Echinofuran, a New Furanosesquiterpene from the Gorgonian Echinogorgia praelonga

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ECHINOFURAN, A NEW FURANOSESQUITERPENE FROM THE GORGONIAN *ECHINOGORGIA PRAELONGA*

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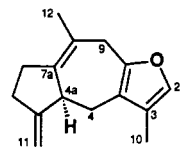
ABSTRACT.—Echinofuran [**1**], which inhibits cell division of fertilized sea urchin eggs, was isolated from the gorgonian *Echinogorgia praelonga*. The structure was determined from spectroscopic data and the absolute configuration by transformation into a known derivative.

Guaiazulenes and related sesquiterpenes are recognized metabolites of some species of gorgonians (1–5). We have reported from *Acalycigorgia* sp. 2,3-dihydrolinderazulene, which exhibited cytotoxicity, antifungal activity, and immunostimulatory activity (6). In this paper we describe the isolation and structure elucidation of a new related compound, echinofuran [**1**], which inhibits cell division of fertilized sea urchin eggs 90% at 1 μg/ml.

A fresh sample of *Echinogorgia praelonga* Ridley (Family Paramuriceidae) was extracted with MeOH. After concentration the resulting aqueous suspension was extracted with EtOAc to furnish a dark oil. The oil was chromatographed on Si gel to give a pure sample of echinofuran [**1**] as an unstable oil in a yield of 32% of the crude extract. As it gradually decomposed on standing, an analytical sample was prepared by hplc immediately before use.

The molecular formula $C_{15}H_{18}O$ of **1** was deduced from hreims. The ^{13}C-nmr spectrum showed signals for 15 carbon atoms, including eight sp^2 carbons, suggesting the presence of four double bonds. One was an exomethylene [δ 105.3 (t), 156.7 (s)], and two others [δ 118.1 (s), 124.6 (s), 135.3 (d), 147.9 (s)] were indicated to be part of a furan ring. The presence of a furan ring was also supported by ir absorptions at 1110, 1130, and 3070 cm^{-1} and by the ^1H-nmr signal at δ 6.98 (1H, s). The ^1H-nmr spectrum showed resonances (δ 1.76, 1.88) for two vinyl methyls, one (δ 1.88) being attached to the β-carbon of the furan ring as evidenced by long-range coupling with the α-proton (δ 6.98) in a COSY spectrum. These spectral data suggested that **1** was a tricyclic sesquiterpene related to linderazulene [**2**]. This was confirmed by dehydrogenation of **1** to **2** by heating with Pd/C. Thus, echinofuran is a tetrahydrolinderazulene. The remaining tetra-substituted double bond [δ 120.8 (s), 140.3 (s)] and the exomethylene could be placed in two ways, giving rise to either **1** or **3** as a possible structure of echinofuran.

The choice of **1** over **3** was made by transformation of echinofuran into derivatives. Partial hydrogenation of **1** over PtO_2 gave dihydroechinofuran [**4**]. The structure of **4** was secured by assign-



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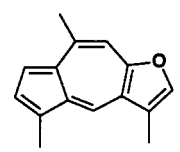

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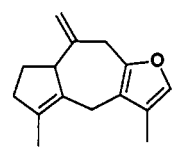

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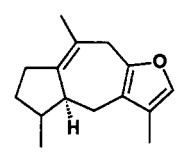

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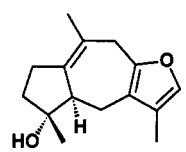

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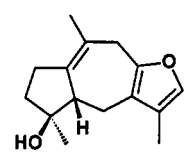

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ment of all proton signals (see Experimental) through decoupling experiments with a 500 MHz nmr instrument. Although stereochemistry of **4** could not be assigned, the decoupling established the connectivity between C-4 and C-5 through C-4a, thus supporting the structure **1** for echinofuran. Furthermore, treatment of **1** with mercuric acetate followed by reduction with $NaBH_4$ afforded a tertiary alcohol **5** that was shown to be identical with (+)-curcumafuranol [**6**] (7,8) in all respects except for the sign of the rotation. Optical rotation of (+)-curcumafuranol is reported as $[\alpha]_D$ +124°, while that of **5** was measured to be −140°. The two compounds are thus enantiomers, and the absolute configuration of **1** must be *S*.

