

Echinolactone A and B: Isolation and Structures of Two Novel Lactones from the Marine Coelenterate *Echinopora lamellosa*

R. Sanduja,^a A. J. Weinheimer,^a M. Alam,^{a*} M. B. Hossain,^b and D. van der Helm^{b*}

^a Department of Medicinal Chemistry, College of Pharmacy, University of Houston, Houston, Texas 77004, U.S.A.

^b Department of Chemistry, University of Oklahoma, Norman, Oklahoma 73019, U.S.A.

Two novel pentacyclic triterpenes were isolated from the marine coelenterate *Echinopora lamellosa*; their structures and absolute configuration (determined from spectroscopic and X-ray diffraction data) are reported.

Marine invertebrates, particularly coelenterates, have been shown to contain a large number of novel compounds.¹ The extracts of one such coelenterate, *Echinopora lamellosa* (class Anthozoa, family Scleractinidae), collected from the waters of Korolevu, Fiji, was found to contain two novel pentacyclic triterpenes which have not so far been encountered in marine invertebrates. In this communication we report the isolation, structure elucidation, and absolute configuration of echinolactone A (**1**) and echinolactone B (**2**) from *E. lamellosa*.

The propan-2-ol extract of the coelenterate upon concentration and lyophilization gave a residue, which after usual partitioning,² gave a CHCl₃ extract. Flash chromatography³ followed by h.p.l.c. gave a group of fractions consisting of two compounds, which were then combined and separated by preparative t.l.c. to give (**1**) and (**2**).[†]

Compound (**1**) crystallized from MeOH to give colourless needles, m.p. 245 °C, C₃₂H₄₆O₅ (calcd. 510.3345, found 510.3381).[‡] The i.r. spectrum of (**1**) showed the presence of an α,β-unsaturated ketone (1670 cm⁻¹), an acetoxy group (1720 and 1265 cm⁻¹), and a γ-lactone (1770 cm⁻¹) moiety. The 400 MHz ¹H n.m.r. spectrum of (**1**) showed the presence of seven methyl groups attached to tertiary carbons, an acetate moiety (δ 2.02), a vinylic proton (δ 6.06), and a single proton (δ 4.48) attached to an acetate-bearing carbon. The multiplicity of the absorption at δ 4.48 (C-3H) indicated the presence of two hydrogen atoms on the adjacent carbon atom. The absorptions at δ 2.55 and 1.82 (mutually coupled, double resonance), and δ 2.29 were assigned to geminal protons on

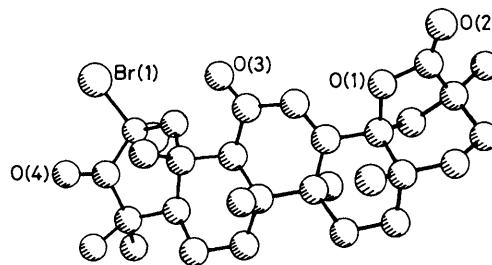
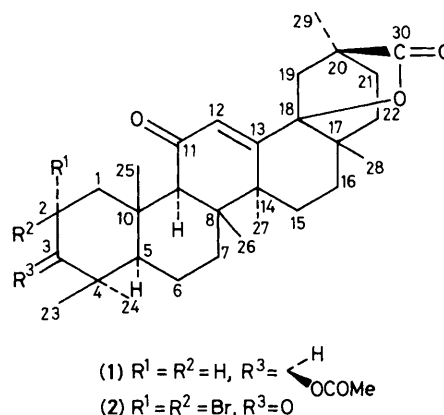


Figure 1. A perspective view of the molecular structure of echinolactone B.

[†] H.p.l.c. 10μ silica gel cartridge used with a radial compression module, Waters Associates, Milford, Massachusetts. Solvent: benzene-ethyl acetate (80:20 v/v); t.l.c.: silica gel 60 (E. Merck); solvent: CHCl₃-MeOH (97:3 v/v); R_F: 0.55 (**1**) and 0.35 (**2**).

