STRUCTURE AND STEREOCHEMISTRY OF HAZUNTAMINE, A NEW BISINDOLE ALKALOID FROM *HAZUNTA MODESTA* VAR. *METHUENII* SUBVAR. *METHUENII*

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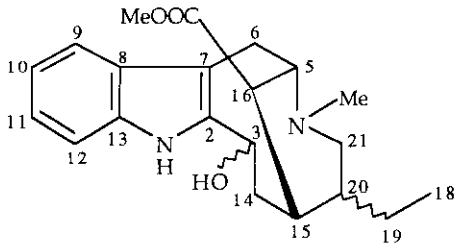
Abstract – The complete stereostructure of hazuntamine, a new bisindole alkaloid from *Hazunta modesta* var. *methuenii* subvar. *methuenii*, has been determined mainly by the use of different spectroscopic means.

Plants of the genus *Hazunta* (Apocynaceae) are native to different regions of Madagascar. In a continuation of our studies on alkaloids of different *Hazunta* plants,[1] we have now examined the root bark of *Hazunta modesta* var. *methuenii* subvar. *methuenii* growing in a region different from where a previously investigated species of the same botanical variety was collected.[1a]

Direct crystallization as well as repeated chromatography of the CH_2Cl_2 extract of the air-dried root bark afforded two newly isolated alkaloids, namely, dregamin-3α-ol and hazuntamine, besides the previously isolated known compounds[1,2] silicine, ibogamine, 19',20'-dihydrotabernamine, methuenine, tabernaemontanine, dregamine, 6-oxosilicine, dihydroellipticine, and 16-*epi*-methuenine. We describe here the identification of dregamin-3α-ol and the structure elucidation of hazuntamine which was found to be a new bisindole alkaloid.

Dregamin-3α-ol (**1**), a monomeric indole base, $C_{21}H_{28}N_2O_3$ ($M^{+\cdot}$ 356.2092, Calcd 356.2100), $[\alpha]_D^{20°}$ +17° (*c* = 1, EtOH), was isolated as a pale yellow amorphous compound. Its structure was established through comparison of its physical and spectral data with those of dregamin-3β-ol (**2**) and tabernaemontanin-3β-ol (**3**), which are obtained by $NaBH_4$ reduction of dregamine and tabarnaemontanine, respectively.[3,4] Whereas the uv (λ_{max}) absorption and mass spectral fragmentation (*m/z* 324, 196, 182)[3,5] confirmed its analogy with dregamin-3β-ol (**2**) and tabernaemontanin-3β-ol (**3**), this newly isolated base differed from either of them by the nmr (^1H and ^{13}C) chemical shifts as well as by the tlc Rf value. In particular, the coupling constants of 3-H (*J* = 12 and 4 Hz) of this compound are different from those observed for the 3-H of dregamin-3β-ol (*J* = 4 and 1 Hz) or tabernaemontanin-3β-ol (*J* = 5 and 2 Hz). From the ^1H nmr chemical shift of its CO_2Me (δ 2.59), the configuration at C-16 was found to be the same as that of dregamin-3β-ol (δ 2.72) or tabernaemontanin-3β-ol (δ 2.63). On the other hand, the observed chemical shift values of H_2-19 (δ 1.38 – 1.45) and H-20 (δ 1.80) along with the C-16 chemical shift (δ 50.0) pointed to the 20*R* configuration (20α-Et)[6] for this alkaloid as in dregamin-3β-ol on the basis of the previously

published data by Bombardelli *et al.*[7] All these observations indicated that this alkaloid should be represented as dregamine-3α-ol (**1**) which fully accounted for the ^{1}H and ^{13}C nmr chemical shift and the proton coupling constant values. In conformity with this stereochemical difference at C-3, compared to dregamin-3β-ol (**2**), significant changes in the carbon chemical shift values at this center and its vicinities could also be noted (Table 1). Seemingly, dregamin-3α-ol (**1**) was previously isolated from *Tabarnaemontana elegans*, but its reported identity[8] with the $NaBH_4$ reduction product of dregamine remains confusing in as much as the compound expected to be obtained under such conditions should be dregamin-3β-ol (**2**).[3,4] Comparison of our own nmr data (see Table 1) and the Si gel tlc Rf values [$CHCl_3$:MeOH (9:1); **1** 0.20; **2** 0.44; **3** 0.53] of the $NaBH_4$ reduction products of dregamine and tabarnaemontanine with those of our isolated material clearly distinguished all these compounds.



