Marine Natural Products: 14-Bromoobtus-1-ene-3,11-diol, A New Diterpenoid from the Sea Hare *Aplysia dactylomela*[1]

Francis J. Schmitz* and Keith H. Hollenbeak

Marine Chemistry Laboratory, Department of Chemistry, University of Oklahoma, Norman, Oklahoma 73019

Daniel C. Carter, M. Bilayet Hossain, and Dick van der Helm*

Crystallography Laboratory, Department of Chemistry, University of Oklahoma, Norman, Oklahoma 73019

Received February 22, 1979

14-Bromoobtus-1-ene-3,11-diol, a bicyclic monobromoditerpenoid exhibiting marginal cytotoxicity, has been isolated from the sea hare *Aplysia dactylomela*, and its structure and absolute configuration (3*S*,6*R*,7*S*,10*R*,11*S*,14*R*) have been determined by X-ray diffraction. Although regular isoprenoid, the new diol belongs to an unusual diterpene skeletal class characterized by the presence of two terminal cyclohexyl rings separated by a three-carbon chain. The compound crystallizes in the monoclinic space group $C2$ with the following crystal data: a = 21.290 (9) Å, b = 9.706 (13) Å, c = 10.328 (7) Å, β = 105.78 (4)°, V = 2053.8 Å^3, Z = 4, at −135 ± 2 °C. Three-dimensional diffractometer data were collected by using Cu K$\bar{\alpha}$ radiation at low temperature. The structure was obtained by the heavy-atom method and refined by least-squares methods to a final R factor of 0.0545 for all 2254 reflections. The absolute configuration of the molecule was determined by the R method.

Previously we have shown that the sea hare *Aplysia dactylomea* contains a variety of sesquiterpenoids[2] and other natural products,[3] several of which are halogenated and all of which are presumably of algal origin.[4] In our continuing study of this mollusc for bioactive compounds we have now isolated a new, marginally cytotoxic, brominated diterpene diol 1 and report herein its structure elucidation by X-ray diffraction. Diol 1 has an uncommon carbon skeleton which was first encountered only recently in obtusadiol, 2, a metabolite from the alga *Laurencia obtusa*.[5,6]

The new diterpene diol was isolated from the digestive glands of *A. dactylomela*. Concentrated isopropyl alcohol extracts of the glands were diluted with water and extracted with methylene chloride. The organic solubles were partitioned according to the scheme described by Kupchan,[7] and the chloroform solubles were chromotographed over Sephadex LH-20. One of the resulting cytotoxic fractions was chromotographed several times over silica gel, using high-pressure liquid chromatography (LC), to give 1: mp 134–135 °C; $[\alpha]_D$ +20° (c 0.3, EtOH). Combustion data indicated the formula $C_{20}H_{35}O_2Br$, and this was confirmed by high-resolution mass spectral data: $C_{20}H_{33}OBr$ ($M^+ - H_2O$) m/e(obsd) 368.1699, 370.1675; m/e(calcd) 368.1715, 370.1694. The infrared spectrum showed a broad hydroxyl absorption at 3400 cm^{-1}. The ^{1}H NMR spectrum (220 MHz) showed signals for one secondary methyl (δ 0.87, d) and four quaternary methyl groups (δ 1.05, 1.11, 1.16, 1.27), for one proton deshielded by bromine (δ 4.00, dd, J = 13, 5 Hz), and for two cis-oriented olefinic protons (δ 5.50, 5.63, doublets, J = 10 Hz).