[‡] Compound (**1**): *m/z* 510 (*M*⁺, C₃₂H₄₆O₅), 495 (C₃₁H₄₃O₅), 465 (C₃₁H₄₅O₃), 450 (C₃₀H₄₂O₃), 301 (C₁₉H₂₅O₃), 232 (C₁₅H₂₀O₂), 205 (C₁₃H₁₇O₂), 188 (C₁₄H₂₀), 165 (C₁₃H₉), 163 (C₁₁H₁₅O), 149 (C₁₀H₁₃O), 148 (C₁₁H₁₆), and 135 (C₉H₁₁O). Compound (**2**): *m/z* 544 (C₃₀H₃₉⁸¹BrO₄), 542 (C₃₀H₃₉O₄⁷⁹Br; *M*⁺ - HBr), 464 (C₃₀H₄₀O₄), 420 (C₂₉H₄₀O₂), 419 (C₂₉H₃₉O₂), 301 (C₁₉H₂₅O₃), 257 (C₁₈H₂₅O), 232 (C₁₅H₂₀O₂), 188 (C₁₄H₂₀), 165 (C₁₃H₉), 163 (C₁₁H₁₅O), 149 (C₁₀H₁₃O), and 135 (C₉H₁₁O).

§ ¹H N.m.r., 400 MHz (CDCl₃): (**1**) δ 6.068 (1H, s, C-12H), 4.480 (1H, dd, *J* 10.2 and 4.8 Hz, C-3H), 2.762 (1H, d, *J* 13.4 Hz), 2.555 (1H, d, *J* 11.8 Hz, C-19βH), 2.287 (1H, s, C-9H), 2.024 (3H, s, acetate CH₃), 1.822 (1H, d, *J* 11.8 Hz, C-19αH), 1.166, 1.151, 1.151, 1.128, 0.937, 0.855, and 0.850 (3H each, s, 7 × CH₃). Irradiation of the doublet at δ 2.762 (*J* 13.4 Hz) caused a change between δ 1.4 and 1.5 in the methylene envelope. (**2**) δ 6.147 (1H, s, C-12H), 4.443 (1H, d, *J* 16.4 Hz, C-1βH), 3.152 (1H, d, *J* 16.4 Hz, C-1αH), 2.578 (1H, d, *J* 11.9 Hz, C-19βH), 2.381 (1H, s, C-9H), 1.822 (1H, d, *J* 11.9 Hz, C-19αH), 1.532, 1.414, 1.256, 1.249, 1.193, 1.184, and 0.963 (3H each, s, 7 × CH₃). Irradiation of the doublet at δ 4.443 (*J* 16.4 Hz) changed the doublet at δ 3.152 (*J* 16.4 Hz) to a singlet and *vice versa*. Similarly, irradiation at δ 2.578 caused the doublet at 1.822 to collapse to a singlet.

C-19 and to the C-9 proton, respectively. The n.m.r. data indicated the presence of a pentacyclic triterpenoidal structure in (**1**) with the possibility of a carbonyl group at C-11 and a lactone ring in ring E.⁴ The position of the α,β-unsaturated carbonyl group was corroborated by the mass fragments at *m/z* 301 and 205 which characterize c-ring cleavage in 12-en-11-ones, e.g., glycyrrhetic acid, *m/z* 301 and 207.⁵ The presence of the lactone ring in (**1**) was supported by the fragments *m/z* 466 and 465 (*M* - CO₂ and *M* - CHO₂). On the basis of the ¹H n.m.r. and mass spectral data, and correlation with crystal and spectral data of (**2**), (**1**) was identified as (3β,20β)-3-acetoxy-18-hydroxy-11-oxo-olean-12-en-29-oic acid, γ-lactone. Thus echinolactone A is structurally related to isogabrolide[¶] which has the alternative fusion of the (18,30) lactone ring.

¶ Canonica *et al.*⁶ have reported the isolation of isogabrolide from *Glycyrrhiza glabra*. The stereochemistry of the lactone ring in isogabrolide is α, while in echinolactone A and B the lactone ring has a β stereochemistry.

Compound (2) crystallized from MeOH to give m.p. 225 °C; field desorption mass spectrum: 622, 624, and 626 (isotopic abundance 51 : 100 : 47); electron impact high resolution mass spectrum (e.i.h.r.m.s.) 542.20213 (calcd. for $C_{30}H_{39}^{79}BrO_4$ 542.20316, $M - HBr$) and 545.20863 (calcd. for $C_{30}H_{40}^{81}BrO_4$ 545.20895, $M - ^{79}Br$). The i.r. spectrum of (2) showed the presence of a lactone moiety (1745 cm^{-1}), an α,β -unsaturated ketone (1660 cm^{-1}), and a carbonyl group (1720 cm^{-1}). The 400 MHz 1H n.m.r. spectrum§ of (2) was similar to that of (1) with the exception that (2) had a doublet at δ 4.44 which was coupled to another doublet at δ 3.15. The doublet at δ 2.57 was coupled with the absorption at δ 1.82. The absence of a methine absorption between δ 3.2 and 4.3 supported the assumption that the C-3 acetate in (1) was replaced by the 3-oxo group (i.r. 1720 cm^{-1}). The electron impact mass spectrum of (2)‡ showed the base peak at m/z 464 ($C_{30}H_{40}O_4$) and the diagnostic fragment at m/z 301, indicating an olefin-12-en-11-one type of molecule.⁵ Although the spectral data supported the 2,2-dibromo-3,11-dioxo-18 β -hydroxyolean-12-en-30-oic acid (30,18 β) lactone structure for (2), the positions of the two bromine atoms and the absolute configuration of the lactone ring could not be determined from the spectral data. In order to establish the structure and stereochemistry unequivocally, the X-ray structure was undertaken.

