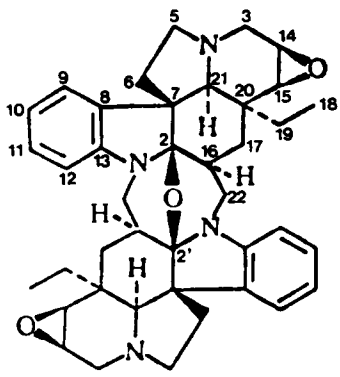
Journal of Natural Products
Vol. 49, No. 2, pp. 321-325, Mar-Apr 1986

STRUCTURE OF HAZUNTIPHYLLINE, A NOVEL SYMMETRICAL BISINDOLE ALKALOID FROM *HAZUNTA MODESTA* VAR. *MODESTA* SUBVAR. *DIVARICATA*

ANNE-MARIE BUI, BHUPESH C. DAS,* ERIC GUITTET, JEAN-YVES LALLEMAND, and PIERRE POTIER

Institut de Chimie des Substances Naturelles du CNRS, 91190 Gif-sur-Yvette, France

Previously, we reported the isolation of several known indole alkaloids from the diethyl ether extract of different parts of the plant *Hazunta modesta* var. *modesta* subvar. *divaricata* Mgf. (Apocynaceae) (1). In the course of our studies, the presence of five unknown bisindole alkaloids were noted in the leaves of this plant (1). One of these alkaloids, given the name hazuntiphylline, was isolated in ca. 1.1×10^{-2}% yield after purification by Sephadex LH20 chromatography followed by silica gel column and preparative tlc. Herein we propose structure **1** for hazuntiphylline, established from an analysis of its ^{1}H- and ^{13}C-nmr spectral properties, together with the consideration of its mass spectral fragmentation pattern.

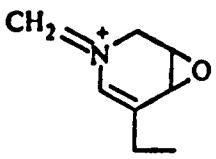


1

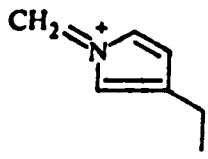
Hazuntiphylline was obtained as a white amorphous solid, $[\alpha]^{20}D$ +14° (c=1, $CHCl_3$); ir ν max ($CHCl_3$) 1605, 1480, 1460, 1380, 1310, 800, 730 cm^{-1}; uv λ max (EtOH) 255 (log ϵ 4.27) and 304 (log ϵ 3.83) with no shift in alkali, characteristic of a nonphenolic, indoline-type alkaloid. High resolution eims established the molecular formula as $C_{40}H_{46}N_4O_3$ ($M^{+\cdot}$ 630.3575; calcd. 630.3569), indicating a bisindole structure for hazuntiphylline. The 400 MHz ^{1}H-nmr spectrum, however, integrated for only 23 protons of which four belonged to the aromatic region (δ 6.35-7.13), while a three-proton triplet (δ 0.63) was indicative of the presence of an ethyl chain. A symmetrical bisindole structure, in which the aromatic unit and the ethyl side-chain remained unsubstituted, was therefore suspected, and this was confirmed by the ^{13}C-nmr spectrum, which displayed 20 carbon signals instead of 40, as would have been expected from its molecular formula.

Besides the prominent molecular ion peak at *m/z* 630, the occurrence of fragment ions at *m/z* 138 (ion *a*, $C_8H_{12}NO$), 108 (ion *b*, $C_7H_{10}N$), and 492 ($M^{+\cdot}-138$, $C_{32}H_{34}N_3O_2$) in the mass spectrum of hazuntiphylline attested to the presence of a 14,15-epoxy substituted *Aspidosperma*-type framework as found in lochnericine (**2**) (2), pachysiphine (**3**) (3,4,5), and hazuntinine (**4**) (4,6).

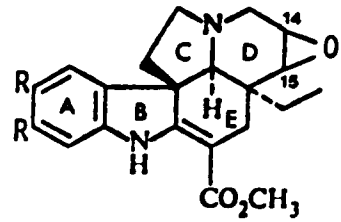
The absence of any >NH, -OH or >C=O bands in the ir spectrum, along with the consideration of the above spectral data, suggested a symmetrical bisindole structure for hazuntiphylline composed of two identical subunits possessing a 14,15-epoxy *Aspidosperma*-type framework whose points of attachment must involve the indoline nitrogens. The structure must also account for the third oxygen atom in the molecule which necessarily forms an ether bridge. All these molecular features of hazuntiphylline can be accommodated in the structural representation **1** which received support from an analysis of its ^{13}C-nmr data. Resonances due to the 40



ion *a*, *m/z* 138



ion *b*, *m/z* 108



2 R=H; 14,15α-oxide
3 R=H; 14,15β-oxide
4 R=OMe; 14,15β-oxide

carbons of hazuntiphylline appeared as 20 distinct signals in its ^{1}H-decoupled ^{13}C-nmr spectrum. A signal due to a quaternary carbon at 94.22 ppm revealed that this carbon is attached to two heteroatoms and must therefore be the C-2 of an *Aspidosperma*-type skeleton. Such a functional group distribution is reminiscent of that found in the bisindole alkaloids folicangine (**5**) (4) and ervafoline (**6**) (8,9). Comparison of the ^{13}C-nmr chemical shift values (Table 1)

