimeric Indole Alkaloid, *Tabernaemontana chippii*, *Tabernaemontana dichotoma*, Antimicrobial Activity

From the rootbark of *Tabernaemontana chippii* and the stembark of *T. dichotoma* a novel dimeric indole alkaloid was isolated which was named monogagine. It was assigned structure **1** on the basis of spectral data. It showed antimicrobial activity against *Bacillus subtilis*.

Introduction

Recently we have reported on the tertiary alkaloids occurring in *Tabernaemontana chippii* [2] and *T. dichotoma* [3] (Apocynaceae). From the rootbark of *T. chippii*, collected in Ivory Coast, 45 indole alkaloids belonging mainly to the corynanthean, ibogan and dimeric corynanthean-ibogan classes were isolated [2]. From the stembark of the Sri Lankan *T. dichotoma*, 22 alkaloids were isolated belonging primarily to the same classes [3].

One minor alkaloid was independently isolated from both species. In previous publications it was provisionally given the code names 6F and 20A for *T. chippii* and *T. dichotoma* respectively. Approximately 3 mg of monogagine was isolated from *T. chippii* and approximately 1 mg from *T. dichotoma*. The spectral data (UV, EI-MS and ¹H NMR at 300 MHz) which were available for this alkaloid at the time of writing the previous publications did not allow its structure elucidation although it could be concluded then that it was not a known alkaloid. Since then more spectral data (FD-MS, high resolution EI-MS, CD, 500 MHz ¹H NMR, 2D COSY ¹H NMR at 500 MHz, NOE difference experiments and ¹³C NMR) have become available and with the help of the new data it has been possible to propose a structure for the novel dimeric indole alkaloid. The

new alkaloid was named monogagine after the collection site of *T. chippii* in Ivory Coast. In the following we wish to report about the structure elucidation of monogagine.

Results and Discussion

The alkaloid gave a gold colour with the ferric chloride perchloric acid spray reagent which changed via dark blue to grey-black upon heating. Such a colour behaviour is typical for dimeric *T.* alkaloids [4]. The UV spectrum showed maxima at 222 and 288 nm and shoulders at 282 and 297 nm and is thus purely indolic. The EI-MS showed the molecular ion at *m/z* 600 which was confirmed by a FD-MS. Further intense fragments were observed at *m/z* 492, 491, 490, 182, 180 (100%), 133 and *m/z* 122. The three fragments at *m/z* 182, 180 and *m/z* 122 are typical for a 3'-vobasinyll half (**3**). However, the 500 MHz ¹H NMR spectrum (Fig. 1) did not confirm the presence of such a half. All the other dimeric indole alkaloids isolated from *T. chippii* and *T. dichotoma* do possess such a half which is readily recognized by certain characteristic signals such as the N-methyl group around 2.6 ppm, the shielded carbomethoxy group around 2.45 ppm, the H16 around 2.75 ppm, H21_s around 2.9 ppm and H14 around 1.9 ppm. In all the dimers containing a vobasinyll half isolated so far the observed coupling constants and the chemical shifts of these vobasinyll protons are very constant. However, none of these signals could be observed at their usual position. Therefore it was concluded that

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either a vobasiny half was present but in a different conformation with a simultaneous change of the chemical shifts and coupling constants or that no vobasiny half was present at all. The EI-MS data favoured the former possibility. Other striking features of the 500 MHz ^1H NMR, some of which were not readily observable at 300 MHz, were the pre-

sence of two unsubstituted aromatic rings, only one indolic NH, two ethylidene side chains, two AB doublets ($J = 17.7$ Hz) at 4.80 and 4.45 ppm, a carbomethoxy group at 3.47 ppm and an N-methyl group at 2.45 ppm. The two unsubstituted aromatic rings indicated a different way of attachment between the two halves than is normal for dimeric *T.* alkaloids

[5]. This and the AB doublets at 4.80 and 4.45 ppm reminded us of the alkaloid vobparicine (**5**) which was also isolated from *T. chippii* [6]. Vobparicine, a dimer of the new apparicine-vobasinal type also showed its molecular ion at m/z 600 and a high resolution MS of monogagine showed that the brutto molecular formula of both alkaloids was identical ($C_{39}H_{44}N_2O_2$). The rest of the aliphatic part of the 1H NMR spectrum was complex although there was relatively little overlap at 500 MHz (Fig. 1). The determination of all chemical shifts, coupling constants and proton-proton connectivities was possible by means of a 2-dimensional COSY 1H NMR spectrum at 500 MHz (Fig. 2). Apart from the methyl groups the proton signals of the following spin systems were found to be present in the region from 4.2 to 1.5 ppm: one large $CH_2-CH-CH-CH-CH_2-CH-CH_2$ spin system, one smaller CH_2-CH_2-CH spin system and two isolated CH_2 systems. The large spin system strongly suggested a 3'-vobasiny half albeit in a different conformation and with an extra methylene group attached to C3. Also it ruled out the presence of a rearranged ervatamine system. The chemical shifts and especially the coupling constants of the smaller spin system resembled strongly the C3/C14/C15 spin system of O-acetyl-vallesamine (**6**) (Table I) while the two isolated CH_2 spin systems are typical for two different pairs of geminal H21 protons.

