Emmottene, from the Gorgonian *Briareum polyanthes*, Contains the First Naturally Occurring *trans*-Bicyclo[5.1.0]octane[1a,b]

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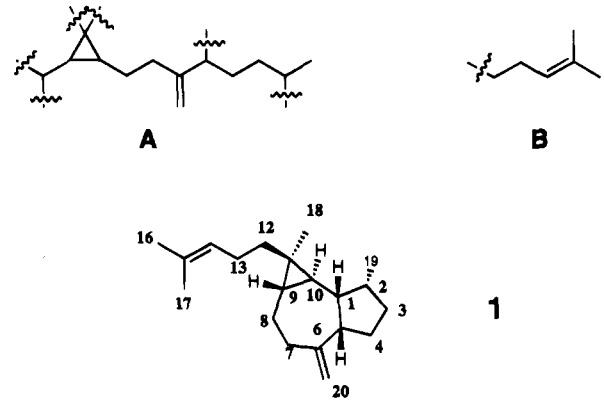
Emmottene, a novel diterpene hydrocarbon isolated from the nonpolar extracts of the Bermudian gorgonian *Briareum polyanthes*, is the first example of a naturally occurring *trans*-bicyclo[5.1.0]-octane ring system. The basic carbon skeleton of emmottene has only been reported once before, in the cneorubin class of diterpenes isolated from the secretory cells on leaves of the terrestrial plant *Cneorum tricoccon*.

Our extensive investigation of the Bermudian gorgonian *Briareum polyanthes* has yielded several briarane diterpenes and the unique pyranone bissetone.[2–5] A recent report by Guerriero et al.[6] on the occurrence of both briarane and cembrane diterpenes in the nudibranch *Armina maculata*, as well as the octocoral *Veretillum cynomorium*, has provided support to earlier speculation that the briarane diterpenes are biogenetically derived from a cembranoid precursor.[7] This finding prompted us to examine the hexane soluble extracts of *Briareum polyanthes* for the presence of these putative biosynthetic precursors to the briaranes. During the course of that study, we isolated and characterized a new diterpene, which we named emmottene (**1**),[1a] representing a skeletal class not previously encountered in marine organisms and containing the first naturally occurring *trans*-bicyclo[5.1.0]octane from any source.

Results and Discussion

Briareum polyanthes was collected, extracted, and partitioned as previously described.[3] Emmottene was isolated from the hexane solubles (24.62 g) by vacuum liquid chromatography on silica gel using hexane as the eluant. The colorless eluate preceding the first yellow band was further separated by a sequence of gel permeation chromatography steps (BioBeads S-X8, S-X4). Final purification, employing HPLC on a cyano-bonded phase column and hexane as the eluant, yielded compound **1**.

High and low resolution mass spectrometry, along with ^{13}C-DEPT spectra, established a molecular formula of $C_{20}H_{32}$, indicating five sites of unsaturation. Two of these sites could be assigned to di- and trisubstituted olefins on the basis of ^{13}C-NMR data. Emmottene was, therefore, tricyclic. From ^{1}H–^{1}H-COSY, ZQCOSY, and HMQC spectral data, recorded in both C_6D_6 and $CDCl_3$ (see Table 1), two fragments were assembled (**A** and **B**). The residual methyl group (0.92 ppm, $CDCl_3$) displayed no multiplicity and hence had to be placed on the quaternary carbon of the cyclopropane ring. HMBC experiments, using various delay values ($^1/_2$J) for the carbon–hydrogen coupling constants (from 3.52 to 4.00 ms), provided unambiguous connection of fragments **A** and **B** to yield the tricyclic hydrocarbon skeleton of **1**.



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The relative configuration of emmottene was determined from proton coupling constants and by NOE difference spectroscopy at 273 and 303 K. The cis ring juncture of the perhydroazulene system was established from an NOE between the two ring juncture protons (H_1 and H_5, 6.6%). The H_1 ring juncture proton was coupled by 10 Hz to the vicinal cyclopropane proton and had no observable NOE with that proton; a trans relationship was therefore assigned. The stereochemistry at C_2 was defined by an NOE (3.3%) between its resident methyl group, H_{19}, and H_{10}. The 10.2 Hz coupling and lack of an observable NOE between the two cyclopropane ring protons (H_9 and H_{10}) suggested a trans relationship. This unusual geometry and the relative configuration of the remaining chiral centers were confirmed by additional NOE relationships between H_{10} and the methyl group (H_{18}) attached to C_{11} (4.3%), and between H_1 and the H_9.

