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HAZUNTIPHYLLIDINE AND ANHYDROHAZUNTIPHYLLIDINE, TWO NEW BISINDOLE ALKALOIDS FROM *HAZUNTA MODESTA* VAR. *MODESTA* SUBVAR. *DIVARICATA*

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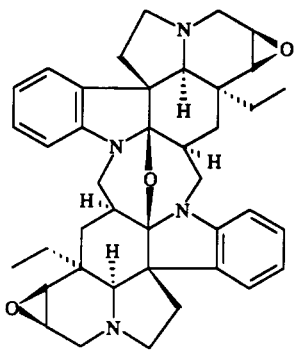
ABSTRACT.—Two new *Aspidosperma–Aspidosperma*-type bisindole alkaloids, hazuntiphyllidine and anhydrohazuntiphyllidine [**4**], have been isolated from the leaves of *Hazunta modesta* var. *modesta* subvar. *divaricata*. Their structures were established by spectroscopic means. In solution, hazuntiphyllidine was found to occur in two distinct structural forms **2** or **3** depending upon the solvent used.

Previously, we described the structural determination of hazuntiphylline [**1**], an *Aspidosperma–Aspidosperma*-type symmetrical bisindole alkaloid, from the leaves of *Hazunta modesta* var. *modesta* subvar. *divaricata* Mgf. (Apocynaceae), a plant endemic to the Nosy-Bé region of Madagascar (1). We now report the isolation and structure elucidation of two closely related new bisindole alkaloids, hazuntiphyllidine and anhydrohazuntiphyllidine [**4**], from the same source. In solution, hazuntiphyllidine is found to exist in two different structural forms depending upon the solvent employed. Thus, structure **2** has been established for hazuntiphyllidine in C_6H_6-d_6 solution, while the open form **3** occurs in DMSO-d_6 solution.



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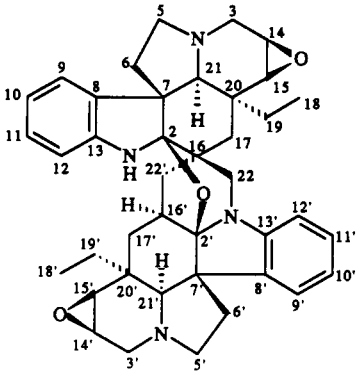

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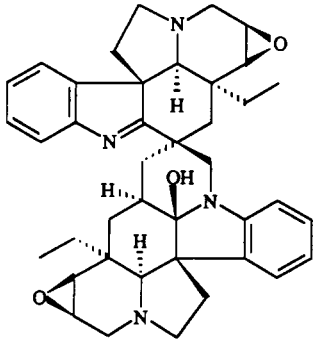

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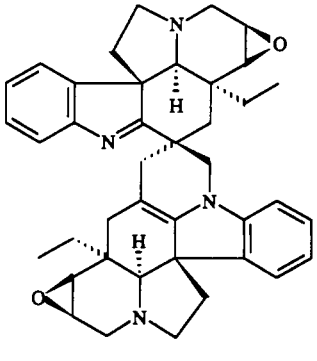

4

The Et_2O extract of the leaves of *H. modesta* was fractionated by using a Sephadex LH-20 column. The earlier fractions, eluted with $CHCl_3$-MeOH (1:4) yielded, on repeated Si gel chromatographic purification, hazuntiphyllidine and anhydrohazuntiphyllidine.

Hazuntiphyllidine, the major alkaloid, was obtained as colorless crystals from MeOH: mp above 300°; $[\alpha]^{22}D$ −28° (c = 0.66, $CHCl_3$); ir ν max (Nujol) 3400, 1605, 1460, 1380, 1240, 860, 750, 740 cm^{-1}; uv λ max (EtOH) 256 (ϵ 15,000) and 304 (ϵ 7,760) nm with no appreciable shift in alkali, characteristic of a nonphenolic indoline-type alkaloid. Its mass spectrum exhibited a molecular ion peak at *m/z* 630 that analyzed for $C_{40}H_{46}N_4O_3$ (found *m/z* 630.3575; calcd 630.3569), isomeric with hazuntiphylline [**1**]. Furthermore, the occurrence of fragment ions at *m/z* 492 $[M-138]^+$, 138, and 108, characteristic of a 14,15-epoxyaspidospermane framework, indicated a close structural similarity to **1** (1).