Fig. 2. COSY spectrum of monogagine (**1**).

Based upon the following considerations: a) the chemical shifts and coupling constants of all protons, b) the absence of the C16–C22 double bond which is present in vobparicine (**5**) and thus, c) the necessity of two bonds between the two halves to account for the same number of protons as in vobparicine, d) the missing of one indolic NH and e) biosynthetic reasoning the basic structure **7** was proposed for monogagine. No stereochemical relationships are indicated in **7** except for H15 and H15'. These two protons were assumed to possess the same absolute configuration as in secologanin and all other non-rearranged monoterpenoid indole alkaloids. The only difference with vobparicine (**5**) is the absence of the C16–C22 double bond and the formation of a new bond between the vobasiny nitrogen and the apparicine C16. In the ^{13}C NMR spectrum which was subsequently recorded, all the carbons appeared at values where they could be more or less expected (Table II). The assignment of certain signals could not be confirmed by an off-resonance spectrum because the amount isolated precluded the recording of such a spectrum. Interesting was the 3 ppm downfield shift of the carbons of the carbomethoxy group if compared with the values of these carbons in a normal vobasiny half. Together with the 1 ppm downfield shift of the methyl protons of the carbomethoxy group in the 1H NMR spectrum this indicated that the carbomethoxy group no longer lies above the indole nucleus. Additional evidence for the substitution at the indole nitrogen of the vobasiny half was found in the unusual non-indole-like shifts of the aromatic protons of this half (7.51, 6.97, 6.79 and 6.55 ppm). Similar shifts (7.46, 7.01, 6.84 and 6.55 ppm) were recently published for the dimeric indole alkaloid kopsoffine which possesses a similar N-substituted indole [7].

The most likely MS fragmentation pattern which accounts for the formation of the intense fragments at m/z 492, 491 and m/z 490 is presented in Fig. 3. An alternative mechanism is the initial breaking of the C6–N4 bond followed by the breaking of the C15–C16 bond, also resulting in the loss of the unsubstituted ethylpiperidine moiety of the apparicine half.

By means of Dreiding models, the determined coupling constants (Table I) and the Karplus equation the relative stereochemistry of all carbon atoms with the exception of the spiro atom C16 and the conformation of most of the rings could be deter-

Table I. ^1H NMR data monogagaine, O-acetyl-vallesamine and 3'-vobasiny half of vobparicine (CDCl_3 , 500, 300 and 500 MHz respectively).

