the ^1H chemical shifts along with the splitting pattern of H-14[13] of 1 and 2 were similar to each other (in $CDCl_3$: 1: δ_H 4.59, dd, J = 11.7 and 4.9 Hz; 2: δ_H 4.60, dd, J = 11.6 and 4.4 Hz), the tridebromo derivative (2) was suggested to be also B/C cis. The relative configurations of 1 at C-8 and C-14 were deduced by the NOESY correlations for H-14/H-15a and H-6α/H-8, indicating that H-14 and C-15 methylene group were oriented to the same side of ring C. Thus the structure of theoneberine was concluded to be 1,[17] and this structure was consistent with the fragment ion peaks observed in the FABMS of 1. The intense peaks at m/z 566, 564, 562, and 560 (ca. 1:3:3:1) were ascribed to the fragment ions generated by loss of the benzyl group ($C_8H_8BrO_2$) at C-8, which was characteristic of 1-benzylisoquinolines,[18] whereas the fragment peaks at m/z 338, 336, and 334 (ca. 1:2:1) corresponded to the A and B rings resulting from the cleavage of C-13/C-14 and N-7/C-8 bonds via retro-Diels-Alder fragmentation.[4]

Theoneberine (1) is the first tetrahydroprotoberberine alkaloid isolated from marine organisms. Theoneberine (1) is also the first example as a naturally-occurring tetrahydroprotoberberine alkaloid with substitution by bromine atoms. Biogenetically theoneberine (1) seems to be classified as a hybrid between 1-benzylisoquinolines and protoberberines, which has been hitherto unknown from natural sources. Theoneberine (1) exhibited antimicrobial activity against Gram-positive bacteria (MIC values: *Staphylococcus aureus*, 16 μg/mL; *Sarcina lutea*, 2 μg/mL; *Bacillus subtilis*, 66 μg/mL; *Mycobacterium* sp. 607, 4 μg/mL).[19] Compound 1 also showed cytotoxicity against murine lymphoma L1210 and human epidermoid carcinoma KB cells in vitro with the IC_{50} values of 2.9 and ca. 10 μg/mL, respectively.

Experimental Section[20]

Isolation. The sponge *Theonella* sp. was collected off Ie Island, Okinawa, and kept frozen until used. The sponge (1 kg, wet weight) was extracted with methanol (1 L × 2). After evaporation under reduced pressure, the residue (39 g) was partitioned between ethyl acetate (400 mL × 3) and 1 M NaCl aqueous solution (400 mL). The ethyl acetate soluble fraction (1.0 g) was subjected to a silica gel column (Wako gel C-300, Wako Pure Chemical, 2.3 × 38 cm) with $CHCl_3$/MeOH (95:5). The fraction eluting from 230 to 320 mL was further separated by a reversed-phase column (YMC gel ODS-A 120-S30/50, 2.3 × 10 cm) with MeOH/H_2O (90:10). The fraction eluting from 430 to 500 mL was then purified by a reversed-phase HPLC [YMC-Pack ODS-AM, 5 μm, 10 × 300 mm; flow rate, 2.0 mL/min; UV detection at 254 nm; eluent, MeOH/H_2O (85:15)], followed by purification through a Sephadex LH-20 column (Pharmacia Fine Chemcials, 2 × 100 cm) with $CHCl_3$/MeOH (1:1) to give theoneberine (1, 3.6 mg, 0.0004% wet weight).

Theoneberine (1): colorless solid; mp 128 °C; $[\alpha]^{20}_D$ -53° (c 0.6, $CHCl_3$); UV λ_{max} (MeOH) 284 nm (ϵ 5300), (0.01 N HCl-MeOH) 286 nm (ϵ 5300), and (0.01 N NaOH-MeOH) 300 nm (ϵ 6800); IR (KBr) ν_{max} 3450, 2920, 1565, 1480, 1455, 1420, 1410, 1240, and 995 cm^{-1}; IR ($CHCl_3$) ν_{max} 3675, 3610, 3000, 1520, and 1420 cm^{-1}; ^1H and ^{13}C NMR (Table I); FABMS m/z 784, 782, 780, 778, 776 $(M + H)^+$, 566, 564, 562, 560 $(M - C_8H_8BrO_2)^+$ and 338, 336, 334; HRFABMS m/z 779.8447 $(M + H)^+$, calcd for $C_{27}H_{28}{}^{79}Br_2{}^{81}Br_2NO_6$ 779.8452.

