Journal of Natural Products
Vol. 58, No. 7, pp. 1139-1142, July 1995

8b-HYDROXYPTILOCAULIN, A NEW GUANIDINE ALKALOID FROM THE SPONGE *MONANCHORA ARBUSCULA*

R. TAVARES, D. DALOZE, J.C. BRAEKMAN,*

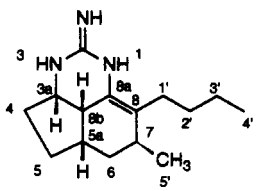
Laboratory of Bioorganic Chemistry, Faculty of Sciences, University of Brussels, 50 Av. F. Roosevelt, 1050 Brussels, Belgium

E. HAJDU, and R.W.M. VAN SOEST

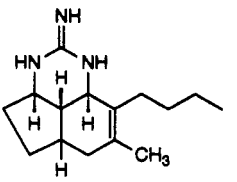
Institute of Systematics and Population Biology, University of Amsterdam, P.O. Box 94766, 1090-GT, Amsterdam, The Netherlands

ABSTRACT.—(+)-Ptilocaulin [**1**] and (+)-8b-hydroxyptilocaulin [**3**] have been isolated from a methanolic extract of the marine sponge *Monanchora arbuscula*. (+)-8b-Hydroxyptilocaulin is a new compound, the structure of which was elucidated by comparison of its spectral properties with those of **1**. The complete assignment of all the ^{1}H- and ^{13}C-nmr signals of **1** and **3** is presented. The taxonomic implications of these results are discussed.

Recently, we reported the isolation from the MeOH extract of the sponge *Monanchora arbuscula* (Duch. & Mich., 1864) (Esperiopsidae) collected off Farol da Barra, Salvador, Brazil, of crambescidin 800 (1), a toxic pentacyclic guanidine alkaloid previously known from the Mediterranean sponge *Crambe crambe* Schmidt (2,3). Further guanidine derivatives were present in the extract, but due to the small amounts of material available, they could not be characterized accurately. Thus, a larger sample of *M. arbuscula* was collected at Cat Cay Lagoon on the barrier reef of Belize. The new MeOH extract was found to be identical by tlc to that of the Brazilian sample and subsequent fractionations of this extract afforded two further guanidine alkaloids. The less polar one, obtained as its crystalline nitrate salt, was identified as (+)-ptilocaulin [**1**], an antimicrobial and cytotoxic tricyclic guanidine alkaloid isolated in 1981 together with isoptilocaulin [**2**] from the Caribbean sponge *Ptilocaulis* aff. *P. spiculifer* (Lamarck, 1814) by Harbour *et al.* (4). The second derivative is a new compound and was obtained as a colorless oil by reversed-phase chromatography of the mother liquors of (+)-ptilocaulin nitrate. From the spectral properties of its hydrochloride, the compound was de-



1



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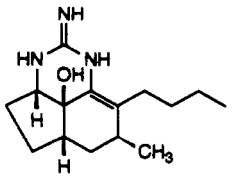

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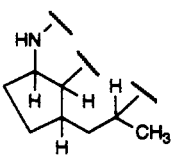

A

duced to be 8b-hydroxyptilocaulin [**3**]. In this paper we report the elucidation of its structure.

The molecular formula of compound **3** was determined by hreims. A parent ion was observed at *m/z* 263.1990 corresponding to a molecular formula of $C_{15}H_{25}N_3O$ (calcd 263.1998) and indicating that **3** differed from ptilocaulin (**1**, $C_{15}H_{25}N_3$) by the presence of an oxygen atom. Moreover, the observed fragment ions at *m/z* 246 (M^+-OH), 204 ($MH^+-C_3H_7-OH$) and 202 ($M^+-C_3H_7-H_2O$) in the mass spectrum of **3**, when compared to the base peak at *m/z* 204 ($M^+-C_3H_7$) in that of **1**, suggested that the former could be a hydroxyptilocaulin.