H-nr	Monogagaine		O-Acetyl-vallesamine	
	δ	J (Hz)	δ	J (Hz)
1	7.40	bs	8.72	bs
3 _R	2.94	14.3(3 _S), 7.0(14 _R), ~1(14 _S)	2.88	13.7(3 _S), 6.8(14 _R), ~1(14 _S), ~1(21 _S)
3 _S	3.07	14.3(3 _R), 12.4(14 _R), 6.1(14 _S)	3.00	13.7(3 _R), 12.7(14 _R), 5.3(14 _S)
6 _R	4.80	17.7(6 _S)	4.83	17.6(6 _S)
6 _S	4.45	17.7(6 _R)	4.18	17.6(6 _R)
9	7.50	7.7(10), 1.3(11)	7.48	8.0(10), 1.2(11), <1(12)
10	7.08	7.7(9), 7.7(11), 1.3(12)	7.10	8.0(9), 7.0(11), 1.2(12)
11	7.12	1.3(9), 7.7(10), 7.7(12)	7.22	1.2(9), 7.0(10), 8.0(12)
12	7.05	1.3(10), 7.7(11)	7.32	<1(9), 1.2(10), 8.0(11)
14 _R	2.85	7.0(3 _R), 12.4(3 _S), 13.8(14 _S), 1.8(15)	2.33	6.8(3 _R), 12.7(3 _S), 14.7(14 _S), 2.6(15)
14 _S	1.83	~1(3 _R), 6.1(3 _S), 13.8(14 _R), 10.9(15)	2.03	~1(3 _R), 5.3(3 _S), 14.7(14 _R), 11.4(15)
15	3.54	1.8(14 _R), 10.9(14 _S)	3.86	2.6(14 _R), 11.4(14 _S), ~0.5(19?)
18	1.87	6.9(19), 2.2(21 _R)	1.77	6.9(19), 2.2(21 _R)
19	5.75	6.9(18)	5.65	~0.5(15?), ~1.5(21 _S), 6.9(18)
21 _R	3.79	2.2(18), 15.4(21 _S)	3.63	2.2(18), 15.5(21 _S)
21 _S	3.86	15.4(21 _R)	3.78	~1(3 _R), 1.5(19), 15.5(21 _R)
22 _R	2.90	12.2(22 _S), 6.8(3')	—	—
22 _S	2.39	12.2(22 _R), 11.0(3')	—	—
Monogagaine			3'-Vobasiny half of vobparicine	
3'	4.07	6.8(22 _R), 11.0(22 _S), 10.8(14 _R '), 1.6(14 _S ')	4.53	10.1(22), 12.1(14 _R '), 3.2(14 _S ')
5'	3.60	5.6(6 _R '), 6.2(6 _S '), 6.9(16')	4.00	8.2(6 _R '), 10.4(6 _S '), 3.1(16')
6 _R '	3.27	5.6(5'), 16.7(6 _S ')	3.21	8.2(5'), 14.7(6 _S ')
6 _S '	3.33	6.2(5'), 16.7(6 _R ')	3.38	10.4(5'), 14.7(6 _R ')
9'	7.51	8.0(10')	7.53	7.5(10')
10'	6.97	8.0(9'), 8.0(11')	7.09	7.5(9'), 7.5(11')
11'	6.79	1.0(9'), 8.0(10'), 8.0(12')	7.12	7.5(10'), 7.5(12')
12'	6.55	8.0(11')	7.20	7.5(11')
14 _R '	2.10	10.8(3'), 14.8(14 _S '), 10.8(15')	2.64	12.1(3'), 15.1(14 _S '), 12.6(15')
14 _S '	2.00	1.6(3'), 14.8(14 _R '), 7.8(15')	1.89	3.2(3'), 15.1(14 _R '), 6.8(15')
15'	3.62	10.8(14 _R '), 7.8(14 _S '), 3.6(16')	3.74	12.6(14 _R '), 6.8(14 _S '), 3.1(16')
16'	3.21	6.9(5'), 3.6(15')	2.74	3.1(5'), 3.1(15')
18'	1.63	6.8(19')	1.67	6.8(19')
19'	5.25	6.8(18')	5.41	6.8(18'), 1.9(21 _R ')
21 _R '	3.42	14.9(21 _S ')	3.77	1.9(18'), 13.8(21 _S ')
21 _S '	3.01	14.9(21 _R ')	2.99	13.8(21 _R ')
CO ₂ Me'	3.47	s	2.47	s
NMe'	2.45	s	2.61	s

Note: To distinguish between the two non-equivalent protons of a methylene group the prochirality rules of Hanson [8] were used.

mined. The configuration of C16' was found to be the same as in vobasinyol (**4**) or vobparicine (**5**) because of the coupling constant of 6.9 Hz between H-5' and H16'. If the configuration at C16' were the opposite, the coupling constant would be 1–3 Hz. The piperidine ring of the vobasiny half no longer possesses the usual chair conformation but is now in a boat like conformation. This explains the difference in chemical shifts and coupling constants of the various protons in monogagaine and vobparicine (Table I). Dreiding models also showed that H3' has

the same configurations as in vobparicine (**5**) and all other alkaloids possessing a vobasiny half, *i.e.* the β -configuration. If the H3' were to have been in the α -position several coupling constants would have been different. The piperidine ring of the apparicine half is thought to have the same conformation as in O-acetyl-vallesamine (**6**) which also lacks the C16–C22 double bond, because of the similar coupling constants (Table I). As mentioned before the absolute configuration of H15 and H15' of monogagaine was deduced by biosynthetic reasoning. This settles

Table II. ^{13}C NMR data of monogagaine, O-acetyl-vallesamine and 3'-vobasiny half of vobparicine (CDCl_3 , 75.2, 25.15 and 75.2 MHz respectively).