1 3α–OH; 20β–H

2 3β–OH; 20β–H

3 3β–OH; 20α–H

Table 1. ^{1}H- and ^{13}C–Nmr Chemical Shift Assignments Based on DQF–COSY, NOESY and ^{1}H–^{13}C Correlation 2D Spectra of Dregamin-3α-ol (**1**), Dregamin-3β-ol (**2**) and Tabernaemontanin-3β-ol (**3**). *J*-Values (Hz) are given in parentheses. A few ^{13}C assignments here differ from previous attributions.[7]

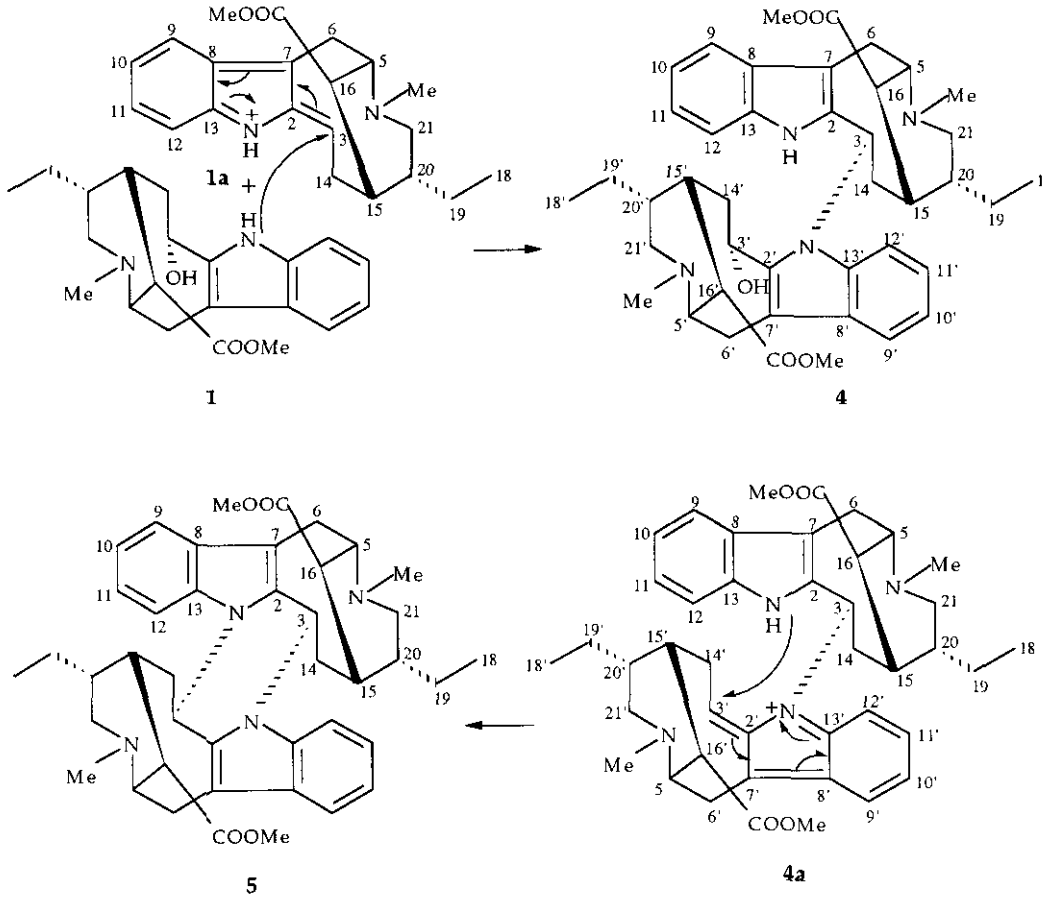
H	1	2	3	C	1	2	3
3	5.07 dd (4, 12)	5.20 dd (4, 1)	5 01 dd (5, 2)	2	137.1[a]	135 6[a]	137.3[a]
5	3.93 dd (9, 3)	4.10 dd (7.5, 1)	3.89 dd (9, 2.5)	3	68.2	66.9	67.1
6	3 18 d (9)	3.03 dd (10.5, 7.5)	3.03 d (9)	5	59.8	59.2	59.3
	3.28 d (9)	3.26 d (10.5)		6	24 4	23.7	25.7
				7	109.8	107 7	108 3
9	7.28 d (7)	7.43 d (7)	7.32 d (7)	8	130.3	129.3	129.3
10	7.03 t (7)	7.22 t (7)	7.09 t (7)	9	118.9	118 0	118 0
11	7.12 t (7)	7.14 t (7)	7.02 t (7)	10	119.6	119.3	119.5
12	7 52 d (7)	7.58 d (7)	7.49 d (7)	11	123.0	122.1	122.3
14	2.00 ddd(13, 4, 7)	2.22 ddd (11, 4, 4)	2 16 ddd (15.5, 5, 5)	12	111 4	110 8	110 6
	2.40 ddd (13, 12, 13)	2.46 ddd(11, 1, 9)	2.72ddd(15.5,2,13.5)	13	137.8[a]	137.3[a]	135.6[a]
15	2.70 dddd (7, 13, 3, 7)	2.82 dddd (4,9,1,1)	2 60 ddd (5,13.5,2 5)	14	33 6	30 7	38.3
16	2.50 dd (3, 3)	2.73 dd (1, 1)	2 78 t (2 5)	15	31 6	28.9	30.0
18	1.00 t (7)	1.13 t (7)	0.97 t (7)	16	50.0	50 2	42.8
19	1.38 m and 1.45 m	1.50 m	1 50 m	18	11.1	11.6	12.8
	1.45 m		1.66 m	19	20 1	19 5	18.2
20	1.80 m	1.94 m	1.50 m	20	42.3	42 5	42.8
21	2.50 dd (12, 4)	2 70 dd (9, 3)	2.62 d (13)	21	50 6	49.5	47.5
	2.78 dd (12, 12)	2.88 dd (9, 9)	3.13 dd (13,4)	CO	172.7	175 1	176.8
NMe	2.41 s	2.51 s	2.38 s	NMe	44.2	43.8	43 9
OMe	2.59 s	2.72 s	2.63 s	OMe	50 0	50.6	50.6