Echinolactone B crystallizes in two different crystal systems (orthorhombic and monoclinic) from the same solvent system (methanol). The structures of both the forms were determined and found to be almost identical.

Crystal data: (2), orthorhombic form, $C_{30}H_{40}Br_2O_4$, $M = 624.5$, orthorhombic, space group $P2_12_12_1$, $a = 11.363(4)$, $b = 26.081(19)$, $c = 9.336(3)$ Å, $U = 2766.8$ Å³, $Z = 4$, $D_c = 1.499\text{ g cm}^{-3}$ at 138 K, $\mu = 28.8\text{ cm}^{-1}$ (Mo- K_{α}). The unit cell parameters were obtained from least-squares fit to $\pm 2\theta$ of 48 reflections measured at 138 K using Mo- $K_{\alpha 1}$ radiation. Intensities were recorded for 3235 unique reflections with $2\theta_{\text{max}}$, 53° on an Enraf-Nonius CAD-4 automatic diffractometer fitted with a liquid nitrogen low temperature device and using graphite-monochromated Mo- K_{α} radiation ($\lambda = 0.71069$ Å) at 138 ± 2 K. The structure was determined by the heavy atom method and refined by a full-matrix least-squares routine.⁷ Anisotropic thermal parameters were given to Br and O atoms and all other atoms were refined isotropically. Refinement converged for 2284 observed reflections [$I \geq 2\sigma(I)$] to $R_w = [\sum w\Delta^2 / \sum w|F_o|^2]^{1/2} = 0.078$ and $R = 0.075$.

Crystal data: (2), monoclinic form, monoclinic, space group $P2_1$, $a = 15.368(11)$, $b = 11.586(10)$, $c = 7.831(6)$ Å, $\beta =$

$102.35(7)^\circ$, $U = 1362.1$ Å³, $Z = 2$, $D_c = 1.522\text{ g cm}^{-3}$. The structure was determined from 2178 unique reflections (Mo- K_{α} , $2\theta \leq 53^\circ$). All light atoms were refined isotropically (no hydrogen atoms were included). Final R 0.079 and R_w 0.082 for 1648 observed reflections [$I \geq 2\sigma(I)$]. The highest peak in the final difference Fourier map is 0.8 e/Å^3 .**

The absolute configuration of the molecule was ascertained by the R method of Hamilton⁸ (R factor for the enantiomer was 0.088 for 2284 reflections) and is shown in Figures 1 and 2. Figure 1 is a perspective view of the molecule showing the atom numbering scheme and Figure 2 shows the endocyclic torsion angles of the five six-membered rings. The rings A/B and B/C are *trans*-fused and D/E are *cis*-fused. A γ -lactone is *cis*-fused with ring E at C-18 and C-20. Excluding the bicyclo lactone part, the rest of the molecule is flat. The rings B, D, and E are in a chair conformation, while ring C with the ethylenic double bond assumes a twist conformation. These observations are consistent with the conformation of the basic olefin-12-ene skeleton found in hederagenin,⁹ $3\beta_{\text{eq}}$ -acetoxyolean-12-en-28-oic acid,¹⁰ cantoniensistriol, and sophoradiol.¹¹ It appears that γ -lactone fusion at C-18-C-20 only causes slight distortion of the chair conformation of ring E, whereas in abruslactone A,⁴ γ -lactone fusion at C(20)-C(22) causes the ring E to assume a boat conformation. The most notable feature of the echinolactone B molecule is that ring A adopts a boat conformation as has been reported for 2,2-dibromo-4,4-dimethylcholestanone.¹² It is noteworthy that in the monoclinic form of echinolactone B, ring A torsion angles, in particular C(3)-C(2)-C(1)-C(10) and C(2)-C(1)-C(10)-C(5), differ by as much as 30° from the corresponding angles observed in the orthorhombic structure. The mean difference in all other torsion angles in the two structures is *ca.* 5°. The bond distances and bond angles are normal. The difference in C-Br (axial) distance of 2.006(13) Å and C-Br (equatorial) distance of 1.930(13) Å appears to be significant. Echinolactone B (2) was thus identified as (20 β)-2,2-dibromo-18-hydroxy-3,11-dioxo-olean-12-en-29-oic acid, γ -lactone.