Although it is not uncommon to find a large amount of a single metabolite from a marine organism, the high content of echinofuran, 32% of the crude extract or 0.2% of a wet sample of the gorgonian, suggested that it might have a defensive role for the organism. However, although it inhibited the cell division of fertilized sea urchin eggs, echinofuran showed no ichthyotoxicity up to 10 ppm against guppies. Echinofuran showed no antimicrobial activity at 100 μg/8 mm disc against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Aspergillus* sp., and *Cladosporium*.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were measured on an Atago AA-5 or on a Jasco DIP-181 polarimeter. Uv spectra were recorded on a Jasco 610 spectrophotometer and ir on a Hitachi 260-10 or 260-30 spectrophotometer. ^1H- and ^{13}C-nmr spectra were taken on a JEOL FX-90Q or GX-500 nmr spectrometer. Mass spectra were measured on a JEOL JMS-D300 instrument.

EXTRACTION AND ISOLATION.—The gorgonian *Ech. praelonga* was collected at Cape Hedo, Okinawa in August, 1987. Taxonomic identification was made by Dr. K. Muzik. A voucher specimen has been deposited at the Department of Marine Sciences, University of the Ryukyus. A fresh sample (2 kg) was extracted by steeping in MeOH. The extract was concentrated, and the resulting aqueous suspension was extracted with EtOAc to give 14.3 g of a dark oil. A part (1.8 g) of this oil was chromatographed on a Si gel column by eluting with hexane to furnish 0.58 g of echinofuran [**1**] as a colorless oil. As it darkened on standing, analytical samples could be obtained by hplc (μ-Porasil, herxane) purification immediately before use.

Echinofuran [**1**].—$[\alpha]^{20}_D$ −91° (c = 0.138, $CHCl_3$); uv (MeOH) λ max 290 nm (ε 1100); cd (MeOH) Δε −7.3 at 225 nm; ir (CCl_4) ν max 3070, 2900, 2850, 1440, 1390, 1130, 1110, 890 cm^{-1}; ^1H nmr ($CDCl_3$) δ 6.98 (1H, s, H-2), 4.96 (1H, s, H-11), 4.89 (1H, s, H-11), 3.68 (1H, br d, J = 18 Hz, H-9), 3.33 (1H, br d, J = 12 Hz, H-4a), 2.98 (1H, d, J = 18 Hz, H-9), 2.54 (2H, m, H-4, -6), 2.41 (2H, m, H-6, -7), 2.29 (2H, m, H-4, -7), 1.88 (3H, s, H-10), 1.76 (3H, s, H-12); ^{13}C nmr ($CDCl_3$) δ 156.7 (s), 147.9 (s), 140.3 (s), 135.3 (d), 124.6 (s), 120.8 (s), 118.1 (s), 105.3 (t), 45.7 (d), 33.4 (t), 32.6 (t), 30.1 (t), 29.5 (t), 21.6 (q), 8.0 (q); eims *m/z* (%) $[M]^+$ 214 (100), 199 (29), 185 (24), 157 (16), 109 (32), 91 (15); hreims *m/z* 214.1363 ($C_{15}H_{18}O$ requires 214.1358).

DEHYDROGENATION OF ECHINOFURAN [**1**] TO LINDERAZULENE [**2**].—A mixture of **1** (10 mg) and 10% palladium on charcoal (30 mg) was blended by adding a small amount of hexane in a long test tube. After evaporation of the solvent by a stream of N_2, the mixture was heated at 300° on a sand bath for 5 min. The reaction mixture was cooled, taken up in hexane, filtered, and concentrated to give a residue. The residue was separated by hplc (μ-Porasil, hexane) to furnish 0.7 mg (7%) of a blue pigment and 4.2 mg (42%) of the starting material. The pigment was identified as linderazulene [**2**] by comparing R_f values on tlc and the ir and uv spectra with those of an authentic sample.