[e] Assignments within a vertical column may be interchanged.

The new bisindole compound, named hazuntamine, was obtained as colorless crystals from MeOH, mp 254°, $[\alpha]_D^{20}$ –88° (c = 0.7, C_5H_5N). The ir spectrum indicated the presence of an *N*H/*O*H group (a broad absorption between 3400–3300 cm^{-1}) and an ester group (1730, 1320, 1280, 1180 cm^{-1}) while the uv spectrum showed maxima at 225 (ε 27.750), 285 (ε 8.900) and 292 (ε 8.500) nm suggesting the presence of an indole chromophore. Its ei mass spectrum exhibited a weak molecular ion peak at *m/z* 694 that analyzed for $C_{42}H_{54}N_4O_5$ (Found *m/z* 694.4041; Calcd 694.4094) The presence of a hydroxyl group in hazuntamine was revealed by an intense [$M^{+\cdot} - H_2O$] peak at *m/z* 676 and this was confirmed by the formation of a monoacetate ($C_{44}H_{56}N_4O_6$, $M^{+\cdot}$ *m/z* 736; intense $M^{+\cdot}$ – AcOH peak at m/z 676) the ir spectrum of which showed a residual *N*H band (3320 cm^{-1}). The molecular weight of hazuntamine was further confirmed by its fabms (MH^+ at *m/z* 695). The ei mass spectrum of hazuntamine showed peaks at *m/z* 196, 182 and 124 which, along with the complementary peaks at *m/z* 494 [$M^{+\cdot} - H_2O - 182$] and 480 [$M^{+\cdot} - H_2O - 196$], are of particular diagnostic importance since these are characteristic [3,5] of 19,20-dihydrovobasine alkaloids such as dregamine and tabernaemontanine or their corresponding 3-ols. A 3-deoxy-19,20-dihydrovobasinol moiety in hazuntamine was indicated by the peaks observed at *m/z* 339 and 338, whereas the rest of the molecule was suspected to be a 19,20-dihydrovobasinol unit from the complenentary peaks at *m/z* 355 and 356. By analogy with known bisindole alkaloids containing similar structural units,[4,7] hazuntamine was thus considered to be constituted of a 3-deoxy-19,20-dihydrovobasinol unit inked at its 3α position with the rest of the molecule. The fabms of hazuntamine monoacetate recorded in glycerol-d_3 matrix demonstrated the presence of only one exchangeable hydrogen (Md_1+D^+ at *m/z* 739) in its molecule thereby indicating that one of the indole nitrogens in hazuntamine must be involved as a point of attachment between the two structural units present in it. These observations as well as the absence of any mass spectral peaks characteristic of an indole skeleton other than the 19,20-dihydrovobasine framework led us to consider for hazuntamine the overall structure (**4**) which accounted for all the data described so far. This structure of hazuntamine including the stereochemistry could be independently inferred from a detailed analysis of the different nmr data obtained for this alkaloid and its anhydro product.