Tridebromotheoneberine (2). A solution of theoneberine (1, 1.0 mg) in MeOH (0.5 mL) containing 10% Pd-C (0.8 mg) was stirred at room temperature under H_2 for 1 day. After removal of the catalyst by filtration and evaporation of the filtrate, the residue was purified by a silica gel column (Wako gel C-300, 0.5 × 5 cm) with $CHCl_3$/MeOH (95:5) to give tridebromotheoneberine (2, 0.3 mg, 51%): colorless solid; $[\alpha]^{26}_D$ -24° (c 0.05, $CHCl_3$); UV λ_{max} (MeOH) 283 nm (ϵ 5100), IR (film) ν_{max} 3400, 2920, 1490, and 1270 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.98 (1 H, d, J = 2.0 Hz, H-17), 6.79 (1 H, dd, J = 8.3, 2.0 Hz, H-21), 6.77 (1 H, d, J = 11.7 Hz, H-11), 6.72 (1 H, d, J = 8.3 Hz, H-20), 6.62 (1 H, d, J = 11.2 Hz, H-12), 6.58 (1 H, s, H-4), 4.60 (1 H, dd, J = 11.6, 4.4 Hz, H-14), 4.12 (1 H, dd, J = 8.3, 4.4 Hz, H-8), 3.90 (3 H, s, H_3-23), 3.88 (3 H, s, H_3-22), 3.85 (3 H, s, H_3-24), 3.14 (1 H, dd, J = 17.6, 4.4 Hz, H-13β), 3.03 (1 H, m, H-6β), 2.96 (2 H, m, H_2-15), 2.67 (1 H, m, H-13α), 2.58 (2 H, m, H-5α and H-6α), and 2.33 (1 H, m, H-5β); FABMS m/z 544, 542 $(M + H)^+$, 464 $(M + H - Br + H)^+$, and 406, 404 $(M + H - C_8H_{10}O_2)^+$; HRFABMS m/z 542.1177 $(M + H)^+$, calcd for $C_{27}H_{29}{}^{79}BrNO_6$ 542.1179.

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Supplementary Material Available: All spectra of compounds 1 and 2 (14 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(16) Bohlmann, F. *Chem. Ber.* **1958**, *91*, 2157-2167.
(17) The absolute configurations of 1 remained undefined, although the CD spectrum of 1 in MeOH was recorded only to give no characteristic curve observed.
(18) Dyke, S. F.; Kinsman, R. G. In *Heterocyclic Compounds, Isoquinolines*; Grethe, G., Ed.; Wiley: New York, 1981; Vol. 38, Part 1, pp 25-26.
(19) Compound 1 was inactive against fungi or Gram-negative bacteria. The antimicrobial activity of tetrahydroberberine was also examined for reference to show fairly weak activity (MIC values: *Staphylococcus aureus*, 133 μg/mL; *Sarcina lutea*, 266 μg/mL; *Bacillus subtilis*, >266 μg/mL; *Mycobacterium* sp. 607, 266 μg/mL).
(20) The general procedures are the same as described in the previous papers.[8]

Conformational Analysis of 1,2:5,6:9,10-Tribenzododeca-1,5,9-triene by ^1H NMR at 173 K