Because the complete ^1H- and ^{13}C-nmr assignments of ptilocaulin nitrate in $CDCl_3$ have not been reported previously in the literature, they are included in Table 1. They were based upon the analysis of its one-dimensional (broad-band proton decoupling, DEPT and NOEDS) and two-dimensional (HMQC and COSY) nmr spectra at 600 and 150.87 MHz. The COSY spectrum was particularly relevant. It clearly indicated the presence of two independent spin-systems, one attributable to the butyl chain and the other to the substructure A. Conspicuous couplings could be observed between H-3a and H-8b as well as between H-8b and H-5a. In the ^1H-nmr spectrum recorded in CD_3OD, the exchangeable NH absorptions at δ 8.90 (1H), 8.36 (1H), and 7.45 (2H) were absent. Concomitantly, H-3a appeared as a ddd (J=6, 8, and 10 Hz). Because H-8b appeared as a dd (J=6 and 6 Hz), this implied that $J_{3a,8b}=J_{8b,5a}=6$ Hz, in accordance with the cis orientation of these three protons in **1**. Moreover, significant nOes were observed between H_3C-5′ and H-5a, H-5a and H-3a, and H-3a and H-8b, again in agreement with the location of these atoms on the same side of the tricyclic

TABLE 1. Nmr Data of Ptilocaulin Nitrate (**1**.HNO_3) and 8b-Hydroxyptilocaulin Hydrochloride (**3**.HCl).[a]

Position	δ ^{13}C (150 MHz)		δ ^1H (600 MHz)	
	1.HNO_3	**3**.HCl	**1**.HNO_3	**3**.HCl
C-2	151.7	151.5	—	—
HC-3a	53.2	57.5	3.77 (m)	3.77 (m)
H_2C-4	32.2	29.6	2.05, 1.40 (m)	2.08, 1.22 (m)
H_2C-5	24.6	20.2	1.67, 1.43 (m)	1.70, 1.35 (m)
HC-5a	33.9	39.6	2.40 (m)	2.27 (m)
H_2C-6	33.0	29.4	1.70, 1.47 (m)	1.68 (m)
HC-7	27.7	27.2	2.37 (m)	2.36 (m)
C-8	121.0	122.2	—	—
C-8a	127.0	129.8	—	—
HC-8b	36.5	—	2.48 (dd, 6,6)	—
C-8b	—	70.1	—	—
H_2C-1′	26.7	26.9	2.33, 2.02 (m)	2.43, 2.03 (m)
H_2C-2′	29.6	29.4	1.40, 1.29 (m)	1.41, 1.27 (m)
H_2C-3′	22.4	22.3	1.25 (m)	1.27 (m)
H_3C-4′	13.9	14.0	0.85 (t, 7)	0.89 (t, 7)
H_3C-5′	19.5	19.3	1.05 (d, 7)	1.10 (d, 7)
HN-1	—	—	8.90 (s)[b]	9.25 (s)[b]
HN-3	—	—	8.36 (d,4)[b]	8.20 (s)[b]
H_2N^+	—	—	7.45 (br s)[b]	7.20 (s)[b]
HO	—	—	—	5.30 (br s)[b]

[a]Recorded in $CDCl_3$ with TMS as internal standard. Multiplicities (J) expressed in Hz.
[b]Signals suppressed in the spectrum taken in CD_3OD solution.

system. The ^{13}C-nmr chemical shifts of **1**.HNO_3 ($CDCl_3$) were identical to those reported by Walts and Roush for (−)-ptilocaulin (5).