Table I. Positional Parameters of Nonhydrogen Atoms[a]

atoms	$x/a \times 10^4$	$y/b \times 10^4$	$z/c \times 10^4$
Br (1)	1443.7 (3)	−1002 (2)	1395 (1)
O(1)	4719 (3)	−3814 (7)	13634 (5)
O(2)	656 (2)	−1291 (6)	5627 (5)
C(1)	3360 (3)	−1936 (8)	11762 (6)
C(2)	3886 (3)	−2137 (7)	12767 (6)
C(3)	4517 (3)	−2765 (7)	12633 (6)
C(4)	4403 (3)	−3430 (7)	11246 (6)
C(5)	4002 (3)	−2508 (7)	10145 (6)
C(6)	3315 (3)	−2300 (7)	10314 (5)
C(7)	2879 (3)	−1246 (7)	9345 (5)
C(8)	2659 (3)	−1804 (8)	7904 (6)
C(9)	2107 (2)	−966 (9)	6963 (5)
C(10)	1790 (3)	−1765 (7)	5643 (6)
C(11)	1110 (3)	−2375 (7)	5634 (6)
C(12)	849 (3)	−3231 (8)	4377 (7)
C(13)	816 (3)	−2421 (9)	3106 (7)
C(14)	1489 (3)	−1907 (8)	3157 (6)
C(15)	1795 (2)	−945 (9)	4339 (5)
C(16)	5065 (3)	−1706 (10)	12906 (7)
C(17)	3184 (4)	172 (8)	9411 (7)
C(18)	1161 (3)	−3265 (8)	6882 (7)
C(19)	1441 (3)	438 (7)	4219 (7)
C(20)	2518 (3)	−682 (7)	4382 (6)

[a] Standard deviations for the last digit are given in parentheses.

The detailed structure and the absolute configuration of the new bromo diol were determined by single-crystal X-ray diffraction, and the results are depicted in formula 1. A stereoview of a single molecule of (3*S*,6*R*,7*S*,10*R*,-11*S*,14*R*)-14-bromoobtus-1-ene-3,11-diol is shown in Figure 1. The molecule has an unrearranged isoprene skeleton identical with that of obtusadiol (2),[5] with cyclohexane and cyclohexene rings joined by a methyl-substituted trimethylene chain. The cyclohexane ring is in the chair conformation with the bromine in an equatorial position and the hydroxy group axially disposed. The trimethylene chain is almost fully stretched (torsion angles in Table II). The interplanar angle between the least-squares planes

(1) This investigation was supported by Grants CA-17256 and 17562 awarded by the National Cancer Institute, DHEW.

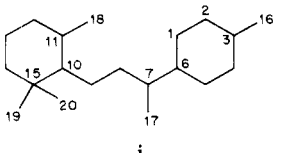
(2) (a) F. J. Schmitz, F. J. McDonald, and D. J. Vanderah, *J. Org. Chem.*, **43**, 4220 (1978); (b) F. J. Schmitz, K. H. Hollenbeak, and D. J. Vanderah, *Tetrahedron*, **34**, 2719 (1978); (c) K. H. Hollenbeak, F. J. Schmitz, M. B. Hossain, and D. van der Helm, *ibid.*, **35**, 541 (1979).

(3) F. J. McDonald, D. C. Campbell, D. J. Vanderah, F. J. Schmitz, D. M. Washecheck, J. E. Burks, and D. van der Helm, *J. Org. Chem.*, **40**, 665 (1975); D. J. Vanderah and F. J. Schmitz, *ibid.*, **41**, 3480 (1976).

(4) Cf. P. J. Scheuer, *Isr. J. Chem.*, **16**, 52 (1977); C. Ireland, M. O. Stallard, D. J. Faulkner, J. Finer, and J. Clardy, *J. Org. Chem.*, **41**, 2461 (1976), and references cited therein; D. J. Faulkner and C. Ireland in "Marine Natural Products Chemistry", D. J. Faulkner and W. H. Fenical, Eds., Plenum Press, New York, 1977, p 23.

(5) B. M. Howard and W. Fenical, *Tetrahedron Lett.*, 2453 (1978).

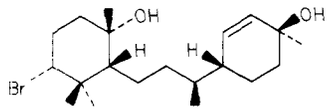
(6) We suggest that the saturated hydrocarbon skeleton i common to 1 and 2 be called obtusane. Accordingly 1 would be 14-bromoobtus-1-ene-3,11-diol.