A more detailed understanding of the structure of hazuntiphyllidine was gained from an analysis of its ^1H- and ^{13}C-nmr spectra. Initially, attempts to analyze the nmr data obtained in $CDCl_3$ solution were rendered ineffective due to apparent doubling of most of the signals. The existence of an equilibrium mixture in $CDCl_3$ solution was suspected, and this was confirmed when the spectrum was recorded in C_6H_6-d_6 or DMSO-d_6 solution, in either of which the equilibrium was shifted entirely in favor of a single but different molecular arrangement. Thus, the ^{13}C-nmr spectra, obtained in C_6H_6-d_6 or DMSO-d_6, displayed in each case all the 40 carbons as distinctly separated resonances, although their chemical shift values are slightly but significantly different in the two solvents employed. In particular, the appearance of a signal at δ 190.7 in DMSO-d_6 was indicative of the presence of an indolenine system (2) that disappeared in C_6H_6-d_6 solution, in which the signal due to the corresponding carbon appeared at δ 102.2. The 400 MHz 2D (DQF-COSY) ^1H-nmr spectrum in C_6H_6-d_6 showed signals due to eight aromatic protons (δ 6.5–7.5) and two ethyl side chains (t, δ 0.72 and 0.86, 3H each). Other readily assignable signals were those of H-21, H-21′ (s, δ 2.43 and 2.24, 1H each), and the mutually coupled protons belonging to the 14,15- and 14′,15′-oxido systems together with the 3- and 3′-methylene protons (Table 1). On the basis of these data, it became clear that hazuntiphyllidine, like hazuntiphylline [**1**], must be constituted of two 14,15-oxidoaspidospermane-type moieties in which the aromatic rings and the ethyl side chains remained unsubstituted. The principal difference resided in the loss of symmetry in hazuntiphyllidine, in which the points of linkage between the two moieties must therefore be different from that found in hazuntiphylline [**1**]. A significant feature of the ^1H-nmr spectrum (in C_6H_6-d_6 solution) of hazuntiphyllidine, in contrast to that of **1**, is the upfield shift of two methylene protons (δ 1.68, H_2-22′) next to a methine proton at δ 2.23 (H-16′), which in turn is coupled with two protons of another methylene group (δ 1.50 and 2.73, H_2-17′) attached to a tetrasubstituted carbon (C-20′). Also, the presence of one N-CH_2 group (δ 2.97 and 5.77, H_2-22) without being coupled to any neighboring proton was noteworthy.

All these elements could be accommodated in structure **2** for hazuntiphyllidine. Additional support for this structure of hazuntiphyllidine was obtained from an analysis of its ^{13}C-nmr in C_6H_6-d_6 solution. Fourteen signals (8 × CH and 6 × C) were observed in the lower field region of the spectrum (above 90 ppm), while the higher field region (below 90 ppm) displayed twenty-six resonances belonging to 2 × Me, 12 × CH_2, 7 × CH, and 5 quaternary carbons. Together they accounted for all the 40 carbons in the molecule of hazuntiphyllidine. Of the 14 lower-field signals, 12 (8 × CH and 4 × C) belonging to two aromatic rings could be readily recognized, whereas the two quaternary carbon resonances at δ 102.2 and 94.9 suggested that they should be due to carbons linked to two heteroatoms and thus attributed to C-2 and C-2′, respec-

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 Hazuntiphyllidine (**2** in C_6H_6-d_6; **3** in DMSO-d_6) and Anhydrohazuntiphyllidine [**4**] (C_6H_6-d_6). *J* values (Hz) are given in parentheses.

