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THE STRUCTURE DETERMINATION OF A XENICANE DITERPENE FROM *XENIA GARCIAE*

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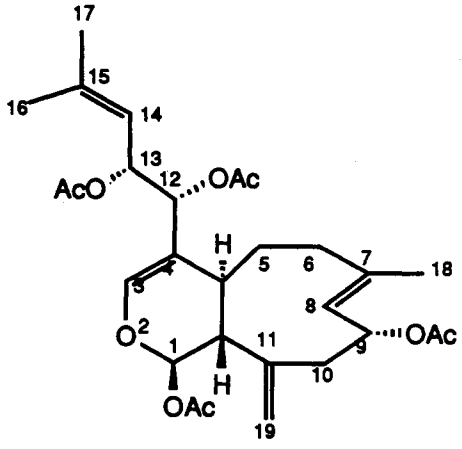
ABSTRACT.—The structure determination of a xenicane diterpene **2** derived from *Xenia garciae* has been secured by single crystal X-ray analysis revealing its relative and absolute stereochemistry as (1*R*,4a*S*,7*S*,8*R*,9*R*,11a*R*,12*R*,13*S*)-desoxyhavannahine. This metabolite thus possesses the opposite configuration at C-7 to that determined for havannahine [**3**]. The diterpene **2** was shown to inhibit the growth of the alga *Ceramium codii*, a common benthic fouling organism.

Soft corals of the genus *Xenia* have produced a range of xenicane diterpenes of which xenicin [**1**] was the first reported example (1). In the course of our continuing investigations of xeniid soft corals (2), we secured a specimen of *Xenia garciae* Bourne (Octocorallia, Alcyonacea) from New Caledonia. After CH_2Cl_2 extraction and rapid chromatography on Si gel (3), a highly crystalline diterpene **2** was obtained as the major and only significant terpenoid component. High field ^1H- and ^{13}C-nmr analysis, including COSY-45, ^1H-^{13}C short- and long-range shift correlated 2D-nmr correlation experiments (4), enabled its structure to be deduced, although full stereochemical detail could not be ascertained. A single crystal X-ray structure determination was carried out (by MFM and JMG), which confirmed our structure deductions and afforded the relative stereochemistry of **2**. Subsequent to this study, a report appeared (5) on the isolation of **2** and **3** from a New Caledonian specimen of *Xenia membranosa*. This included an



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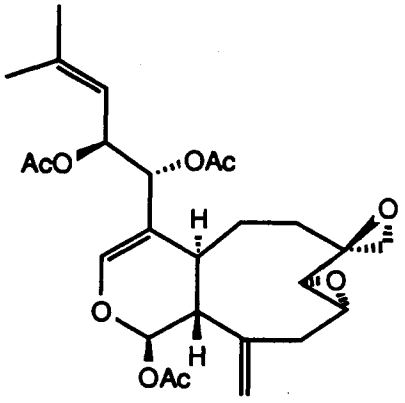

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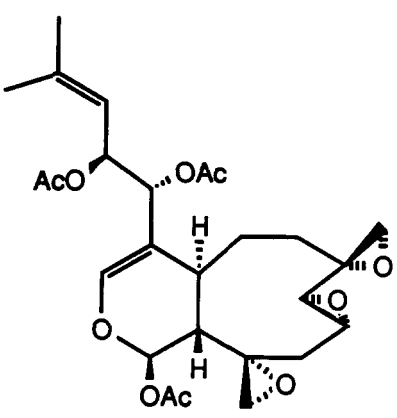

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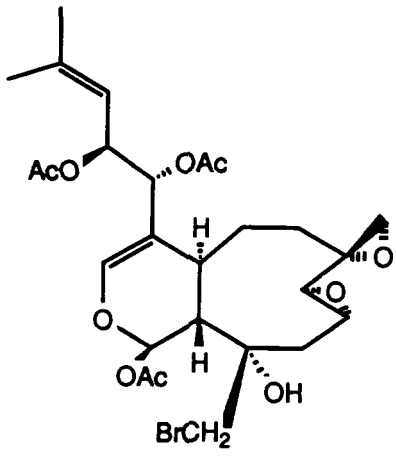

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X-ray structure determination of **3** and a tentative structure assignment and partial characterization of **2**. A more recent publication from the same group (6) has reported several related metabolites and, as a result of a companion X-ray study, determined the absolute stereochemistry of **3**.