The ^{1}H- and ^{13}C-nmr data of **3**.HCl are reported in Table 1. As for ptilocaulin nitrate, the assignments were based upon the analysis of the one-dimensional (broad-band proton decoupling and NOEDS) and two-dimensional (HMQC and COSY) nmr spectra. Comparison of the nmr data clearly confirmed that both compounds had the same basic C,N-skeleton. The most significant differences between the spectra of **1**.HNO_3 and **3**.HCl were the absence in the nmr spectra of **3**.HCl of signals attributable to H-8b, the presence in the ^{13}C-nmr spectrum of **3**.HCl of a quaternary carbon atom at δ 70.1, and the change of multiplicity of the signal at δ 3.85 attributed to H-3a (dd, J=8 and 10 Hz instead of ddd, J=6, 8, and 10 Hz) in the ^{1}H-nmr spectra recorded in CD_3OD. In addition, irradiation of the H_3C-5′ resonance in **3**.HCl simplified the H-7 signal into a dd with $J_{7,6trans}$=10 Hz, $J_{7,6cis}$=6 Hz. Typically, the same decoupling experiment carried out upon the H_3C-5′ resonance in **1**.HNO_3 simplified the H-7 signal into a ddd with $J_{7,6trans}$=9 Hz, $J_{7,6cis}$=6 Hz, and $J_{7,8b}$=2 Hz.

All these results are consistent with the proposal that **3** is 8b-hydroxy-ptilocaulin. The relative configuration of the latter at the stereogenic carbon atoms C-3a, C-5a, and C-7 are proposed to be identical to those of ptilocaulin based on the similarities of the ^{13}C-nmr chemical shifts and of the coupling constants between H-3a and both H_2-4 as well as between H-7 and both H_2-6. NOe difference experiments performed to assign the relative configuration of the hydroxyl group at C-8b were inconclusive, presumably due to the small amount of material available. Nevertheless, based on great similarities of chemical shifts between ptilocaulin and 8b-hydroxy-ptilocaulin, we tentatively assign to **3** the same C-8b configuration as **1**. Indeed, a change in the configuration of this center would impose conformational changes that would have significant effects on the spectral data of **3**.

The cooccurrence in *M. arbuscula* of crambescidin-, crambescin-, and ptilocaulin-type derivatives supports the point of view that these three groups of polycyclic guanidine alkaloids derive from related biogenetic pathways. Indeed, their polycyclic part can be visualized as resulting from the combination of an adequately modified fatty acid with a substituted guanidine moiety. Both crambescin- and crambescidin-type alkaloids have been isolated from MeOH extracts of the Mediterranean red sponge *C. crambe* (2, 3, 6–8). These chemical similarities reinforce the recent opinion based on morphological characters that the distinction between the genera *Crambe* Vosmaer, 1880 and *Monanchora* Carter, 1883 is questionable (9,10).

Moreover, a crambescidin-type alkaloid, namely ptilomycalin-A, as well as ptilocaulin [**1**] and isoptilocaulin [**2**] were reported from the Caribbean sponge, *Ptilocaulis* aff. *spiculifer* (4,11). Ptilomycalin-A is also known from a red sea sponge, *Hemimycale* sp. (11). We have re-examined the voucher specimen of *Ptilocaulis* aff. *spiculifer* (Harbor Branch collection) in which ptilomycalin-A was found, and it does not conform to *Ptilocaulis*, because its spicules are thin anisostrongyles coring a highly developed spongin skeleton, which is distinct from the skeleton of stout styles found in proper *Ptilocaulis* (12). Through its thin strongyles, the voucher specimen seems to conform to the poecilosclerid genus *Batzella* Topsent, 1891, which is possibly closely related to the genera *Crambe* and *Monanchora*.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were taken on a Philips PU 8700 uv-vis spectrometer. Eims measurements were performed on a VG Micromass 7070F and hreims on a VG

Autospec 6F. The ^{1}H- and ^{13}C-*nmr spectra* were recorded in $CDCl_3$ at 600 MHz and 150.87 MHz, respectively, using a Varian Unity 600 instrument. The chemical shifts are reported in ppm from internal TMS and the coupling constants in Hz. Optical rotations were measured on a Perkin-Elmer 141 polarimeter at 589 nm (Na D line) in a 1-dm cell. Flash liquid chromatography (13) was performed over Macherey-Nagel Si gel (0.04–0.063 mm), and tlc analysis conducted on Polygram $SilG/UV_{254}$ *precoated plates (0.25 mm). Hplc was* performed on a Waters LCM1 instrument.