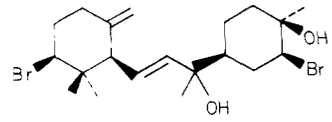
i

(7) S. M. Kupchan, R. W. Britton, M. F. Ziegler, and C. W. Sigel, *J. Org. Chem.*, **38**, 178 (1973).

0022-3263/79/1944-2445$01.00/0



1 (3*S*,6*R*,7*S*,10*R*,11*S*,14*R*)



2

through the two rings is 64°. The bond lengths and bond angles involving the nonhydrogen atoms are shown in Figures 2 and 3, respectively. The Br–C distance of 2.000 (6) Å is significantly longer than the expected value of 1.937 Å[8] but similar to the C–Br distances of 1.975 Å found in deodactol.[2c] On the basis of this correlation, we think that 1.97–2.00 Å is more representative for C(sp^3)–Br distances. The C(10)–C(11) and C(10)–C(15) bonds are lengthened. This is caused by the fact that they are bonds between tertiary (C(10)) and quaternary (C(11) and C(15)) carbon atoms. There are two short intermolecular contacts involving the two hydroxyl oxygens: O(1)–O(2) ($^1/_2 - x$, $-^1/_2 + y$, $2 - z$) = 2.708 Å and O(2)–O(2) ($-x$, y, $1 - z$) = 2.744 Å.

An interesting feature of the ^{1}H NMR spectrum of 1 is the downfield position of the C(13) axial proton signal (δ 2.40, dq, J = 14, 5 Hz), attributable to deshielding by the axial OH group at C(11).

Since 1 and 2 are the only diterpenoids reported to date to have the obtusane skeleton,[6] it is of interest to note that both have the same relative stereochemistry for like substituents on the *gem*-dimethyl substituted cyclohexane rings. The relative stereochemistry for the substituents on the bromohydrin-containing ring in 2 has not been established. The absolute configuration has thus far only been established for diol 1.

In the National Cancer Institute's in vitro bioassays for cytotoxicity,[9] 1 showed marginal inhibitory activity against the KB (ED_{50} 4.5 μg/mL) and PS (ED_{50} 10 μg/mL) cell lines.

The metabolites isolated from *A. dactylomela* thus far[2,3] appear to come from a variety of algal sources and indicate a diversified diet for this mollusc.

Experimental Section[10]

Isolation of 14-Bromoobtus-1-ene-3,11,-diol (1). Sea hares were collected at Bimini, Bahamas, in May 1975, and their digestive glands were excised and macerated in isopropyl alcohol. The alcohol was decanted, filtered, and concentrated at reduced pressure. The concentrate was suspended in water (final volume 1200 mL) and extracted with dichloromethane continuously for 24 h. Evaporation of the dichloromethane yielded a dark green oil (388 g), fraction A. The dry weight of the digestive glands after exhaustive extraction with dichloromethane and methanol was 459 g.

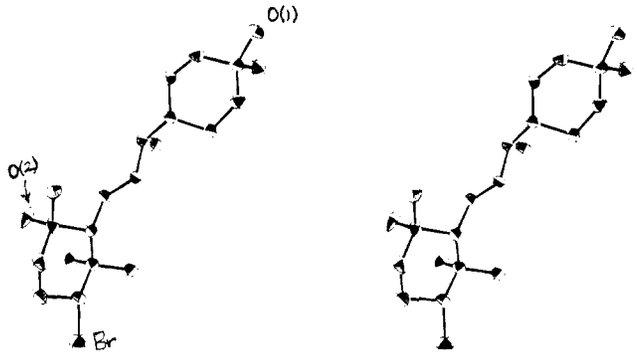


Figure 1. Stereoview of a single molecule of 14-bromoobtus-1-ene-3,11-diol.

