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CHEMISTRY OF SPONGES, X.[1] NEW SESQUITERPENES FROM A MARINE SPONGE OF THE GENUS *EURYPON*

M.R. KERNAN, R.C. CAMBIE,*

Department of Chemistry, University of Auckland

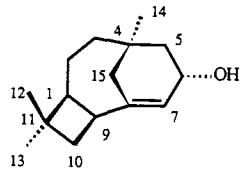
and PATRICIA R. BERGQUIST

Department of Zoology, University of Auckland, Auckland, New Zealand

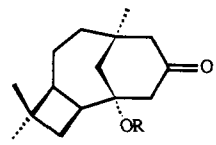
ABSTRACT.—Three new sesquiterpenes **5–7** with a tricyclo[6.3.1.$0^{2,5}$]dodecane skeleton have been isolated from the Axinellid sponge *Eurypon* sp. along with the known metabolites **1** and **2**.

Recently the isolation of the sesquiterpenes **1–4** from a New Zealand marine sponge of the genus *Eurypon* was reported by other workers (1). During a collection of marine sponges at the Mercury Islands, New Zealand, we obtained a specimen of the same species and have isolated some different but related compounds. Separation of the crude hexane extract, which had significant antimicrobial activity, resulted in the isolation of the antimicrobial sesquiterpene **1**, together with the inactive sesquiterpenes **2** and **5–7**. The structures of the new sesquiterpenes **5–7** were determined by spectroscopic methods, but their absolute configurations were not determined. The known compounds **1** and **2** were identified by comparison of their spectral properties with literature values (1,2).

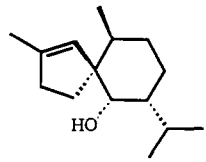
The molecular formula of **5** was determined to be $C_{15}H_{24}O$ by hrms. Bands in the ir spectrum at 3400 and 1180 cm^{-1} and a peak (*m/z* 202) due to loss of H_2O in the ms required the presence of a hydroxyl group. Inspection of the ^1H- and ^{13}C-nmr spectra of **5** indicated the same carbon skeleton as in **1**. Both spectra showed significant line broadening at room temperature that was reduced by conducting nmr experiments at 253K.



1



2 R=H
3 R=Me



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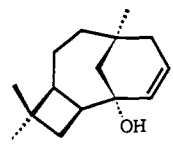

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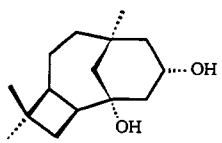

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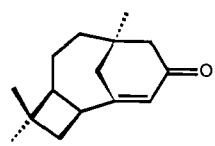

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[1]For Part IX, see M.R. Kernan, R.C. Cambie, and P.R. Bergquist, *J. Nat. Prod.*, **53**, 724 (1990).

A complete assignment of the ^{1}H-nmr spectrum was obtained after COSY and NOEDS experiments which gave results that were consistent with the proposed structure. NOEDS experiments (Table 1) were crucial in the assignment of the position of the 6,7 double bond: irradiation of the signal due to the C-4 methyl protons (δ 0.95) resulted in enhancement of the signals assigned to H-5 [δ 1.92 (br dd, J = 17.3, 1.7 Hz, 1.8% enhancement); 1.72 (br dd, J = 17.3, 5.6 Hz, 4.8% enhancement)].

standing in $CDCl_3$ solution, a sample of **6** was converted into **1**.

The sesquiterpene **7** was obtained as an unstable oil and had a molecular formula $C_{15}H_{22}O$ from the hrms. The ir spectrum of **7** showed absorption at 1680 cm^{-1} that was assigned to an α,β-unsaturated ketone. The ^{1}H- and ^{13}C-nmr spectra were similar to those of **1**, except that the signals due to the allylic alcohol in **1** were replaced by signals due to an α,β-unsaturated ketone [δ 5.44 (td, J = 3, 1 Hz, H-7), 198.0 (s, C-6),

TABLE 1. Nuclear Overhauser Enhancement Data for Compounds **5** and **6**.[a]

Irradiated proton	Compound	
	5	**6**
H-6	—	H-15 (4.5)
H-7	no enhancement	—
Me-12	H-9 (7.4), H-1 (1.5), Me-13 (5.3)	Me-13 (5.3)
Me-13	H-2 (2.2), H-3 (4.0), H-10 (4.1), Me-12 (2.4)	—
Me-14	H-2′ (1.4), H-3′ (1.5), H-5′ (1.8), H-15′ (5.5)	—

[a]Data are observed nOe's (%).