Proton	Compound			Carbon	Compound		
	2	**3**	**4**		**2**	**3**	**4**
H-3	2.15 d (12)	2.70 d (12.7)	2.32 d (12.5)	C-2	102.2[a]	190.7	188.9
	3.50 d (12)	3.65 d (12.7)	3.57 d (12.5)	C-3	53.1	50.4	52.4
H-5	2.37 ddd (8, 7, 13)	2.26 ddd (10, 10, 10)	2.51 ddd (7, 6, 12)	C-5	55.3	51.9	54.8
	3.24 dd (8, 8)	3.05 dd (10, 4)	3.27 dd (7, 7)	C-6	34.4	32.3	36.2
H-6	2.21 ddd (13, 13, 8)	1.10 ddd (13, 10, 4)	1.80 dd (14, 6)	C-7	56.8[b]	60.4	61.0
	4.02 dd (13, 7)	2.86 dd (13, 10)	2.98 ddd (14, 12, 7)	C-8	136.6[c]	147.1[a]	144.7
H-9	7.16 d (7)	7.62 d (7)	7.36 d (7)	C-9	121.9	127.4	127.4
H-10	6.95 t (7)	7.29 t (7)	7.35 t (7)	C-10	118.1	119.9	121.5[a]
H-11	7.11 t (7)	7.39 t (7)	7.37 t (7)	C-11	128.0	125.6	125.7
H-12	6.53 d (7)	7.54 d (7)	7.98 d (7)	C-12	107.6	120.9	120.8[a]
H-14	3.14 d (3.5)	3.30 d (3.5)	3.11 d (3.5)	C-13	148.0[d]	152.5	154.5
H-15	2.92 d (3.5)	2.79 d (3.5)	2.80 d (3.5)	C-14	52.8	52.1	53.4
H-17	1.95 d (14)	1.43 d (14)	2.23 d (17)	C-15	56.4	55.6	57.0
	2.26 d (14)	2.85 d (14)	3.61 d (17)	C-16	36.3[e]	43.6	41.3
H-18	0.86 t (7)	0.64 t (7)	0.68 t (7)	C-17	39.8	35.9	41.8
H-19	1.34 m and 1.47 m	0.78 m	1.05 m	C-18	7.6	7.3	8.1
H-21	2.43 s	2.54 s	2.64 s	C-19	28.1	26.7	28.9
H-22	2.97 d (10)	3.39 d (13.2)	3.10 d (11)	C-20	34.8	36.4	38.8
	5.77 dd (10, 3)	4.20 d (13.2)	4.97 d (11)	C-21	67.5	72.3	72.0
				C-22	51.3	49.7	54.5
H-3′	2.10 d (12.5)	2.45 d (13)	2.53 d (12)	C-2′	94.9[a]	93.1	148.4[b]
	3.37 d (12.5)	3.41 d (13)	3.57 d (12)	C-3′	51.8	52.5	51.6
H-5′	2.04 m	2.87 m	2.43 m	C-5′	53.1	52.6	52.6
	3.04 m	3.35 m	2.85 m	C-6′	32.9	32.4	44.2
H-6′	1.50 m	1.62 m	1.82 m	C-7′	55.0[b]	54.6	50.1
	3.37 m	2.69 m	2.43 m	C-8′	138.5[c]	136.1	140.0
H-9′	7.25 d (7)	7.19 d (7)	7.35 d (7)	C-9′	122.8	122.0	121.7[a]
H-10′	6.99 t (7)	6.69 t (7)	7.06 t (7)	C-10′	119.8	117.2	120.0
H-11′	7.24 t (7)	7.11 t (7)	7.35 t (7)	C-11′	128.5	126.8	127.5
H-12′	6.61 d (7)	6.82 d (7)	6.80 d (7)	C-12′	110.2	108.3	108.6
H-14′	3.19 d (3.5)	3.37 d (3.5)	3.19 d (3.5)	C-13′	147.3[d]	147.8[a]	148.6[b]
H-15′	2.91 d (3.5)	2.96 d (3.5)	3.09 d (3.5)	C-14′	54.5	52.4	52.6
H-16′	2.23 m	2.05 d (12)		C-15′	59.4	55.6	56.3
H-17′	1.50 m	0.97 d (10.7)	1.73 d (14.5)	C-16′	31.0	28.1	103.3
	2.73 dd (13.5, 13.5)	2.05 d (10.7)	3.21 d (14.5)	C-17′	30.4	27.4	28.5
H-18′	0.72 t (7)	0.92 t (7)	0.91 t (7)	C-18′	8.7	7.5	8.1
H-19′	1.34 m and 1.65 m	1.43 m and 1.61 m	1.10 m and 1.49 m	C-19′	34.9	27.5	27.0
H-21′	2.24 s	2.54 s	2.50 s	C-20′	33.8[e]	36.4	36.9
H-22′	1.68 m	1.23 d (13)	2.23 d (17)	C-21′	75.7	67.1	75.1
		3.10 dd (13, 12)	3.58 dd (17, 3)	C-22′	37.4	35.5	42.9