TABLE 1. ^{1}H-(400 MHz, C_6D_6) and ^{13}C-(100.61 MHz, $CDCl_3$) nmr Chemical Shift (δ TMS=0) Assignments for Hazuntiphylline (**1**)

Proton	δ (ppm)	Coupling constants (Hz)		Carbon	δ (ppm)
3A	2.01	3A,3B	12.5	2	94.22 s
		3A,14	1.2		
				3	53.13[a] t
3B	3.24	3B,14	1.2		
				5	52.81[a] t
5A	3.10	5A,5B	8.8		
		5A,6A	8.0	6	35.36 t
		5A,6B	3.4		
				7	57.90 s
5B	3.18	5B,6A	7.1		
		5B,6B	12.8	8	137.34 s
6A	2.08	6A,6B	9.3	9	122.51 d
6B	1.43			10	118.89 d
9	6.98	9,10	7.4	11	127.76 d
10	6.76	10,11	6.8	12	108.24 d
11	7.3	11,12	7.4	13	147.98 s
12	6.35			14	52.60 d
14	2.82	14,15	3.9	15	56.61 d
15	2.65			16	33.89 d
16	1.94	16,22A	5.4	17	27.15[b] t
		16,22B	2.7		
				18	7.52 q
17ax	2.81	17ax,17eq	13.8		
		17ax,16	13.8	19	28.02[b] t
17eq	1.14	17eq,16	2.0	20	35.14 s
		17eq,21	0.5		
				21	70.62 d
18	0.63	18,19A	7.0		
		18,19B	7.0	22	46.46 t
19A	1.15	19A,19B	14.5		
19B	1.42				
21	2.24				
22A	3.57	22A,22B	12.2		
22B	3.02				

[a,b]Assignments may be interchanged.