Although compounds of the oleanane type are common in plants, their occurrence in a coelenterate is unprecedented. *E. lamellosa* is unique in the sense that, as well as echinolactones A and B, it has a number of other secondary metabolites (smilagenin, glycyrrhetic acid, and neodunol methyl ether¹³) which are common only to plants. The significance of these metabolites in *E. lamellosa* is unclear.

This work was supported by grants from the Robert A. Welch Foundation, Houston, Texas (to M. A. and A. J. W.) and from the National Cancer Institute, Department of Health and Human Services (to D. van der Helm). Collection of *E. lamellosa* was supported by the N.C.I. The h.r.m.s. were recorded at the Mass Spectrometry facility, Massachusetts Institute of Technology, Cambridge, Mass. 02139, which is supported by a grant from the National Institutes of Health Research Resources Branch. The 1H n.m.r. spectra were recorded at the South Carolina Magnetic Resonance Center, University of South Carolina, Columbia, S.C., supported by the National Science Foundation. We thank Mr. K. L. Loening, Director of Nomenclature, Chemical Abstract

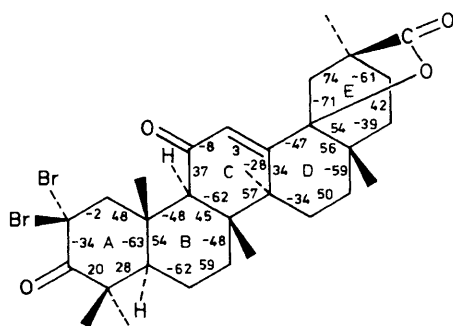


Figure 2. Endocyclic torsion angles of the six-membered rings. Standard deviations of torsion angles range between 1° and 2°.

** The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Rd., Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.

Service, Columbus, Ohio, for the current C.A. index name of (1).

Received, 27th March 1984; Com. 426

References

- 1 'Marine Natural Products,' vol. IV, ed. P. J. Scheuer, Academic Press, New York, 1982, and preceding volumes in this series.
 - 2 A. J. Weinheimer, J. A. Matson, M. B. Hossain, and D. van der Helm, *Tetrahedron Lett.*, 1977, 2923.
 - 3 N. M. Targett, J. P. Kilcoyne, and B. Green, *J. Org. Chem.*, 1979, **44**, 4962.
 - 4 H-M. Chang, T-C. Chiang, and T. C. W. Mak, *J. Chem. Soc., Chem. Commun.*, 1982, 1197.
 - 5 H. Budzikiewicz, J. M. Wilson, and C. D. Djerassi, *J. Am. Chem. Soc.*, 1963, **85**, 3688.
 - 6 L. Canonica, B. Danieli, P. Manitto, and G. Russo, *Gazz. Chim. Ital.*, 1966, 843.
 - 7 G. M. Sheldrick, SHELX 76, Program for Crystal Structure Determination, University of Cambridge, 1976.
 - 8 W. C. Hamilton, *Acta Crystallogr.*, 1965, **18**, 502.
 - 9 R. Roques, D. Druet, and L. Comeau, *Acta Crystallogr., Sect. B*, 1978, **34**, 1634.
 - 10 R. Roques, J. P. Declercq, and G. Germain, *Acta Crystallogr., Sect. B*, 1978, **34**, 2367.
 - 11 T. C. W. Mak, T-C. Chiang, and H-M. Chang, *J. Chem. Soc., Chem. Commun.*, 1982, 785.
 - 12 N. L. Allinger, J. G. D. Carpenter, and M. A. DaRooge, *J. Org. Chem.*, 1965, **30**, 1423; G. R. Chaudhry, T. G. Halsall, and E. R. H. Jones, *J. Chem. Soc.*, 1963, 806.
 - 13 R. Sanduja, G. E. Martin, A. J. Weinheimer, M. Alam, M. B. Hossain, and D. van der Helm, *J. Heterocycl. Chem.*, in the press.
-