C-nr	Monogagaine δ	O-Acetyl-vallesamine δ	C-nr	Monogagaine δ	3'-Vobasiny half of vobparicine δ
2	132.9 (?)	128.4	2'	143.9	135.1
3	47.0	47.4	3'	40.7	33.9
6	53.6	52.2	5'	59.7	59.7
7	110.7 (?)	110.0	6'	22.0	19.4
8	128.0	127.8	7'	110.7 (?)	109.5
9	118.4	118.5	8'	128.1 (?)	129.9
10	119.3	119.4	9'	117.2	117.7
11	122.8	122.9	10'	118.1	119.8
12	110.7	110.5	11'	121.2	122.2
13	135.7	135.3	12'	110.7	110.0
14	26.8	25.2	13'	137.0	135.8
15	33.7	34.7	14'	26.8	33.7
16	51.5	55.8	15'	33.6	33.4
18	13.7	14.0	16'	46.6	46.0
19	123.8	125.4	18'	12.6	12.3
20	132.4 (?)	132.8	19'	118.7	119.6
21	55.1	54.6	20'	138.8	136.9
22	36.5	—	21'	56.0	52.2
			NMe'	43.0	42.3
			CO ₂ Me'	52.9	50.1
			CO ₂ Me'	173.5	not observed

Note: The assignment of monogagaine signals, which differ less than 3 ppm from each other, may be interchanged. (?): very weak signal.

Fig. 3. Possible MS fragmentation pattern of monogagaine (1).

automatically the stereochemistry of all adjacent carbon atoms. A CD spectrum was recorded to allow a comparison in case monogagaine is isolated from another species. The CD spectrum was similar to the CD spectra of the voacamine type of alkaloids but this could have been a coincidence.

The only carbon atom the relative stereochemistry of which could not be determined from the ^1H NMR data is the spiro atom C16. Dreiding models showed that both configurations at C16 are possible. A distinction between the possible C16 configurations could be made by means of NOE difference spectroscopy by which it is possible to make long range through space connections between various protons. In case of a 16S configuration NOE's were expected between H12' and H6_R/H6_S/H14_R if the 8-membered ring of the apparicine derived half has the same conformation as in O-acetyl-vallesamine (**6**). The alternative conformation of the 8-membered ring in which H6_S lies in the plane of the indole nucleus was considered less likely because the Dreiding models showed that in that case H14_R (δ 2.85 ppm) would be below the indole nucleus and would have been strongly shielded. As it happens just the reverse is true, this proton is somewhat deshielded in comparison with O-acetyl-vallesamine (**6**) (Table I). In case of a 16R configuration NOE's were expected between H12' and H18 and possibly also H1/H15. Irradiation of H6_R gave, apart from a strong NOE for H6_S, only a weak NOE for H9. Irradiation of H6_S gave a strong NOE for H6_R and further only a weak NOE for H9. These NOE's confirmed unambiguously the ^1H NMR assignment of the two aromatic parts. Irradiation of H12' gave an NOE for H11' and an NOE for H14_R. Irradiation of H14_R and, as it was also necessary, H22_R gave strong NOE's for H12', H3', H22_S and H14_S. Irradiation of H18 gave no significant NOE's with exception of its direct neighbour H19. Finally irradiation of H1 gave no significant NOE's at all. All the observed NOE's were positive. The results of the NOE experiments are summarized in Table III.

From the above data, especially the strong NOE between H12' and H14_R it follows that C16 has the S configuration. However, from the absence of an NOE between H6_R/H6_S and H12' it must be concluded that the 8-membered ring of the apparicine derived half does not have the conformation predicted by the Dreiding model but a version of this conformation strained in such way that H6_R lies on

Table III. NOE connectivities.

Proton irradiated	NOE observed for
H1	—
H6 _R	H6 _S (++) , H9 (+)
H6 _S	H6 _R (++) , H9 (+)
H14 _R and H22 _R	H14 _S (++) , H22 _S (++) , H3' (++) H12' (++)
H18	H19 (++)
H12'	H14 _R (++) , H11' (++)

Note: (++) : strong NOE; (+) : weak NOE.

one side of the indole plain and H6_S on the other side instead of being on the same side. This would also fit the smaller chemical shift difference between H6_R and H6_S in monogagaine as compared to O-acetyl-vallesamine (0.35 ppm and 0.65 ppm respectively). Supporting evidence for the 16S configuration was obtained from the chemical shift of H14_R (2.85 ppm) and H14_S (1.83 ppm). In O-acetyl-vallesamine the same protons have shifts of 2.33 and 2.03 ppm respectively. These deshielding and shielding effects can be explained by the position of these protons near the indole nucleus of the vobasiny half. H14_R (2.85 ppm) which is close to H12' according to the NOE experiments lies in the plain of the indole nucleus and is therefore 0.5 ppm deshielded. H14_S (1.85 ppm) lies somewhat above the indole nucleus and is thus 0.2 ppm shielded. If C16 possessed the R configuration the H14 protons would be far away from the indole nucleus and would probably possess shifts not greatly different from those found for O-acetyl-vallesamine. Thus monogagaine is thought to have the configuration depicted in **1**.