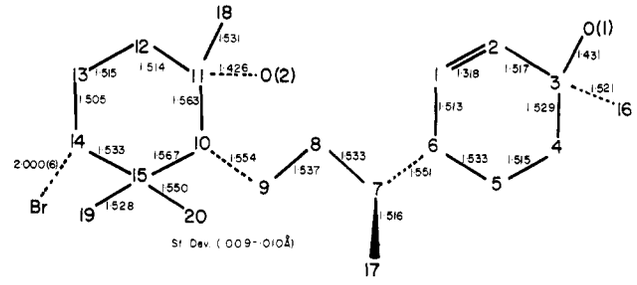


Figure 2. Bond lengths involving nonhydrogen atoms.

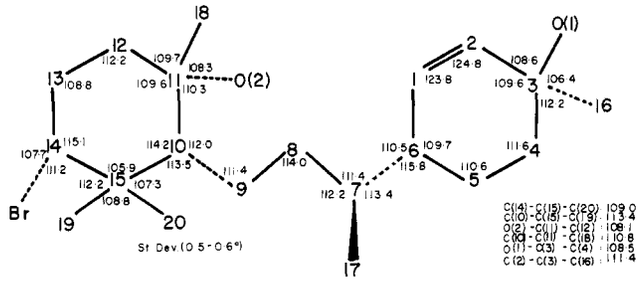


Figure 3. Bond angles involving nonhydrogen atoms.

The aqueous phase from above was further extracted three times with 1-butanol (500, 300, 300 mL) and then lyophilized. The combined 1-butanol layers were evaporated to give 16.8 g of residue, fraction B. The residue from lyophilization was triturated several times with methanol at room temperature to give methanol-soluble fraction C, 108 g, and methanol-insoluble fraction D, 38.8 g.

Following the procedure of Kupchan et al.,[7] 100 g of fraction A was dissolved in 1500 mL of methanol–water (9:1) and then extracted with hexane three times (1500, two 700-mL portions). The hexane extracts were combined to give fraction E, 77.5 g. The methanol–water phase was diluted with 150 mL of water and extracted three times with carbon tetrachloride (1500, two 700-mL portions). The combined carbon tetrachloride extracts contained 43.9 g, fraction F. After dilution with 220 mL of water, the aqueous methanol phase was extracted three times with chloroform (1500, two 700-mL portions), fraction G, 5.7 g. The aqueous methanol phase was concentrated and lyophilized, fraction H, 253 g.

A 3.6-g portion of fraction G was chromatographed on 450 g of Sephadex LH-20 (2 in. × $3^1/_4$ ft column), using chloroform–methanol (1:1) and collecting 65-mL fractions. The eleventh fraction yielded 447 mg, 32 mg of which was fractionated by LC on a Partisil-10 column, using ethyl acetate–dichloromethane (15:85), to give 12 mg of 1, mp 134–135 °C, from benzene–hexane: IR (KBr) 3400 cm^{-1} brd; ^{1}H NMR data not discussed in the text 0.9–2.18, complex multiplets, ~16 H; mass spectrum m/e (percent; composition confirmed by high resolution) 368, 370 (3, 3, $C_{20}H_{33}OBr$, $M^+ - H_2O$), 350, 352 (8.5, 8, $C_{20}H_{31}Br$), 271 (6, $C_{20}H_{31}$), 132, 133, 134, 135 (34, 24, 40, 18, $C_{10}H_{12}$–$C_{10}H_{15}$), 119, 120, 121

(8) "International Tables for X-ray Crystallography", Vol. III, Kynoch Press, Birmingham, 1974, p 275.

(9) R. I. Gueran, N. H. Greenberg, M. M. Macdonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, 3, No. 2 (Sept 1972). Effective doses (ED_{50}) in the tissue culture tests are expressed as concentrations in μg/mL of test material in the growth medium that cause 50% inhibition of cell growth. "Active" materials display an $ED_{50} \leq 20$ μg/mL. PS and LE refer to the National Cancer Institutes cell cultures of P388 lymphocytic leukemia and L1210 lymphoid leukemia.