The ^1H nmr data of hazuntamine are compatible with the two dregamine-like units linked together as presented in **4** . Besides two three-proton triplets near δ 1.01 (2 x Et) and eight aromatic protons between δ 6.9 and 7.5, the 400 MHz ^1H nmr spectrum was characterized by the presence of four three-proton singlets at δ 2.40, 2.45, 2.60, and 2.66 which fall in the region where the *N*Me and CO_2Me groups of vobasine-type alkaloids resonate. Two low-field protons at δ 6.20 and 5.48 are compatible with H-3 and H-3', respectively, while the two one-proton signals at δ 4.11 and 3.93 should be appropriate for H-5 and H-5'. The β-orientation of both H-3 and H-3' followed from their coupling constants (H-3, J = 12 and 2 Hz; H-3', J = 10 and 4 Hz) with the neighbouring H_2-14 and H_2-14', respectively. Incidentally, these values are comparable with those observed for the H-3 proton of dregamin-3α-ol (**1**). The high-field region of the spectrum revealed two sets of two-proton multiplets near δ 1.25 and δ 1.42–1.47 which could be attributed to H_2-19 and H_2-19'. These values together with the chemical shifts of H-20 and H-20' observed as a two-proton signal near δ

1.85 indicated[7] the dregamine-like configuration at C-20 and C-20'. The chemical shift values of the remaining protons in hazuntamine could also be assigned on the basis of structure (**4**).



The proton decoupled ^{13}C nmr spectrum of hazuntamine exhibited signals for the 42 carbon atoms in the molecule. As expected for structure (**4**), the nmr data revealed the presence of 8 aromatic methine carbons and 10 quaternary carbons (of which two belonged to the ester carbonyls) in the sp^2 region, whereas the sp^3 region showed 6 methyl (2 x *C*Me, 2 x *N*Me and 2 x *O*Me), 8 methylene and 10 methine groups. Comparison with the ^{13}C nmr chemical shift values of dregamin-3α-ol (**1**) as well as of tabernaelegantines C and D,[7] which have a 3α substituted 3-deoxydregaminol moiety, together with their carbon multiplicites allowed direct signal matching and unambiguous assignments (Table 2) for all the carbons in **4** . In particular, the methine signals at δ 51.6 and 65.7 could be readily attributed to C-3 and C-3', respectively, while the resonances associated with C-14, C-14', C-16, and C-16' indicated, on the basis of the arguments developed previously by Bombardelli *et al.*,[7] that the C-20 and C-20' ethyl chains in hazuntamine should have α-orientation as in dregamine.[6] Further support for the structure (**4**) of hazuntamine was provided by the nmr data of its anhydro

product. Treatment of hazuntamine with trifluoroacetic acid resulted in the isolation of a compound named anhydrohazuntamine in 18% yield, mp 164°, $[\alpha]_D^{20°}$ + 97° (c = 0.55, $CHCl_3$), showing uv absorption maxima characteristic of indole chromophores. Formation of this compound by the elimination of a molecule of water from the starting alkaloid was inferred from its molecular formula $C_{42}H_{52}N_4O_4$ (hreims $M^{+\cdot}$ 676.3949; Calcd 676.3989; fabms MH^+ 677). A symmetrical