We wish to present the results of a single crystal X-ray determination of **2**, which enables its relative and absolute stereochemistry to be reported for the first time.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Reichert microscopic hot-stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ solutions with a Perkin-Elmer 141 polarimeter. Ir spectra were determined on KBr plates as films or Nujol mulls using a Perkin-Elmer 297 ir spectrophotometer. Uv spectra were recorded in EtOH solutions using a Varian 634 spectrophotometer. ^1H and ^{13}C-nmr spectra were recorded in $CDCl_3$ solution on a Bruker AM300 nmr spectrometer using $CHCl_3$ (δ 7.26) as reference. Microanalysis was performed at the Department of Chemistry, University of Queensland. Si gel, type 60 (Merck), was used for cc, and plastic-backed plates coated with Si gel F254 (Merck) were used for tlc. Plates were visualized by spraying with vanillin/H_2SO_4 and warming.

BIOLOGICAL MATERIAL.—The soft coral *X. garcia* was collected in shallow water (< 5 m) at Kuebini Reef, Touaourou on the east coast of New Caledonia. The museum specimen (C5526) is held in the Northern Territory Museum, Darwin, NT, Australia, by Mr. Phil Alderslade who provided taxonomic verification and curation.

(1*R*,4a*S*,7*S*,8*R*,9*R*,11a*R*,12*R*,13*S*)-*Desoxyhavannahine* [**2**].—The freeze-dried sample (105 g) was crushed and extracted repeatedly with $CHCl_2$. On removal of the solvent, the crude extract (7 g, 6.9%) was rapidly chromatographed on Si gel (3), using increasing proportions of Et_2O in light petroleum ether. The major component **2** crystallized as prisms (150 mg) from the chromatography: mp 138–139° [lit. (5) "amorphe"]; $[\alpha]_D$ +35° (c = 0.005) [lit. (5) +37° (c = 1.04)]; uv λ max (EtOH) 205 nm (2×10^4); ir ν max (film) 1725, 1665, 1440, 1365, 1225, 1160, 1020, 935, 790, 755, 720 cm^{-1}; ^1H nmr spectrum identical (±0.05 ppm) with literature (5); ^{13}C-nmr spectrum identical c = 0.5; (±0.4 ppm) with the literature (5).

X-RAY CRYSTALLOGRAPHY OF **2**.—The ORTEP (7) perspective drawing of **2** appears as Figure 1. Non-hydrogen atomic coordinates of all atoms in **2** appear in Table 1, and selected torsion angles appear in Table 2.[1]

CRYSTAL DATA.—$C_{26}H_{34}O_9$, M 490.55, orthorhombic, space group $P2_12_12_1$, a 12.706(1), b

[1]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, UK.

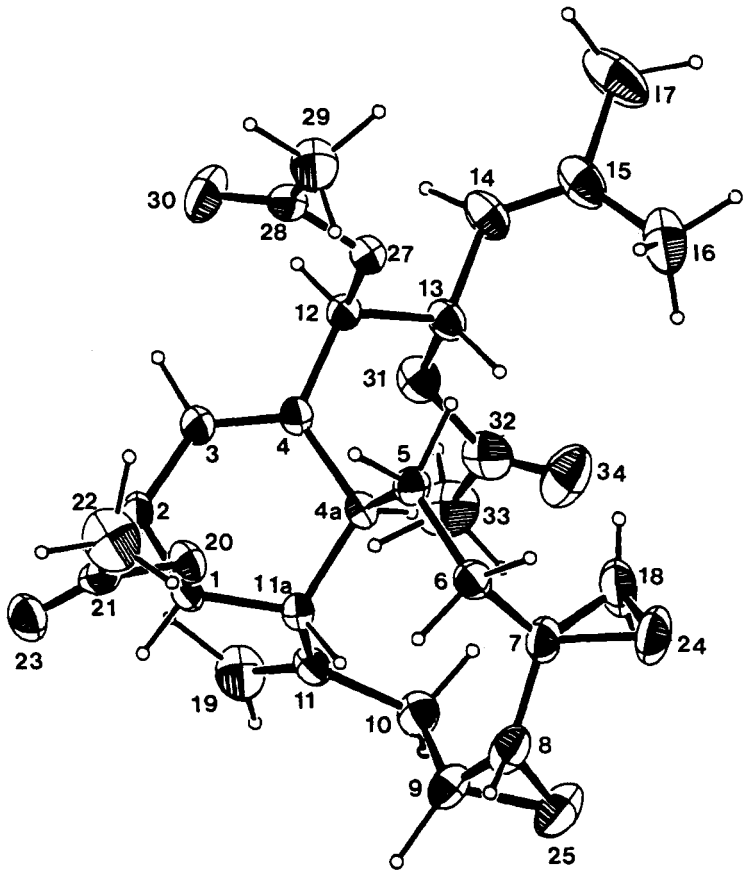

FIGURE 1. ORTEP Plot of **2** showing crystallographer's numbering system.