The sesquiterpene **6** was obtained as a colorless oil. Although a molecular ion was not observed in the ms, a molecular formula $C_{15}H_{26}O_2$ was determined from the ^{13}C- and ^{1}H-nmr spectra (15 carbons, 24 attached protons, 2 hydroxyl groups) and hrms of the $[M-H_2O]^+$ peak. The ir spectrum showed absorptions at 3350, 1190, and 1150 cm^{-1} due to the presence of both secondary and tertiary hydroxyl groups. The ^{13}C- and ^{1}H-nmr spectra were consistent with the proposed structure **6**, which was confirmed by COSY, decoupling, and NOEDS experiments. The observation of an enhancement of one of the signals assigned to H-15 [δ 2.34 (d, J = 14 Hz, 4% enhancement)] upon irradiation of the H-6 proton [δ 3.48 (m, 1H)] in an NOEDS experiment required the stereochemistry at C-6 to be as shown. This was confirmed by the fact that, on

157.0 (s, C-8), 119.8 (d, C-7)]. Sesquiterpene **7** may, in fact, be the unstable, uv-absorbing intermediate reported previously in the conversion of alcohol **1** into the methoxy ketone **3** in MeOH solution (1).

The alcohol **1**, which was reported to by cytotoxic (1), was the only antimicrobial compound, inhibiting the growth of *Staphylococcus aureus* at 100 μg/disk in the standard disk assay.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—These were as in Part VI (3), except as follows. Ir spectra were obtained either on a Shimadzu IR-27G spectrometer or a Bio-Rad Ft-ir spectrometer. All solvents were distilled prior to use. Hplc was carried out with a Shimadzu LC-6A solvent delivery system equipped with a Waters R401 r.i. detector, using a Merck LiChrosorb Si gel column (25 × 1 cm). 2D nmr experiments were performed on a Bruker 400 MHz nmr spectrometer following standard procedures.

SPONGE COLLECTION AND TAXONOMY.—A specimen of the genus *Eurypon* (Order Axinellida, Family Euryponidae) was collected by scuba diving (−10 m) from the Mercury Islands, New Zealand, in December 1988. A portion was stored in 70% EtOH for taxonomic identification (voucher number AUZ 5-03, Department of Zoology, University of Auckland) while the remainder was immediately frozen. Subsequent study of the skeletal composition and organization revealed that this sponge is an undescribed species of *Eurypon* and that it is a second collection of *Eurypon* n. sp. reported on by Barrow *et al.* (1) and identified as new by Bergquist. This new species has a distinctive habit for the genus, being a thick cushion (2.0 cm deep) rather than a very thin (1.0 mm deep) encrustation as in *Eurypon hispida*, the only other described New Zealand species. It is also distinctive in spicule composition, having only rare coarsely spined acanthostyles but many faintly roughened ones and possessing oxeote and stylote modifications of the long main megascleres which in *Eurypon* generally are markedly asymmetric, wavy subtylotes. Habit and spiculation mark this as distinct from the only Australian species, *Eurypon graphidophora* Hentschel. Full taxonomic description awaits a future revision of the New Zealand axinellid fauna.

EXTRACTION AND ISOLATION OF NATURAL PRODUCTS.—The sponge (11.89 g dry wt) was chopped and soaked in MeOH. After filtration and evaporation of the MeOH the residue was partitioned into aqueous-, hexane-, CH_2Cl_2-, and EtOAc-soluble fractions. Only the hexane-soluble portion had antimicrobial activity (*S. aureus*), and it was purified by chromatography on Si gel (Chromatotron, 50% EtOAc/hexane) to give three terpenoid fractions. The major fraction was purified by hplc on μ-Porasil (2.5% EtOAc/CH_2Cl_2) to give the sesquiterpenes **1** (1.2 mg, 0.01%), **5** (16.3 mg, 0.14%), and **7** (13 mg, 0.11%). Two more polar fractions from the Chromatotron were purified by hplc (50% EtOAc/hexane) to give **2** (3.1 mg, 0.03%) and **6** (2.1 mg, 0.02%). The alcohol **1** inhibited the growth of *S. aureus* at 100 μg/disk in the standard disk assay.

TRICYCLO[6.3.1.$0^{2,5}$]DODEC-6-EN-8α-OL [**5**].—The compound crystallized from hexane as colorless needles: mp 115°; found $[M]^+$ 220.1832, $C_{15}H_{24}O$ requires $[M]^+$ 220.1827; $[\alpha]_D$ −6.1° (c = 0.03, $CHCl_3$); ir (KBr) ν max 3500–3000 (br, OH), 1450, 1340, 1320, 1180, 1045, 1020, 985, 780, 710 cm^{-1}; ^1H nmr ($CDCl_3$, 253° K) δ 5.72 (ddd, J = 10, 5.6, 1.7 Hz, H-6), 5.54 (br d, J = 10 Hz, H-7), 2.55 (br dd, J = 10.4, 8.0, 7.7 Hz, H-9), 2.24 (br d, J = 13.2 Hz, H-15′), 1.92 (br dd, J = 17.3, 1.7 Hz, H-5′), 1.82 (m, J = 8.0, 3.6 Hz, H-1), 1.79 (dd, J = 10.7, 10.4 Hz, H-10), 1.72 (br dd, J = 17.3, 5.6 Hz, H-5), 1.58 (ddd, J = 10.7, 7.7, 3.6 Hz, H-10′), 1.48 (br dd, J = 13, 11 Hz, H-3), 1.36 (m, H-2), 1.31 (br dd, J = 13, 7.2 Hz, H-3′), 1.18 (br d, J = 13.2 Hz, H-15), 1.18 (m, H-2′), 1.15 (s, 11α-Me), 0.95 (s, 4α-Me), 0.93 (s, 11β-Me); ^{13}C nmr ($CDCl_3$, 298° K) δ 135.7 (d, C-6), 128.1 (d, C-7), 71.7 (s, C-8), 48.3 (d, C-1), 41.5 (t, C-3 to C-5), 40.8 (t, C-5 or C-3), 39.7 (d, C-9), 34.6 (t, C-10 or C-15), 33.5 (t, C-15 or C-10), 33.7 (s, C-4 or C-11), 32.9 (s, C-11 or C-4), 30.8 (q, C-13 or C-14), 29.1 (q, C-14 or C-13), 24.4 (q, C-12), 20.6 (t, C-2); ms *m/z* 220 (rel. int. 5%), 205 (8), 202 (10), 187 (8), 177 (4), 164 (60), 149 (20), 135 (50), 121 (25), 109 (100), 95 (20), 91 (20), 81 (15), 79 (20), 69 (15), 67 (18), 55 (22), 41 (40).