The *trans*-bicyclo[5.1.0]octane system is unprecedented among natural products. Molecular modeling experiments (BIOSYM Discover)[8] were used to compare energy minima for the cis- and trans-fused cyclopropane alterna-

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Table 1. ^1H and ^{13}C NMR Assignments of 1[a]

C/H number	^{13}C (C_6D_6)[a]	chemical shift ^1H (C_6D_6)[b]	chemical shift ^1H ($CDCl_3$)[b]
1	42.0 (1)	1.72 (m)	1.85 (m)
2	38.2 (1)	1.95 (m)	2.04 (m)
3	31.7 (2)	1.35 (m); 1.70 (m)	1.35 (m); 1.78 (m)
4	28.4 (2)	1.70 (m); 1.95 (m)	1.71 (m); 1.87 (m)
5	51.1 (1)	2.55 (q, J = 8.0)	2.63 (q, J = 7.9)
6	152.0 (0)	–	–
7	36.1 (2)	2.38 (m)	2.29 (m)
8	22.4 (2)	1.18 (m); 1.79 (m)	1.26 (m); 1.78 (m)
9	24.4 (1)	0.59 (dt, J = 6.0, 10.2)	0.59 (dt, J = 5.9, 10.1)
10	23.6 (1)	0.40 (dd, J = 10, 10.2)	0.27 (dd, J = 9.9, 10.1)
11	21.4 (0)	–	–
12	43.7 (2)	1.00 (m); 1.45 (m)	1.00 (m); 1.36 (m)
13	25.7 (2)	2.12 (m)	2.03 (m)
14	125.5 (1)	5.20 (t, J = 7.1)	5.06 (t, J = 7.1)
15	130.6 (0)	–	–
16	17.6 (3)	1.56	1.55
17	25.8 (3)	1.68	1.65
18	13.4 (3)	0.95 (s)	0.92 (s)
19	16.9 (3)	0.95 (d, J = 6.8)	0.94 (d, J = 6.7)
20	110.2 (2)	4.85 (s)	4.69 (s); 4.70 (s)

[a] d (no. of H from DEPT experiment). [b] d (multiplicity, coupling constants in Hz).

Table 2. Calculated Energy Minima of Diastereomers/ Conformers of 1[a]

diastereomer[b]	conformer[c]	energy minima[d]
trans	boat	−21.65
trans	chair	−22.71
cis	boat	−22.06
cis	chair	−23.48

[a] BIOSYM.[8] [b] Ring fusion geometry of cyclopropane. [c] Of cycloheptane ring. [d] kcal/mol.

tives in two conformations (boat and chair) of the cycloheptane ring. With the relative stereochemistry at C_1, C_5, C_6, and C_{10} fixed in each model, there was very little conformational mobility in the the tricyclic core of emmottene, centered mostly in the methylene groups at C_7 and C_8. The results (Table 2) indicated that the trans-fused cyclopropanes were only slightly less stable (<0.8 kcal/mol) than their cis-fused counterparts, and that the chair form of the cycloheptane ring was slightly favored over the boat form. The absolute configuration of **1** has not been determined; that shown is an arbitrary choice.

While the tricyclic hydrocarbon skeleton of **1** has been observed in several sesquiterpenes from the coelenterates,[9-10] only one report of diterpenes with this structural framework has been recorded. Trautmann et al.[11] described a series of diterpenes, the cneorubines, from secretory cells on the leaves of the terrestrial plant *Cneorum tricoccon*. Moreover, we are unaware of any previously reported naturally occurring trans-fused cyclopropanes, except for a few examples of *trans*-bicyclo[8.1.0]undecanes from brown algae[13] and octocorals.[14] While *trans*-bicyclo[5.1.0]octanes have been quite uncommon as synthetic materials as well, two recent communications from the Smith group,[12] following earlier papers from Pirkle[15] and Gassman,[16] have revealed that this ring system is, in fact, accessible and reasonably stable.

Although emmottene was not isolated via our bioassay-guided separation protocols, follow-up biological screening indicated no antimicrobial activity[17] and only threshold brine shrimp toxicity (LD_{50} @ 24 h, 1000 ppm). In our insecticidal screen (against the tobacco hornworm, *Manduca sexta*) emmottene, at 100 ppm, caused 10% mortality and 40% weight reduction relative to controls.

Experimental Section

Emmottene. *Briareum polyanthes* was collected at the eastern end of Bermuda in 1986 and extracted and partitioned as described previously.[3] The hexane soluble portion of the crude extract (24.62 g) was separated by vacuum liquid chromatography on silica gel (124 g) with hexane. The colorless eluate preceding the first yellow band was further separated by a sequence of gel permeation chromatography steps, including BioBeads S-X8 (2.5 × 115 cm), using CH_2Cl_2–hexane (3:2), and BioBeads S-X4 (2.5 × 115 cm) with CH_2Cl_2–hexane–EtOAc (4:3:1). Final purification required HPLC on a cyano-bonded phase column with hexane as the eluant to yield emmottene, (**1**) (fraction 1–111 mg) as a colorless oil: $[\alpha]_D$ −2.3°, (*c* 0.11, $CHCl_3$); ν_{max} (CCl_4) 2926, 2870, 1454, 885, 658 cm^{-1}; HREIMS *m/z* 272.2493, (M^+, calcd for $C_{20}H_{32}$ 272.2456, 58%), 229 (33), 190 (76), 147 (100); NMR data are presented in Table 1.

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Supporting Information Available: The low resolution electron impact mass spectrum, ^1H-NMR spectra in $CDCl_3$ and C_6D_6, and the ^{13}C-NMR spectrum in C_6D_6 of emmottene (**1**) are provided (4 pages). This material is contained in 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.

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