13.041(1), *c* 16.079(1) Å, U 2664.3 (4) Å^3, D_m 1.21(1), D_c (Z = 4) 1.22 g cm^{-3}, F(000) 1048, Cu Kα radiation (graphite-crystal monochromator), λ 1.5418 Å, μ 6.81 cm^{-1}, T 288(1) K.

STRUCTURE DETERMINATION.—Intensity data were measured from a crystal of dimensions ca. 0.12 by 0.39 by 0.40 mm aligned on a Rigaku-AFC diffractometer; the intensities were recorded by an ω–2θ scan. Of 2511 non-equivalent terms measured to a $2\theta_{max}$ 130°, 1999 terms for which $I > 3\sigma I$ were used for the structure refinement; the intensities were corrected for Lorentz and polarization factors but not for absorption. The structure was solved by direct methods with SHELXS 86 (8) and refined using SHELX 76 (9). The sites of the non-methyl hydrogen atoms were located on difference maps and included in the refinement with isotropic temperature factors; their positional coordinates were not varied. As the sites of the methyl hydrogens were not clearly resolved the methyl hydrogens were included at idealized positions and given a common isotropic temperature factor which refined to a value of B_{iso} 21(8) Å^2. Full-matrix least-squares refinement with anisotropic temperature factors given to the carbon and oxygen atoms converged at R 0.053 and R_w 0.046 [weights$(\sigma^2|F_o| + 0.00005|F_o|^2)^{-1}$]. Six large low order terms (0 1 1, 0 2 1, 0 0 2, 0 2 0, 0 4 0, 4 0 0) seriously affected by extinction were omitted from the final refinement. Neutral scattering factors (10,11) were used, those for carbon and oxygen being corrected for anomalous dispersion (12). Figure 1 contains the crystallographer's atom numbering system and was prepared from the output of ORTEP (7).

TERPENE ASSAYS USING ALGAL CULTURES.—*Ceramium codii* (Richards) Mazoyer, held in unialgal culture in the Botany Department of the James Cook University, was selected as the assay organism because a species of the same genus was one of the predominant algae known to overgrow soft corals. Test inocula were grown singly in 10 ml of a simple, inorganic liquid culture medium ["Grundmedium" of von Stosch (13)] in glass Petri dishes (50 mm diam.). Inocula consisting of branch systems (ca. 3–5 mm long) were excised from actively growing cultures. Cultures were maintained at 25° ± 1°, with irradiation of 30 μE·m^{-2}·sec^{-1} provided by "Grolux" fluorescent tubes on a 12:12 h light:dark cycle.

TABLE 1. Final Atomic Coordinates of the Carbon and Oxygen Atoms for $C_{26}H_{34}O_9$ [**2**].[a]

Atom	10^4x	10^4y	10^4z
C-1	−3538(4)	3032(4)	5233(3)
O-2	−2421(2)	3044(3)	5309(2)
C-3	−1883(4)	2751(4)	4602(3)
C-4	−2280(4)	2592(3)	3857(3)
C-4a	−3446(4)	2789(3)	3661(3)
C-5	−4030(4)	1824(4)	3391(3)
C-6	−5219(4)	1977(4)	3244(3)
C-7	−5537(4)	2918(5)	2750(3)
C-8	−6116(5)	3698(5)	3247(4)
C-9	−5665(5)	4632(5)	3654(4)
C-10	−4528(5)	4940(4)	3561(3)
C-11	−3847(4)	4528(4)	4262(3)
C-11a	−3944(4)	3393(3)	4396(3)
C-12	−1534(4)	2238(4)	3190(3)
C-13	−1670(4)	2779(4)	2350(3)
C-14	−833(4)	2491(4)	1725(3)
C-15	−1021(5)	2225(4)	947(3)
C-16	−2117(6)	2059(5)	578(3)
C-17	−91(6)	2056(5)	356(4)
C-18	−5054(5)	3203(6)	1951(3)
C-19	−3227(5)	5145(4)	4709(4)
O-20	−3891(2)	1991(2)	5315(2)
C-21	−3968(4)	1612(4)	6108(4)
C-22	−4330(5)	519(4)	6097(4)
O-23	−3800(3)	2117(3)	6707(2)
O-24	−6072(3)	2687(3)	1960(2)
O-25	−6297(3)	4705(3)	2903(3)
O-27	−1710(3)	1155(2)	3014(2)
C-28	−1258(4)	481(4)	3555(3)
C-29	−1662(5)	−592(4)	3426(3)
O-30	−650(3)	754(3)	4084(2)
O-31	−1505(3)	3852(3)	2565(2)
C-32	−2138(5)	4554(5)	2188(4)
C-33	−1853(5)	5631(5)	2466(4)
O-34	−2834(4)	4319(4)	1717(3)

[a]ESD values are given in parentheses.