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In 1978, Brickwood, Ollis, Stephanidou-Stephanatou, and Stoddart[1] studied the conformational behavior of 1,2:5,6:9,10-tribenzododeca-1,5,9-triene (1) (called by these authors, 5,6,11,12,17,18-hexahydrotribenzo[*a*,*e*,*i*]cyclododecene). By means of proton-decoupled ^{13}C NMR spectroscopy at -104 °C in a 2:1 mixture of CD_2Cl_2-CS_2, they determined that the sole conformation present in solution is that of C_2 symmetry. Only this conformation is consistent with the observation of three methylene signals. Recently,[2] we have determined the X-ray structure

(1) Brickwood, D. J.; Ollis, W. D.; Stephanidou-Stephanatou, J.; Stoddart, J. F. *J. Chem. Soc., Perkin Trans. 1* **1978**, 1398.
(2) Domiano, P.; Cozzini, P.; Claramunt, R. M.; Lavandera, J. L.; Sanz, D.; Elguero, J. *J. Chem. Soc., Perkin Trans. 2* **1992**, 1609.

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Table I. ^{1}H NMR Parameters of Compound 1 in CD_2Cl_2–CS_2 (2:1) at 173 K

atom[a]	δ (ppm)	J (Hz)	vicinal dihedral angle[b]	benzylic dihedral angle[b]	COSY	ROESY
H_{13} (H_{16})	7.304				H_{121} (H_{31})	H_{121} (H_{31})
H_{14} (H_{15})	7.257					
H_{17} (H_{24})	7.276				H_{31} (H_{121})	H_{42} (H_{112})
H_{18} (H_{23})	~7.21					
H_{19} (H_{22})	~7.21					
H_{20} (H_{21})	7.406				H_{71} (H_{82})	
H_{31} (H_{121})	2.716	H_{31}–H_{32} (H_{121}–H_{122}) = −13.3		22.4 (27.7)	H_{17} (H_{24})	
		H_{31}–H_{41} (H_{121}–H_{111}) = 13.2	170.9 (172.6)			
H_{32} (H_{122})	3.170	H_{32}–H_{41} (H_{122}–H_{111}) = 2.7	74.7 (74.0)	41.4 (39.7)		
		H_{32}–H_{42} (H_{122}–H_{112}) = 13.5	170.5 (171.7)			
H_{41} (H_{111})	3.289	H_{41}–H_{42} (H_{111}–H_{112}) = −13.5		35.2 (38.9)		
H_{42} (H_{112})	2.738	H_{31}–H_{42} (H_{121}–H_{112}) = 6.6	56.1 (58.2)	26.2 (26.2)	H_{17} (H_{24})	
H_{71}	2.925	H_{71}–H_{72} = −13.4		21.2 (25.5)	H_{20}	H_{32} + H_{41}
		H_{71}–H_{81} (H_{72}–H_{82}) = 13.2	173.9 (167.6)			
H_{72}	3.120	H_{72}–H_{81} = 6.8	56.6 (54.0)	41.0 (40.9)		
H_{81}	3.120	H_{81}–H_{82} = −13.4		41.0 (40.9)		
H_{82}	2.925	H_{82}–H_{71} = 2.4	68.7 (78.9)	21.2 (25.5)	H_{24}	H_{41} + H_{122}

[a] The equivalent hydrogens are in parentheses. [b] The angles corresponding to the calculated conformation are in parentheses.