Biogenesis. It is speculated that monogagaine is formed in a similar fashion as vobparicine and the dimeric alkaloids of the voacamine type *i.e.* by an attack of the electrophilic C3 of vobasinal (**4**) on an electron rich centre such as in apparicine (**2**) C22. However, in contrast with vobparicine (**5**) the reaction does not stop at this stage and is immediately followed by ring closure between the nitrogen of the 3'-vobasiny half and C16 of the apparicine half (Fig. 4). It is not considered likely that vobparicine (**5**) is a precursor or an intermediate in the biogenesis of monogagaine (**1**). More likely they are merely the different results of a reaction between apparicine and vobasinal which can proceed in two distinctly different ways depending on the conditions.

No attempt to synthesize monogagaine (**1**) was made because under the conditions usually favour-

Fig. 4. Possible biogenetical pathway leading to monogagaine (**1**).

able for the formation of this type of alkaloid the mixture of vobasinol (**4**) and apparicine (**2**) gave only vobparicine (**5**) in detectable amounts [6]. For the same reason it is not considered likely that monogagaine (**1**) is an artefact. In a preliminary screening (biogram method) monogagaine showed antimicrobial activity against *Bacillus subtilis*.

1: Monogagaine.

2: Apparicine $R_1 = H$.

5: Vobparicine $R_1 = 3'$ -vobasinyl.

3: 3'-Vobasinyl.

4: Vobasinol $R_1 = OH$.

6: O-Acetyl-vallesamine.

7: Monogagaine.

Experimental

The botanical details and procedures used for the isolation of monogagaine can be found in the previous publications [2, 3]. UV and CD spectra were recorded in MeOH. 300 and 500 MHz 1H NMR spectra were recorded in $CDCl_3$ on a Bruker WM 300 and WM 500, respectively. All NOE experiments were carried out at 300 MHz. The COSY spectrum was recorded by employing the pulse sequence proposed by Bax *et al.* [9], *i.e.* the second 90° pulse was replaced by a 45° pulse in order to obtain a better resolution near the diagonal. The 75.2 MHz ^{13}C NMR spectrum was recorded in ^{13}C enriched $CDCl_3$ without TMS ($CDCl_3 = 77.0$ ppm) on a Bruker WM 300. The FD-MS was recorded on a VG-

Analytical ZAB-HF mass spectrometer with a combined EI/FI/FD source. The high resolution EI-MS was recorded on a Kratos MS 50.

TLC: For the determination of the R_f value the following systems were used:

S1: toluene – 100% EtOH satd. with NH_3 (19:1)*

S2: (*iso*-Pr) $_2\text{O}$ – 100% EtOH satd. with NH_3 (9:1)*

S3: CHCl_3 – MeOH (9:1)

Spectral data monogagine (**1**): R_f value in S1 0.48, S2 0.31, S3 0.39; Colour with $\text{FeCl}_3/\text{HClO}_4$: gold, changing upon heating *via* dark blue to grey-black; UV λ_{max} : 222, 282 (sh), 288, 297 (sh) nm; CD λ ($\Delta\epsilon$): 208 (0.0), 218 (–45), 226 (0.0), 233 (+73), 263 (+14), 287 (+15), 350 (0.0); FD-MS (18 mA) m/z (rel. int.) 600 (100%); EI-MS (220 °C) m/z (rel. int.) 614 (2), 600 (M^+ , 38), 599 (6), 598 (6), 569 (3),

* Prior to development the plates were left in an atmosphere of NH_3 for 20 min. After development the plates were sprayed with 0.2 M FeCl_3 in 35% HClO_4 , followed by heating with hot air.

568 (4), 493 (10), 492 (32), 491 (35), 490 (74), 489 (14), 460 (7), 459 (9), 458 (18), 405 (8), 309 (13), 295 (18), 277 (11), 206 (13), 183 (10), 182 (72), 181 (26), 180 (100), 149 (19), 133 (42), 129 (13), 124 (12), 123 (11), 122 (46), 120 (10), 111 (14), 110 (15), 109 (12), 105 (11); High resolution MS: 600.3452 (calcd for $\text{C}_{39}\text{H}_{44}\text{N}_4\text{O}_2$ 600.3464); ^1H NMR, see Fig. 1 and Table I; ^{13}C NMR, see Table II.

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- [1] Part 14 in the series “Pharmacognostical studies of *Tabernaemontana* species”. For part 13 see: T. A. van Beek and R. Verpoorte, *Fitoterapia*, accepted for publication.
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