TRICYCLO[6.3.1.$0^{2,5}$]DODECANE-6α,8α-DIOL [**6**].—The compound was obtained as a colorless oil: found $[M-H_2O]^+$ 220.1853, $C_{15}H_{24}O$ requires 220.1827; $[\alpha]_D$ −10.0° (c = 0.002, $CHCl_3$); ir (film) ν max 3500–3100 (br, OH), 2924, 2864, 1462, 1367, 1261, 1216, 1190, 1150, 1120, 1070, 1050, 1010, 956, 876, 800, 757 cm^{-1}; ^1H nmr ($CDCl_3$) δ 3.48 (m, H-6), 2.62 (br s, OH), 2.35 (m, H-9), 2.34 (d, J = 14 Hz, H-15), 2.27 (m, H-5′), 1.81 (m, H-1), 1.80 (t, J = 10 Hz, H-10), 1.75 (m, H-10′), 1.70 (ddd, J = 14, 12, 2 Hz, H-2), 1.62 (m, H-7), 1.55 (m, H-7′), 1.53 (m, H-2′), 1.47 (m, H-3′), 1.29 (br d, J = 14 Hz, H-15′), 1.19 (dd, J = 12, 11 Hz, H-3′), 1.17 (s, 11α-Me), 1.05 (m, H-5), 0.96 (s, 4α-Me), 0.94 (s, 11β-Me); ^{13}C nmr ($CDCl_3$) δ 75.0 (d, C-6), 74.5 (s, C-8), 48.7 (d, C-1), 38.9 (d, C-9), 38.3 (t), 36.4 (t), 34.9 (q, C-14), 34.0 (t), 33.5 (s, C-4 or C-11), 33.3 (t), 32.3 (s, C-11 or C-4), 29.7 (q, C-13), 26.4 (t), 24.4 (q, C-12), 22.9 (t, C-2); ms *m/z* 220 (rel. int. 10%), 205 (6), 182 (23), 179 (72), 164 (24), 123 (100), 109 (46), 107 (20), 95 (32), 81 (33), 69 (50), 55 (62), 41 (88).

TRICYCLO[6.3.1.$0^{2,5}$]DODEC-7-EN-6-ONE [**7**].—The compound was obtained as an unstable, colorless oil: found $[M]^+$ 218.661, $C_{15}H_{22}O$ requires 218.670; ir ($CHCl_3$) ν max 1680 cm^{-1} (α,β-unsaturated CO); ^1H nmr ($CDCl_3$) δ 5.44 (td, J = 3, 1 Hz, H-7), 3.25 (dd, J = 16, 8 Hz, H-9), 2.32 (m, H-5), 2.25 (d, J = 14 Hz, H-15), 2.10 (m, H-5′), 1.98 (t, J = 10 Hz, H-10), 1.82 (m, H-1), 1.5–1.7 (m, 3H), 1.42 (d, J = 14 Hz, H-15′), 1.3–1.42 (m, 2H), 1.21 (s, 11β-Me), 1.03 (s, 4α-Me), 0.96 (s, 11α-Me); ^{13}C nmr ($CDCl_3$) δ 198.0 (s, C-6), 157.0 (s, C-8), 119.8 (d, C-7), 53.5 (t, C-5), 50.1 (d, C-1), 38.9 (d, C-9), 37.4 (t), 36.2 (t), 35.4 (t), 34.6 (s, C-11), 30.3 (q, C-13), 29.7 (s, C-4), 28.5 (q, C-14), 24.2 (q, C-12), 22.5 (t, C-2); ms *m/z* 218 (rel. int. 15%), 203 (4), 181 (19), 180 (12), 179 (17), 123 (100).

LITERATURE CITED

1. C.J. Barrow, J.W. Blunt, and M.H.G. Munro, *Aust. J. Chem.*, **41**, 1755 (1988).
2. F. Bohlmann and J. Ziesche, *Phytochemistry*, **20**, 469 (1981).
3. P. Karuso, R.C. Cambie, B.F. Bowden, and P.R. Bergquist, *J. Nat. Prod.*, **52**, 289 (1989).

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