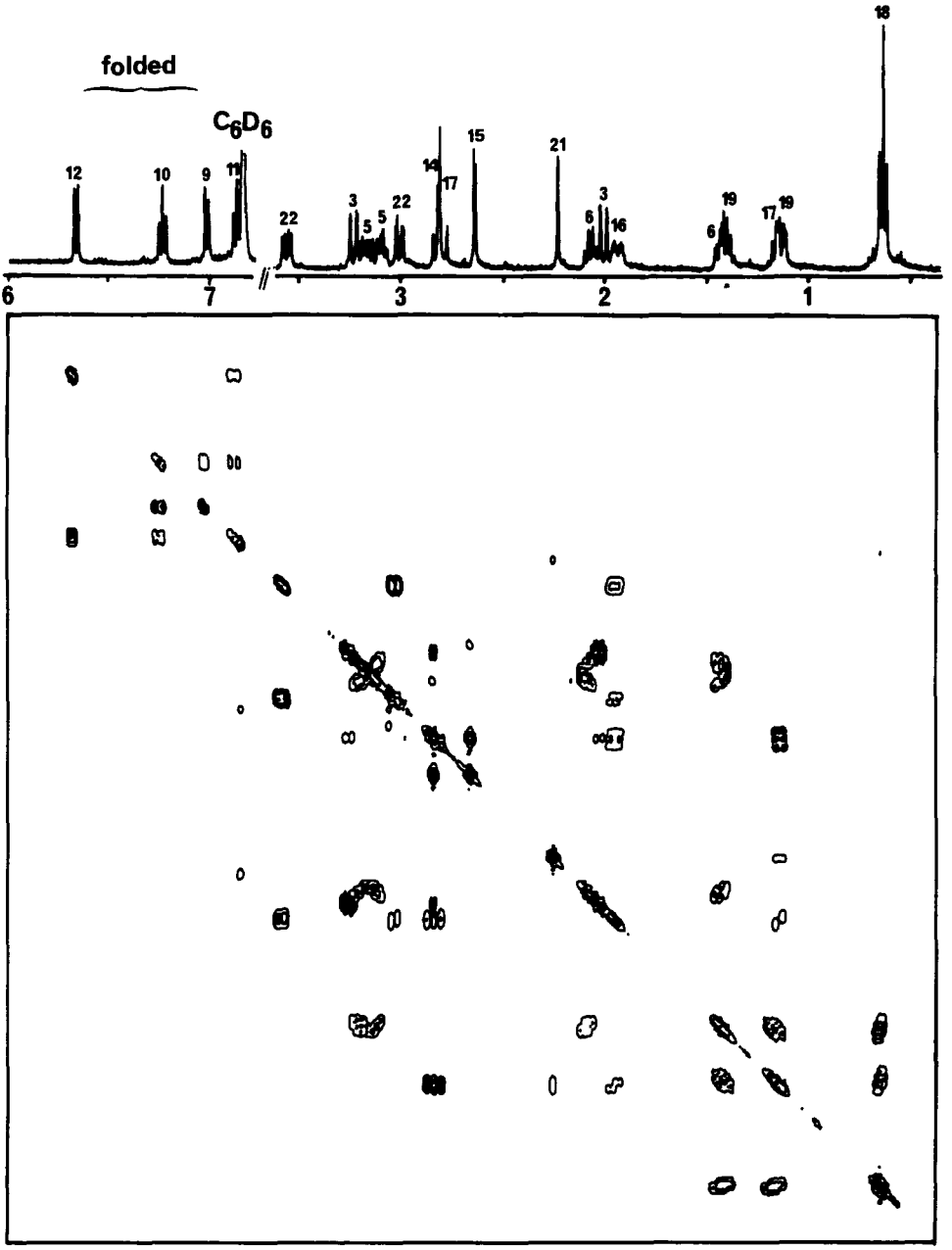


FIGURE 1. ^1H-^1H chemical shift correlated 2-D (45° COSY) nmr spectrum of **1** in C_6D_6 at 400 MHz. In order to reduce the size of the data matrix, the low field region (6.0-7.3 ppm) has been folded in both dimensions.

as well as the single frequency off-resonance multiplicities of hazuntiphylline (**1**) with those of **5** and **6**[1] direct signal matching for all of the carbon atoms of rings A, B, C, D, and E of these bases. The closer correspondence of the ring D carbon chemical shift values of hazuntiphylline (**1**) with those of pachysiphine (**3**), rather than those of lochnericine (**2**), pointed toward a 14,15-β configuration for the oxirane ring (10). Any chemical shift differences observed for some of the

[1]The complete ^{13}C-chemical shift values of **6** were kindly communicated to us by Dr. H.P. Husson (8,9).

similar carbons of these alkaloids could be the consequence of a difference in their overall carbon framework as well as minor conformational changes involved. The depicted stereochemistry in structure **1** was also supported by the proton coupling pattern (Table 1) and the carbon connectivities revealed by a COSY (11) experiment (Figure 1). In particular, an α-axial orientation for H-16 was indicated by its coupling pattern (see Table 1; $J_{16,17ax}$ 13.8 Hz, $J_{16,17eq}$ 2 Hz) with the C-17 methylene protons. Also, the small coupling constants (1.2 Hz each) between H-14 and each of the C-3 methylene protons as well as the large Δδ for C-3 and C-17 methylene protons support the proposed stereochemistry. Further confirmation of the relative stereochemistry of hazuntiphylline was obtained by nOe correlations from a two-dimensional NOESY (Tm=4 sec) experiment (12). The existence of a strong nOe between H-16 and H-21 is clearly indicative of their *syn*-relationship. The nOe observed between H-21 and H-3, H-21 and H-9, and that between H-21 and H-19 are also consistent with our structural proposal for hazuntiphylline. According to the molecular model construction, this stereochemistry of the rings C, D, and E of **1** required that the ether bridge could only be formed through the 2β-oxygen linkage.

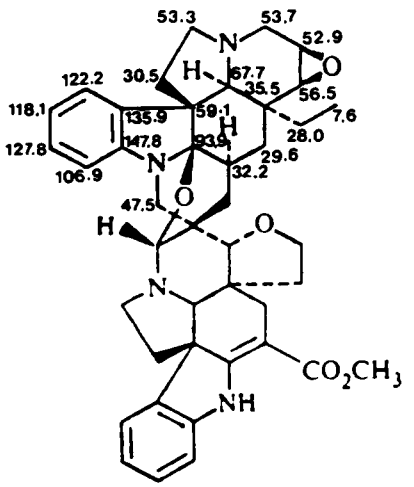
Hazuntiphylline (**1**) is a new example of a symmetrical bisindole alkaloid composed of two *Aspidosperma*-type subunits linked through N-1 and C-22 as well as by an ether bridge between C-2 and C-2′. It thus compares with the *Strychnos* series of symmetrical bisindole alkaloids such as *C*-curarine I and *C*-alkaloid E which consist of two akuammicine-type subunits having a C-2–C-2′ ether bridge (13).