Table 2. ^1H– and ^{13}C–Nmr Chemical Shift Assignments Based on DQF–COSY, NOESY and ^1H–^{13}C Correlation 2D Spectra of Hazuntamine (**4**) and Anhydrohazuntamine (**5**). *J*-Values (Hz) are given in parentheses.

H	4	5	C	4	5
3	6.20 d (12, 2)	5.79 dd (14, 4)	2	132.9[a]	135.7[a]
5	3.93 ddd (4, 7, 2.5)	3.89 dd (6, 6)	3	51.6	49.7
6	3.14 dd (12, 4)	2.71 dd (16, 6)	5	58.2	59.6
	3.26 dd (12, 7)	3.34 d (16)	6	23.4	22.1
9	7.50 d (7)	7.81 d (7)	7	109.9	108 9
10	6.90 t (7)	7.37 t (7)	8	128.8	130.3
11	6.90 t (7)	7.50 t (7)	9	117 6	119.4
12	7.20 d (7)	8.36 d (7)	10	118 6	119.9
14	2.28 dd (12, 2,)	2 28 dd (14, 14)	11	122 0	123.0
	3.15 ddd (12, 12, 12)	3.28 ddd(14, 4, 10)	12	110 2	110.6
15	2.95 ddd (12, 2.5, 7)	2.75 ddd (10, 6, 6)	13	135 8[a]	139.1[a]
16	2.8 dd (2.5, 2.5)	3.35 dd (6, 6)	14	27 9	36.5
18	1 01 t (7)	0.65 t (7)	15	30.1	31.3
19	1.25 m	0.92 m	16	50.1	50 7
20	1.85 m	1.54 m	18	11.1	11.0
21	2.60	1 65 dd (12, 12)	19	19.1	22.0
	2.80	1.82 dd (12, 4)	20	43.5	42.4
NMe	2 60 s	2.63 s	21	49.1	51.8
OMe	2 66 s	3.55 s	2'	134.7	
3'	5.48 dd (4, 10)		3'	65.7	
5'	4.11 ddd(7, 9, 3)		5'	58 6	
6'	3.40 dd(12, 7)		6'	23.4	
	3 42 dd(12, 9)		7'	108.4	
9'	7.50 d (7)		8'	128.8	
10'	6.90 t (7)		9'	118 0	
11'	6.90 t (7)		11'	122 0	
12'	7.00 d (7)		12'	110 6	
14'	2.13 d (3.5)		13'	135.6	
	2.6		14'	33 3	
15'	2.80 (8, 3,)		15'	31.0	
16'	2 70 dd (3, 3)		16'	49.4	
18'	1.01 t (7)		17'	30.4	
19'	1 42 m		18'	11.2	
	1.47 m		19'	19.3	
21'	2 60		20'	43 2	
	2.80		21'	49.1	
NMe	2.45 s		NMe	42.0	42 6
OMe	2 66 s			42.2	
			OMe	49 0 (x 2)	50 2
			CO	172.1; 172.2	170.7

[a] Assignments within a vertical column may be interchanged.

bisindole structure for anhydrohazuntamine followed from its ^1H nmr spectrum which integrated for 26 protons while its ^{13}C nmr spectrum displayed 21 carbon resonances instead of 42 carbons present in the molecule. Thus, anhydrohazuntamine must be represented by the symmetrical structure (**5**) which could be elaborated from **4** by linking *N*-1 and C-3' with concomitant loss of water. The ms and nmr (Table 2) data are fully consistent with this structure. The nmr attributions of structures (**4**) and (**5**) were further confirmed by extensive use of DQF–COSY and 1H-13C heteronuclear correlation spectra.