ANIMAL MATERIAL.—Samples of *Monanchora arbuscula* (Duch. & Mich. 1864) were hand-collected at depths of 1 to 4 meters off Farol da Barra, Salvador, Brazil, and at Cat Cay Lagoon on the barrier reef of Belize, and stored in MeOH. Voucher specimens are deposited in the sponge collection of the Institute of Systematics and Population Biology of the University of Amsterdam.

EXTRACTION AND ISOLATION.—The samples were extracted as reported previously (1). The CCl_4 and *n*-BuOH extracts were combined and chromatographed on Sephadex LH-20 (eluent CH_3OH). The separations were monitored by tlc (*Si gel; vanillin/*H_2SO_4; *lower phase of the mixture* $CHCl_3$-CH_3OH-*i*PrOH-H_2O, 9:12:1:8). One of the resulting fractions was almost homogenous by tlc and its ^{1}H-nmr spectrum indicated that the major constituent was identical to ptilocaulin [**1**]. This fraction was then dissolved in CH_2Cl_2 and the organic phase was treated with 1 M $NaNO_3$, evaporated to dryness under reduced pressure, and the solid residue recrystallized several times from $CHCl_3/CH_3OH$. This afforded colorless crystals of (+)-*ptilocaulin nitrate* [43 mg; mp 183–185°, lit. (4) 183–185°; $[\alpha]_D$ +110° (*c*=0.44, CH_3OH), lit. (5) $[\alpha]_D$ +74.4° (CH_3OH)]; uv (CH_3OH) λ max 224 nm (10500); eims *m/z* 247 (M^+, 45), 232 (73), 218 (20), 204 (100), 190 (41); ^{1}H- and ^{13}C-nmr data, see Table 1.

Flash chromatography on Si gel (eluent: lower phase of the mixture $CHCl_3$-CH_3OH-*i*-PrOH-H_2O, 9:12:1:8) of the mother liquor of crystallization of ptilocaulin nitrate afforded crude 8b-hydroxyptilocaulin which was further purified by semi-prep. hplc (Lichrospher 60, RP Select B, 10 μm, 25×0.6 cm, flow rate 5 ml/min, uv detection at 234 nm, eluent CH_3OH-0.1 M NaCl, 60:40). This yielded pure 8b-hydroxyptilocaulin [**3**], presumably in the form of its hydrochloride [colorless oil; 2.6 mg; $[\alpha]_D$ +77.5° (*c*=0.12, CH_3OH); uv (CH_3OH) λ max 234 nm (6160); eims *m/z* 263 (M^+, 88), 248 (92), 246 (24), 234 (32), 221 (92), 220 (74), 206 (80), 204 (44), 202 (20), 136 (100); hreims molecular ion at *m/z* 263.1990 (calcd for $C_{15}H_{25}N_3O$, 263.1998); ^{1}H- and ^{13}C-nmr data, see Table 1].

ACKNOWLEDGMENTS

This work was supported by FRFC Belgium (2.4570.93). We thank Prof. R. Flammang, University of Mons, for the hreims, Mr. C. Moulard for the eims, Mr. C. Maerschalk for the nmr spectra, and Dr. S. Pomponi, Harbor Branch Oceanographic Institution, for the loan of a voucher specimen of *Ptilocaulis* aff. *spiculifer*. One of us (E.H.) is thankful to the Conselho Nacional de Desenvolvimento Cientifico e Tecnologico, Brazil, and the Coral Reef Ecosystems Program (contribution #435) for providing financial support. We also thank K. Rutzler and K. Smith, National Museum of Natural History, Smithsonian Institution, Washington, DC, for their help in organizing the field trip to Belize.

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Received 26 January 1995