Dihydroechinofuran [**4**].—A mixture of **1** (73 mg) and PtO_2 (10 mg) in MeOH (3 ml) was stirred with H_2 under atmospheric pressure until 10 ml of H_2 was absorbed. The mixture was filtered over a pad of Si gel. After concentration of the filtrate the resulting residue was separated by hplc [Cosmosil 5C18, MeOH-H_2O (20:1)] to give 15.0 mg (20%) of **4** as an oil: $[\alpha]^{20}_D$ −38° (c = 0.242, $CHCl_3$); ^1H nmr ($CDCl_3$) δ 6.99 (1H, br s, H-2), 3.70 (1H, br d, J = 18 Hz, H-9), 2.95 (1H, d, J = 18 Hz, H-9), 2.78 (1H, br dd, J = 13, 6 Hz, H-4a), 2.43 (1H, br dd, J = 12, 12 Hz, H-7), 2.39 (1H, ddd, J = 15, 3, 3 Hz, H-4), 2.25 (1H, m, H-7), 2.16 (1H, dddq, J = 12, 7, 6, 6 Hz, H-5), 2.04 (1H, ddd, J = 15, 13, 2 Hz, H-4), 1.90 (3H, d, J = 1 Hz, H-10), 1.80 (1H, ddd, J = 12, 6, 6 Hz, H-6), 1.74 (3H, s, H-12), 1.43 (1H, dddd, J = 12, 12, 12, 8 Hz, H-6), 1.06 (3H, d, J = 7 Hz, H-11); ^{13}C nmr ($CDCl_3$) δ 148.6,

135.2, 128.2, 124.1, 118.4, 110.0, 44.4, 38.6, 33.6, 32.6, 30.6, 26.6, 21.6, 15.2, 8.2; eims *m/z* (%) $[M]^+$ 216 (75), 201 (32), 187 (31), 173 (22), 159 (59), 145 (42), 109 (100); hreims *m/z* 216.1501 ($C_{15}H_{20}O$ requires 216.1511).

(−)-Curcumafuranol [**5**].—To a stirring suspension of mercuric acetate (223 mg) in THF (5 ml) and H_2O (5 ml) was added a solution of **1** (150 mg) in 4 ml of THF. The mixture was stirred at room temperature for 1.5 h, treated with NaOH solution and then with $NaBH_4$ solution and added NaCl according to the procedure of Brown and Geoghegan (9). The resulting two layers were separated, and the lower layer was extracted with THF. The combined organic layers were filtered and concentrated to give a residue. The residue was chromatographed on Si gel (hexane/EtOAc) to furnish 31.1 mg of crude product and 56.7 mg (38%) of the starting material. Purification of the crude product using hplc [Cosmosil 5C18, MeOH-H_2O (10:1)] gave a pure sample (8.3 mg, 5%) of (−)-curcumafuranol [**5**] as an unstable oil, which showed identical spectral data with those of (+)-curcumafuranol (3). (−)-Curcumafuranol [**5**]: $[\alpha]^{20}D$ −140° (*c* = 0.92, $CHCl_3$); ^1H nmr (C_6D_6) δ 6.97 (1H, br s), 3.63 (1H, d, *J* = 18 Hz), 3.03 (1H, d, *J* = 18 Hz), 2.44 (1H, br d, *J* = 12 Hz), 2.38 (1H, dd, *J* = 15, 3 Hz), 1.88 (1H, dd, *J* = 15, 13 Hz), 1.77 (3H, d, *J* = 1 Hz), 1.55 (3H, s), 1.10 (3H, s); ^{13}C nmr ($CDCl_3$) δ 148.2, 138.9, 135.4, 126.2, 121.1, 117.7, 81.7, 53.0, 38.5, 33.5, 28.3, 25.0, 23.9, 21.7, 8.2.

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