During the partial synthesis of a bisindole alkaloid, dihydrovoacamine, by the condensation of dregamin-3β-ol (**2**) and voacangine under acidic conditions, Büchi *et al.*[4] reported the isolation of an additional bisindole base in which the C-3 of the "dregaminol" portion was substituted from the α-side by the indole nitrogen of voacangine. In light of this precedent and the mechanism suggested therein for the formation of such bisindole bases, the occurrence of hazuntamine (**4**) and its subsequent transformation into anhydrohazuntamine (**5**) can be rationalized in a straightforward manner. Thus, the formation of hazuntamine (**4**) from dregamin-3α-ol (**1**) or from dregamin-3β-ol (**2**) may be envisaged through the intermediacy of an iminium salt (**1a**). The latter should be susceptible to undergo condensation with the nucleophilic indole nitrogen atom of a second dregamin-3α-ol unit from the less hindered α-side leading to hazuntamine (**4**). An intramolecular repetition of the same process under acidic conditions should then furnish anhydrohazuntamine (**5**) through the intermediate iminium salt (**4a**).

EXPERIMENTAL

General Experimental Procedures.—Melting points were obtained on a Kofler hot-stage apparatus. Optical rotation was determined on a Perkin-Elmer model 141 polarimeter. Spectra were recorded with the following instruments: uv, Perkin-Elmer Lambda 5; ir, Nicolet 205 FT-IR spectrometer; ms, AEI MS50 and Kratos MS80; nmr, ^1H (400 MHz) and ^{13}C (100.61 MHz) on a Bruker WM 400. Chemical shifts are given in ppm relative to TMS ($\delta = 0$).

Plant material.—*Hazunta modesta* var. *methuenii* subvar. *methuenii* was collected in the Isalo massif region of Madagascar. A voucher specimen (collection No. AB 203) was deposited at the herbarium of the Muséum National d'Histoire Naturelle de Paris, France.

Extraction and isolation.—The air-dried powdered plant (9 kg) was defatted with petrol ether (50 l; bp 40 – 60 °C) for 72 h and then soaked with 40% aqueous NH_4OH (4 l) and exhaustively extracted with CH_2Cl_2 (60 l) for 2 weeks at room temperature. The alkaloids were partitioned into 2% aqueous HCl and then washed several times with Et_2O. After basification with concentrated NH_4OH, the crude alkaloid mixture (534 g; yellow powder) was obtained by CH_2Cl_2 extraction followed by solvent removal. Direct crystallization from MeOH yielded silicine (131 g). The residue (400 g) obtained after evaporation of solvent was subjected to column chromatography over alumina (Prolabo, France; 10 kg) eluting with Et_2O (fractions 1–15), Et_2O–MeOH (10%) (fractions 16–19),

and Et_2O–MeOH (20%) (fractions 20–21), collecting 1 l fractions. Fraction 1 did not leave any residue after solvent evaporation. Silica gel tlc of fractions 2–3 (4.1 g) revealed the presence of ibogamine as the single component. Similar tlc of fraction 4 (2.8 g) showed three spots which could be fractionated by further column chromatography on alumina (85 g; eluant EtOAc) into ibogamine (0.49 g), a mixture (0.89 g) of ibogamine and dihydrotabernamine of which the latter could be crystallized (0.48 g) from MeOH, and a mixture (1:1; 0.72 g) of dihydrotabernamine and silicine. Fraction 5 (16.5 g) was composed of dihydrotabernamine, methuenine and silicine, while fraction 6 (33.8 g) indicated the presence of methuenine and silicine. Fractions 7–12 (163.4 g) furnished methuenine (100 g) by direct crystallization from Et_2O and further crystallization from MeOH provided silicine (30.5 g). Fractions 13–15 (87.2 g) revealed by tlc the presence of a mixture of silicine and tabernaemontanine from which pure silicine (50 g) could be crystallized from MeOH. Crystallization from MeOH of the residue (22 g) obtained after solvent evaporation of the fractions 16–17 gave dregamine (14.7 g); the alkaloid content of the mother liquor was further purified by the usual sequence of acid-base treatment thereby providing 3.6 g of a basic material revealing by tlc the presence of five spots among which were identified dregamine and methuenine. Addition of $CHCl_3$ to this material left a precipitate (1.4 g) which was the new compound hazuntamine almost in a pure form. The $CHCl_3$ soluble portion (2.2 g) on further chromatography over alumina (Merck, activity II-III) eluting with C_6H_6, C_6H_6–Et_2O, Et_2O and finally with Et_2O–MeOH (2%) followed by crystallization of different fractions provided further quantities of dregamine (150 mg), a new alkaloid, hazuntamine (**4**; 750 mg), and methuenine (630 mg). Fractions 18–19 (4.4 g) showed the presence of four compounds on Si gel tlc. Preparative Si gel chromatography ($CHCl_3$:MeOH/9:1) of this mixture (200 mg) furnished 6-oxosilicine (30 mg), methuenine (45 mg), silicine (85 mg) and a more polar new compound dregamin-3α-ol (**1**; 35 mg). The residue (29 g) of the fractions 20–21 was subjected to further purification through acid-base fractionation sequence followed by crystallization thereby furnishing dihydroellipticine (3 g) and 16-*epi*-methuenine (*ca.* 6 g).