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

tively. Furthermore, the upfield shift of a methylene carbon (δ 37.4, C-22′) and the appearance of a new tetrasubstituted carbon (δ 36.3, C-16), compared to the corresponding carbons of hazuntiphylline [**1**], could only be accounted for by the structural representation **2** for hazuntiphyllidine. This structure accommodates all the chemical shifts (Table 1) along with their multiplicity patterns observed in the ^{13}C-nmr spectrum.

In DMSO-d_6 solution, the 400 MHz 2D (DQF-COSY) ^{1}H-nmr spectrum of hazuntiphyllidine showed essentially the same connectivity pattern represented in structure **2**. However, the ^{13}C-nmr spectrum recorded in the same solvent displayed a low-field quaternary carbon at δ 190.7 characteristic of an indolenine functionality (2), thereby indicating the structure **3** in this solvent. As shown in Table 1, all other carbon chemical shift values could also be readily assigned in terms of this structure. It may be noted that the nmr spectra of hazuntiphyllidine, recorded with a freshly prepared solution in C_6H_6-d_6, corresponded predominantly to structure **3** and only after several hours did the equilibrium shift entirely in favor of structure **2** in this solvent. This observation suggested that the initial structural form of hazuntiphyllidine should possibly be represented by the chemical arrangement **3**.

Treatment of hazuntiphyllidine with trifluoroacetic acid at reflux temperature gave anhydrohazuntiphyllidine [**4**], whose identity with the second new alkaloid isolated from the leaves of *H. modesta* was established by comparison of their physical characteristics and spectral data. Anhydrohazuntiphyllidine crystallized from hexane: mp 260°; $[\alpha]^{22}D$ −175° (c = 0.57, $CHCl_3$); ir ν max (Nujol) 1600, 1540, 1360, 1350, 830, 740, 730 cm^{-1}; uv λ max (EtOH) 269 (ϵ 13.710) nm. Hrms established the molecular formula $C_{40}H_{44}N_4O_2$ (found *m/z* 612.3458; calcd 612.3464) for anhydrohazuntiphyllidine, while its 400 MHz 2D ^{1}H-nmr spectrum and ^{13}C-nmr data (Table 1) are in complete agreement with the structure **4**.

On the basis of these considerations, structures **2** and **3** are proposed for hazuntiphyllidine, while **4** should represent anhydrohazuntiphyllidine. All the chemical shifts and couplings from the DQF-COSY and ^{1}H-^{13}C correlation 2D spectra, as well as the nOe correlations from NOESY experiments, fully support these structures. While the depicted overall stereochemistry in structures **2**, **3**, and **4** followed from the generally accepted representation of the *Aspidosperma–Aspidosperma*-type bisindole alkaloids (3), the indicated configuration at the spiro carbon C-16 was based on the observed nOe between H-22 (δ 5.77 for **2**, δ 4.20 for **3**, δ 4.97 for **4**) and H-6 (δ 4.02 for **2**, δ 2.86 for **3**, δ 2.98 for **4**) in these alkaloids. Also, the configurations at C-15 and C-15′ were further supported by the existence of a strong nOe between H-15 and H-18 as well as between H-15′ and H-18′. The stereochemistry at C-2′ and C-16′ of **2** and **3** remains to be considered. According to molecular model construction, the stereochemistry of rings C, D, and E required that the ether bridge in **2** could only be formed through the 2β,2′β-oxygen linkage, which also required an α-axial orientation for H-16′. This was supported by the observed large diaxial coupling constant (J = 12 Hz) between H-16′ (δ 2.05) and H-22′ at δ 3.10 (see Table 1) of **3**.

An interesting aspect of the ^{1}H-nmr spectrum of hazuntiphyllidine [**2**] is the occurrence of a long range spin-spin coupling ($^4J_{H,H}$ = 3 Hz) between H-22 at δ 5.77 and one of the H-22′ at δ 1.68, which can be clearly explained by a four-bond "W" shape planar arrangement as noted in the molecular model. Similar observations were also made in the spectra of **3** and **4**, although the corresponding coupling constants were much smaller (< 1.5 Hz).