EXPERIMENTAL

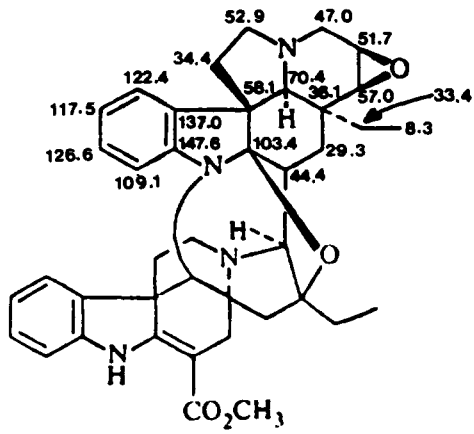
GENERAL EXPERIMENTAL PROCEDURES.—Optical rotation was determined on a Perkin-Elmer model 141 polarimeter. Spectra were recorded with the following instruments: uv, Bausch and Lomb 505; ir, Perkin-Elmer 297; ms, AEI MS50; nmr, ^1H (400 MHz, C_6D_6) and ^{13}C (100.61 MHz, $CDCl_3$) on a Bruker WM 400; chemical shifts are given in ppm relative to TMS (δ=0); carbon multiplicities expressed by the abbreviations s, d, t, and q in Table 1 refer to singlet, doublet, triplet, and quartet, respectively.

PLANT MATERIAL.—The *H. modesta* var. *modesta* subvar. *divaricata* Mgf. 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.

EXTRACTION.—The ground air-dried leaves (1 kg) were soaked with 25% aqueous NH_4OH (150 ml) and then exhaustively extracted with Et_2O in a Soxhlet apparatus. The alkaloids were partitioned into dilute HCl (0.1 N) and then washed several times with Et_2O. After basifica-



5



6

tion with concentrated NH_4OH, the crude alkaloids (12 g) were obtained by successive Et_2O and $CHCl_3$ extraction.

ISOLATION OF HAZUNTIPHYLLINE.—The alkaloid mixture (11 g) was subjected to Sephadex LH20 (400 g) column chromatography, eluting with $CHCl_3$-MeOH (20:80) (30 ml fractions). The bisindole alkaloids (5 g), found in fractions 11-14, were rechromatographed with Et_2O on a silica gel column (700 g) and 150 ml fractions were collected. Fractions 3-4 (1 g) contained hazuntiphylline, which could not be separated from the mixture by column chromatography. Pure hazuntiphylline was isolated by preparative tlc on several Al_2O_3 plates (CH_2Cl_2) as a white amorphous solid (0.1 g; 1.1×10^{-2}%), Rf 0.6 (Al_2O_3, CH_2Cl_2); Rf 0.5 (silica gel, CH_2Cl_2-MeOH, 95:5).

LITERATURE CITED

1. A.M. Bui, P. Potier, M. Urrea, A. Clastres, D. Laurent, and M.M. Debray, *Phytochemistry*, **18**, 1329 (1979).
2. B.K. Moza, J. Trójanek, A.K. Bose, K.G. Das, and P. Funke, *Lloydia*, **27**, 416 (1964).
3. M.B. Patel and J. Poisson, *Bull. Soc. Chim. Fr.*, 427 (1966).
4. Y. Rolland, N. Kunesch, J. Poisson, E.W. Hagaman, F.M. Schell, and E. Wenkert, *J. Org. Chem.*, **41**, 3270 (1976).
5. N. Kunesch, C. Miet, and J. Poisson, *Bull. Soc. Chim. Fr.*, II, 285 (1982).
6. P. Potier, A.M. Bui, B.C. Das, J. Le Men, and P. Boiteau, *Ann. Pharm. Fr.*, **26**, 621 (1968).
7. E. Wenkert, D.W. Cochran, E.W. Hagaman, F.M. Schell, N. Neuss, A.S. Katner, P. Potier, C. Kan, M. Plat, M. Koch, H. Mehri, J. Poisson, N. Kunesch, and Y. Rolland, *J. Am. Chem. Soc.*, **95**, 4990 (1973).
8. A. Henriques, C. Kan, A. Chiaroni, C. Riche, H.P. Husson, S. Kan, and M. Lounasmaa, *J. Org. Chem.*, **47**, 803 (1982).
9. A. Henriques, C. Kan, A. Ahond, C. Riche, and H.P. Husson, *Tetrahedron Lett.*, 3707 (1978).
10. N. Kunesch, A. Cavé, E.W. Hagaman, and E. Wenkert, *Tetrahedron Lett.*, **21**, 1727 (1980).
11. W.P. Ave, E. Barthold, and R.R. Ernst, *J. Chem. Phys.*, **64**, 2229 (1976).
12. S. Makura, Y. Huang, and R.R. Ernst, *Biochem. Biophys. Res. Commun.*, **90**, 305 (1979).
13. G.A. Cordell, in: "The Monoterpenoid Indole Alkaloids." Ed. by J.E. Saxton, Wiley-Interscience, New York, 1983, pp. 585-596.

Received 15 July 1985