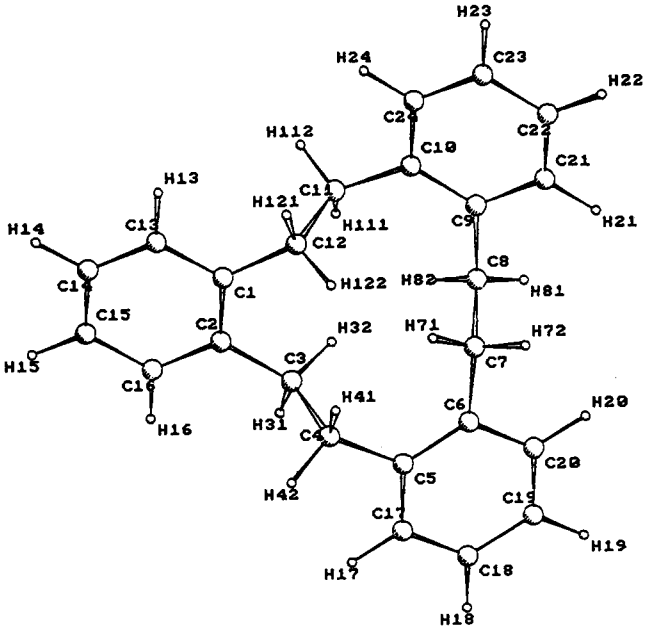

Figure 1. A view of compound 1 with the atomic numbering.

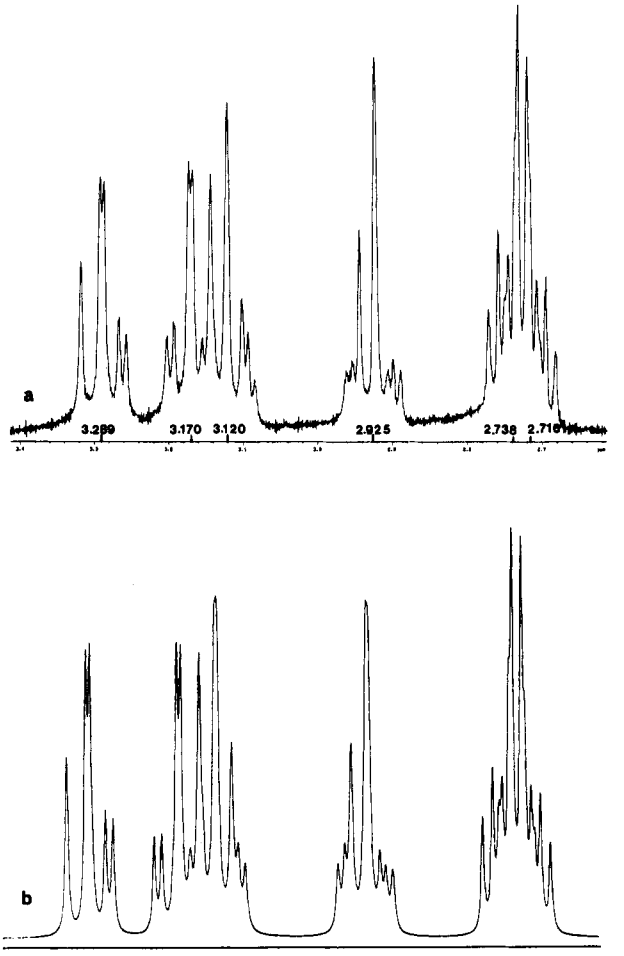

Figure 2. (a) The experimental spectrum (zone of methylene protons). (b) The simulated spectrum using Table I values.

of compound 1, which has the same propeller conformation C_2 that was established for solution.

We report here the ^{1}H NMR study of this compound which provides information not only of the symmetry of the most stable conformation but also of its torsion angles. In a way, the ^{1}H NMR spectrum has more information than the ^{13}C one, but it is more difficult to obtain. We have recorded, at the same temperature and in the same solvent mixture used for the ^{13}C NMR spectrum,[1] the 500-MHz spectrum of compound 1 (our first attempts at 200 MHz were completely unsuccessful, whereas the ^{13}C NMR spectrum was obtained at 25 MHz).

The numbering of the protons is reported in Figure 1, which is the actual X-ray structure.[2] The aliphatic part of the spectrum is represented in Figure 2a (Figure 2b corresponds to the simulated spectrum). The analysis and assignment have been possible by a series of experiments carried out at 173 K: COSY's and ROESY (see the Experimental Section). They result in the values reported in Table I where some significant dihedral angles from the X-ray study are also reported. To verify if these angles correspond to the C_2 minimum energy conformation, an approximate molecular mechanics calculation was carried out.[3] The results, close to the crystallographic values, are also given in Table I (values in parentheses).