Hazuntiphyllidine [**2**, **3**] and anhydrohazuntiphyllidine [**4**] represent a novel series of *Aspidosperma–Aspidosperma*-type bisindole alkaloids in which the site of the spiro carbon is different from that observed in the related vobtusine group (3) of bisindole alkaloids.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—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; nmr, ^{1}H (400 MHz) and ^{13}C (100.61 MHz) on a Bruker WM 400. Chemical shifts are given in ppm relative to TMS (δ = 0); abbreviations s, d, t, and m in Table 1 refer to singlet, doublet, triplet, and multiplet, respectively.

EXTRACTION AND ISOLATION.—Alkaloid extraction of the air-dried leaves of *H. modesta* var. *modesta* subvar. *divaricata* was described previously (1). The plant material used was collected at Nosy-Bé in Madagascar. A voucher specimen (H. 1580D) was deposited at the herbarium of the Muséum National d'Histoire Naturelle de Paris, France.

ISOLATION OF HAZUNTIPHYLLIDINE [**2**, **3**] AND ANHYDROHAZUNTIPHYLLIDINE [**4**].—The alkaloid mixture (11 g) was subjected to Sephadex LH 20 (400 g) fractionation, eluting with $CHCl_3$-MeOH (1:4) and collecting 30-ml fractions. The bisindole alkaloids (5 g), located in fractions 11–14, were rechromatographed on a hydrated Si gel (350 g, 15% H_2O) column by starting the elution with Et_2O; fractions of 50 ml each were collected. The first five fractions, which contained 150 mg nonalkaloidal residue, were rejected. The isolate (1.5 g) of fractions 6–16 furnished hazuntiphyllidine [**2**] (360 mg) by crystallization from MeOH. Similar crystallization of fractions 17–34 (860 mg) also yielded pure hazuntiphyllidine

[**2**, **3**] (380 mg). Further 250 fractions collected by continuing the elution with Et_2O containing MeOH in a gradually increasing concentration (from 2% to 50%) gave 1.91 g of a complex mixture of polar alkaloids. Si gel tlc of the mother liquour (450 mg) of fractions 17–34 also revealed the presence of a similar mixture of polar compounds. The residue (970 mg) of the mother liquour of fractions 6–16 gave several distinct spots on Si gel tlc, two of which corresponded to hazuntiphylline and hazuntiphyllidine. This was subjected to further Si gel (40 g containing 13% H_2O) cc. Fractions of 20 ml were collected by eluting in the following manner: *n*-hexane fractions 1–100 (no residue); *n*-hexane–EtOAc (9:1) fractions 1–20 (no residue); fractions 21–32 (80 mg, not investigated); fractions 33–50 (260 mg); fractions 51–66 (145 mg); *n*-hexane–EtOAc (4:1) fractions 67–82 (258 mg; crystallization from MeOH furnished 125 mg hazuntiphyllidine). Preparative Si gel tlc of fractions 33–50 (260 mg) yielded hazuntiphylline [**1**] (35 mg), hazuntiphyllidine [**2**, **3**] (18 mg), and anhydrohazuntiphyllidine [**4**] (53 mg). Similarly, fractions 51–66 (145 mg) provided hazuntiphyllidine [**2**, **3**] (27 mg), R_f 0.42 [Si gel, $CHCl_3$-MeOH (19:1)] and anhydrohazuntiphyllidine [**4**] (31 mg), R_f 0.56 [Si gel, $CHCl_3$-MeOH (19:1)].

DEHYDRATION OF HAZUNTIPHYLLIDINE [**2**, **3**] TO ANHYDROHAZUNTIPHYLLIDINE [**4**].—Hazuntiphyllidine (55 mg) was dissolved in trifluoroacetic acid (0.5 ml), and the orange solution obtained was heated to reflux at 70° for 5 min. After removal of trifluoroacetic acid in vacuo, H_2O was added to the residue. The aqueous phase was adjusted to pH 8.5 with Na_2CO_3 and extracted with CH_2Cl_2. Removal of solvent yielded anhydrohazuntiphylline [**4**] (51 mg).

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