After 24 h, when regeneration from each inoculum was confirmed, the initial branch length was measured and treatment initiated. A stock solution of the diterpene **2** was prepared in DMSO. Measured volumes of DMSO and the appropriate stock solution were added to the culture dishes to give final concentrations of 0 (control), 12.5, 25, and 50 ppm.

The final length of each inoculum was measured at completion of the assay 2 days after treatment. Growth was expressed as the relative growth rate (RGR), according to the formula $RGR = (G/M)/d_n$, where G = increase in length of branch system during assay, M = mean of initial and final length of branch system, and d_n = duration of assay (days).

RESULTS AND DISCUSSION

The results of the X-ray crystallographic study of **2** are shown in Figure 1 and Tables 1 and 2. Careful comparison of the data reported for desoxyhavannahine [**2**] (5) with those obtained for our isolate clearly shows that the compounds are identical; on the basis of the reported data no stereochemical differences are possible. ^{13}C- and ^{1}H-nmr data, including all coupling constant values, are identical, as are the chiroptical properties of the two samples.

Table 2. Selected Torsion Angles (degrees) for $C_{26}H_{34}O_9$ [2]. Values for 4 are included for comparison.[a]

Atoms	Compound		Atoms	Compound	
	2[b]	4		2[b]	4
C-11a–C-1–O-2–C-3	−38.3(5)	−43.0	O-2–C-1–O-20–C-21	80.1(5)	93.4
C-1–O-2–C-3–C-4	9.1(7)	17.0	C-3–C-4–C-12–C-13	−136.0(5)	−101.4
O-2–C-3–C-4–C-4a	4.1(8)	−1.4	C-4a–C-4–C-12–C-13	42.1(6)	74.3
C-3–C-4–C-4a–C-11a	11.9(6)	12.1	C-3–C-4–C-12–O-27	106.2(5)	134.4
C-4–C-4a–C-11a–C-1	−38.7(5)	−35.6	C-4a–C-4–C-12–O-27	−75.7(5)	−49.9
C-4a–C-11a–C-1–O-2	54.4(5)	53.2	C-4–C-12–C-13–C-14	173.6(4)	64.9
C-11–C-11a–C-4a–C-5	−141.5(4)	−139.5	C-4–C-12–C-13–O-31	58.5(5)	−56.1
C-11a–C-4a–C-5–C-6	49.8(6)	46.3	O-27–C-12–C-13–C-14	−65.9(5)	−169.6
C-4a–C-5–C-6–C-7	45.2(5)	49.7	O-27–C-12–C-13–O-31	179.0(4)	69.4
C-5–C-6–C-7–C-8	−112.3(5)	−112.7	C-12–C-13–C-14–C-15	132.5(5)	113.4
C-6–C-7–C-8–C-9	96.7(7)	94.8	O-31–C-13–C-14–C-15	−115.3(6)	−126.5
C-7–C-8–C-9–C-10	3.9(10)	0.0	C-4–C-12–O-27–C-28	−80.5(5)	−119.1
C-8–C-9–C-10–C-11	−93.7(7)	−96.3	C-12–C-13–O-31–C-32	−140.4(5)	−142.2
C-9–C-10–C-11–C-11a	52.8(6)	61.2	C-14–C-13–O-31–C-32	100.3(5)	94.8
C-10–C-11–C-11a–C-4a	69.5(5)	64.9			

[a]Values for 4 are from Almourabit *et al.* (6).
[b]ESD values for 2 are given in parentheses.

As a result of experiments involving the conversion of 3 to its 11α-hydroxy-19-bromo derivative 4 and a subsequent X-ray structure determination which afforded the absolute configuration of 4, the absolute configuration of 3 is available (6). Comparison of the results of our X-ray determination of 2 with that of 3 surprisingly revealed that the relative (and absolute) configuration at C-7 was reversed between the two compounds. The absolute stereochemical description of 2 is (1*R*,4a*S*,7*S*,8*R*,9*R*,11a*R*,12*R*,13*S*)-desoxyhavannahine.

In the course of our studies of the antialgal properties of soft corals (14,15), unialgal cultures of *C. codii* were treated with the diterpene 2 (suspended in DMSO) at three different concentrations. The RGR's were determined after 2 days. These values were then expressed as a percentage of the control RGR values. At 50 ppm of 2, growth was only 4% of control. At 25 ppm it was 21% of control and even at 12.5 ppm, algal growth was only 42% of the control value. Clearly, diterpene 2 is a significant growth inhibitor of the alga.

It is interesting that in *X. membranosa*, epoxidation at the $\Delta^{7(18)}$ position affords compounds 2 and 3 epimeric at C-7. In *X. garciae*, only one epoxide 2 is formed. The mechanism and selectivity of epoxidation reactions in preformed terpenoids clearly requires further study.

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