The molecule in its C_2 conformation has a binary axis which passes through the middle of C_{14}–C_{15}, C_1–C_2, and C_7–C_8 bonds. This conformation is characterized by a mixture of AA′BB′ and ABCD systems: aromatic protons H_{13}–H_{14}–H_{15}–H_{16} form an AA′BB′ system; H_{17}–H_{18}–H_{19}–H_{20} and H_{24}–H_{23}–H_{22}–H_{21} form two identical ABCD systems; aliphatic protons H_{31}–H_{32}–H_{41}–H_{42} and H_{121}–H_{122}–H_{111}–H_{112} form two identical ABCD systems; H_{71}–H_{72}–

(3) PCMODEL 1990, Serena Software, Bloomington, IN.

H_{81}–H_{82} form an AA'BB' system. At room temperature, the spectrum shows three identical AA'BB' systems for the aromatic protons and four identical A_4 systems.[2]

The assignment of the aromatic protons was possible thanks to the small benzylic coupling between the ortho and the methylene protons; at room temperature[2] the value of the coupling constant cannot be measured but the ortho protons (H_{13}, H_{16}, H_{17}, H_{20}, H_{21} and H_{24}) are clearly broadened with regard to the meta protons. The ortho protons are the most deshielded (7.32 compared with 7.22 ppm for the meta protons).[2] At low temperature, the COSY experiment relates *some* aromatic and aliphatic protons: H_{13} with H_{121} (and H_{16} with H_{31}) and H_{20} with H_{71} (and the symmetrically related H_{21} with H_{82}). The COSY experiment also connects the AA' and BB' protons, on one hand, and the ABCD protons on the other; thus, the complete series of aromatic protons are assigned. At low temperature the ortho protons (averaged value, 7.329 ppm) are still more deshielded than the meta ones (averaged value, 7.226 ppm).

The assignment of the aliphatic protons is now straightforward. The two spin systems, the AA'BB' and the $[ABCD]_2$, were clearly identified through the COSY experiments, and then the complete spin analysis (neglecting the small benzylic couplings) provided the values of chemical shifts and coupling constants of Table I. With these values, both spin systems have been simulated and added (Figure 2b). The ROESY experiment confirms that protons H_{71} (and H_{82}) are close to protons H_{32} (H_{122}) and H_{41} (H_{111}), identifying them as the protons "inside" the cavity.

The chemical shifts of the aliphatic protons can be classified in two groups, those which appear at an averaged value of 2.79 ppm (H_{31}, H_{121}, H_{42}, H_{112}, H_{71} and H_{82}) and those which appear at 3.19 ppm (H_{32}, H_{122}, H_{41}, H_{111}, H_{72} and H_{81}) (at room temperature, all aliphatic protons appear at 3.03 ppm).[2] These values are probably related in a complex manner to the relative positions of aliphatic protons and aromatic rings (the three aromatic rings) since the dihedral angles of these protons with the adjacent aromatic rings also belong to two groups, with values of 23.3° (26.5°) and 39.2° (39.8°), respectively.

The geminal coupling constants, −13.4 Hz, are normal. The vicinal coupling constants follow a Karplus relationship,[4] with the vicinal dihedral angles: $J_{trans} \approx 13.3$ Hz for an angle of about 172.3°, where the equation gives 13.15 Hz. There are two kinds of J_{gauche} values, one at about 2.6 Hz and the other at about 6.7 Hz. They are related to dihedral angles of 71.7° (76.4°) and 56.4° (56.1°). This is consistent with the Karplus relationship, although the empirically generalized Karplus-type equation we have used[4] gives for 71.7° and 56.4° values of ^3J = 1.3 and 3.5 Hz (6.7/2.6 = 2.6; 3.5/1.3 = 2.7). Due to limitations of this model,[4] it has been necessary to use a simple carbon atom to represent the phenyl rings. This approximation, which gives an excellent result for J_{trans} is not entirely satisfactory for J_{gauche}. Nevertheless, the J_{gauche} values support the propeller C_2 conformation for compound 1 at 173 K.