Compound identifications.—All known compounds were identified by tlc, ^1H nmr, and, when appropriate, ^{13}C-nmr comparison with our previously isolated samples.[1a] The identification of dregamin-3α-ol (**1**) and the structure elucidation of the new alkaloid hazuntamine (**2**) rested mainly on the analysis of their different spectral data.

Dehydration of hazuntamine to anhydrohazuntamine.—Hazuntamine (**4**, 55 mg) was dissolved in ice-cold trifluoroacetic acid (0.5 ml) and left for 3 h at 0° with magnetic stirring. After removal of trifluoroacetic acid in vacuo, the residue was dissolved in water. The aqueous phase was adjusted to pH 8.5 with solid K_2CO_3 and extracted with CH_2Cl_2 . Removal of solvent left a residue which showed several spots on tlc. Separation by preparative tlc on silica gel resulted in the isolation of the major component, anhydrohazuntamine (**5**, 10 mg), by eluting with Me_2CO–MeOH (70:30).

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REFERENCES

1. a) A.-M. Bui, M.-M. Debray, P. Boiteau, and P. Potier, *Phytochemistry*, 1977, **16**, 703; b) A.-M. Bui, P. Potier, M. Urrea, A. Clastres, D. Laurent, and M.-M. Debray, *ibid.*, 1979, **18**, 1329; c) A.-M. Bui, B. C. Das, and Potier, *ibid.*, 1980, **19**, 1473; d) M. Urrea, A. Ahond, A.-M. Bui, and P. Potier, *Bull. Soc. Chim. Fr. II*, 1981, 147; e) A.-M. Bui, B. C. Das, E. Guittet, J.-Y. Lallemand, and P. Potier, *J. Nat. Prod.*, 1986, **49**, 321; f) A.-M. Bui, B. C. Das, E. Guittet, and P. Potier, *ibid.*, 1991, **54**, 514.
2. B. Danieli and G. Palmisano in "The Alkaloids", Vol. 27, Academic Press Inc., California, 1986, p. 1.
3. U. Renner, D. A. Prins, A. L. Burlingame, and K. Biemann, *Helv. Chim. Acta*, 1963, **46**, 2186.
4. G. Büchi, R. E. Manning, and S. A. Monti, *J. Am. Chem. Soc.*, 1964, **86**, 4631.
5. H. Budzikiewicz, C. Djerassi, and D. H. Williams, 'Structure Elucidation of Natural Products by Mass Spectrometry', Holden-Day, San Franciso, 1964, vol. I, p. 67.
6. A. Husson, Y. Langlois, C. Riche, H.-P. Husson, and P. Potier, *Tetrahedron*, 1973, **29**, 3095.
7. E. Bombardelli, A. Bonati, B. Gabetta, E. M. Martinelli, G. Mustich, and B. Danieli, *J. Chem. Soc., Perkin Trans. I*, 1976, 1432.
8. R. van der Heijden, R. L. Brouwer, R. Verpoorte, T. A. van Beek, P. A. A. Harkes, and A. Baerheim Svendsen, *Planta Med.*, 1986, **52**, 144.

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