In conclusion, the complete analysis and assignment of the 12 aliphatic protons of 1,2:5,6:9,10-tribenzododeca-1,5,9-triene proves that the only conformer present in solution is the propeller one.

Experimental Section

The synthesis of compound 1 has been described.[1,2]

NMR Spectroscopy. ^{1}H NMR spectra were recorded on a Varian UNITY-500 spectrometer operating at 499.84 MHz, using a 2:1 CD_2Cl_2–CS_2 mixture as solvent. Variable-temperature experiments for compound 1 were carried out in the following conditions: pulse angle 90°, acquisition time 3 s, sweep width 4912 Hz, and data size 32 K (digital resolution ± 0.3 Hz). The temperature was varied in the range 173–303 K. The conformational analysis was performed at 173 K, where the signals were narrow enough (bandwidth, ~2 Hz).

The homonuclear 2D chemical shift correlation experiments were carried out with the following conditions: (1) for vicinal correlations a double quantum filter phase-sensitive COSY was used with spectral widths of 1053.1 Hz in both dimensions, a relaxation delay of 1 s, number of increments = 256 and 1024 × 1024 points for the data matrix; (2) for long-range correlations, a relayed-COSY in the absolute mode was used with spectral widths of 2704.5 Hz in both dimensions, a relaxation delay of 1 s, τ delay used to achieve coherence = 100 ms, number of increments = 225 and 1024 × 1024 points for the data matrix.

The 2D phase-sensitive ROESY spectrum was measured covering the spectral width of 2704.5 Hz in both dimensions with a relaxation delay of 1 s, spin lock field strength of 2.1 KHz, and spin lock time of 150 ms, using 16 scans for each of the 225 increments and a final data matrix of 1K × 1K points.

The ^{1}H-NMR iterative analysis of the spectrum was performed using the PANIC program (for the ABCD system, rms error = 0.17, J and δ are given with ±0.1 Hz and for the AA'BB' system, rms error = 0.5, J and δ are given with ±0.5 and ±0.25 Hz, respectively).[5] The simulated spectrum (Figure 2b) has been obtained using a bandwidth of 2.3 Hz.

(4) Cerda-García-Rojas, C. M.; Zepeda, L. G.; Joseph-Nathan, P. *Tetrahedron Comput. Methodol.* **1990**, *3*, 113.

(5) PANIC 86, Bruker Program Library, Germany.

Stereospecific Synthesis of *gem*-Diphenylcyclopropanecarboxamides: Aminolysis of Spiro Cyclopropano Lactones by Acetonitrile and Triethylamine†

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Cyclopropanecarboxylic acid derivatives play an important role as effective agents in agriculture and medicine. Of these, *gem*-diphenylcyclopropanecarboxylic acids and amides are of considerable pharmaceutical interest as coronary vasodilators[1] and in the treatment of Parkinson's disease.[2] In a continuation of our studies on cyclopropanation of 4-ylideneoxazol-5-ones,[3] we report here the preparation of *gem*-diphenylcyclopropanecarboxylic acid amides starting from azalactones in a single step. Thus, treatment of (*Z*)-2-phenyl-4-ylideneoxazol-5-one **1a** with diphenyldiazomethane (DPDM)[4] in acetonitrile containing TEA under reflux gave a colorless solid melting at 164 °C in 90% yield. The structure of the solid was shown to be 1-benzamido-2,2,3-triphenylcyclopropane-1-carboxamide (**3a**) based on elemental analysis and spectral data. The structure of **3a** was further established by comparison with an authentic sample of the amide prepared unambiguously from the spirolactone **2a** and ammonia (Scheme I).

Mechanistically the spirolactone **2a** may be considered as a reasonable intermediate in the formation of **3a**. The initially formed **2a** from **1a** and DPDM could subsequently

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