(10) The melting point is uncorrected. The IR spectrum was taken on a Beckman Acculab 3 spectrophotometer. NMR spectra were acquired on Varian 220- and XL 100-MHz instruments; signals are reported in parts per million (δ) downfield from internal Me_4Si. Mass spectra were obtained on Hitachi RMU-7 and CEC (Du Pont, Monrovia, Calif.) 110 mass spectrometers. A Perkin-Elmer Model 141 polarimeter was used for obtaining the optical rotation. Chromatographic adsorbents used were Mallinckrodt silica AR CC-7 and Whatman, Inc., 10 μm microparticulate silica gel (Partisil 10).

Table II. Torsion Angles (deg)

C(1)-C(2)-C(3)-C(4)	-13.2	C(9)-C(10)-C(11)-C(12)	-175.7
C(2)-C(3)-C(4)-C(5)	+44.5	C(10)-C(11)-C(12)-C(13)	-55.6
C(3)-C(4)-C(5)-C(6)	-64.2	C(11)-C(12)-C(13)-C(14)	+58.6
C(4)-C(5)-C(6)-C(1)	+47.6	C(12)-C(13)-C(14)-C(15)	-60.7
C(5)-C(6)-C(1)-C(2)	-16.8	C(13)-C(14)-C(15)-C(10)	-56.3
C(6)-C(1)-C(2)-C(3)	-0.4	C(14)-C(15)-C(10)-C(11)	-52.1
C(6)-C(7)-C(8)-C(9)	+167.0	C(15)-C(10)-C(11)-C(12)	-53.7
C(7)-C(8)-C(9)-C(10)	-165.9	C(12)-C(13)-C(14)-Br	+174.6
C(8)-C(9)-C(10)-C(11)	+104.4	C(15)-C(6)-C(7)-C(17)	-56.3

(100, 29, 64, C_9H_{11}–C_9H_{13}), 93 (90, C_7H_9).

Thin plate-shaped crystals of the compound were obtained from a methanol solution equilibrated with water at 0 °C. The data crystal was a triangular plate with dimensions 0.31 × 0.08 × 0.04 mm. The crystal data at -135 °C are as follows: $C_{20}H_{35}BrO_2$, mol wt 387.4; monoclinic, a = 21.290 (9) Å, b = 9.706 (13) Å, c = 10.328 (7) Å, β = 105.78 (4)°, V = 2053.8 Å^3, Z = 4, ρ(calcd) = 1.252 g cm^{-3}, space group $C2$, as confirmed by the structure determination; λ(Cu Kα_1) 1.540 51 Å for 2θ data and λ(Cu K$\bar{\alpha}$) 1.5418 Å for intensity data; μ(Cu K$\bar{\alpha}$) = 30.4 cm^{-1}. The cell parameters were obtained from a least-squares fit to the $\pm 2\theta$ values of 20 reflections.

The intensities of all 2254 unique reflections with $2\theta \leq 150°$ were measured on a Nonius CAD-4 automatic diffractometer, using a θ–2θ scan technique. For each reflection the scan width was calculated as (0.85 + 0.14 tan θ)°. A horizontal receiving aperture with a variable width of (3.50 + 0.86 tan θ) mm and a height of 6 mm was located 173 mm away from the crystal. A reflection was scanned for a maximum of 80 s with two-thirds of the time spent in scanning the peak (P) and one-sixth of the time spent on each of the two backgrounds, left (LB) and right (RB). The unscaled intensities were calculated as $I = P - 2(RB + LB)$. A standard reflection was monitored after every 20 min. Three orientation control reflections were centered after every 200 measurements and a new orientation matrix was automatically obtained if there was an angular change greater than 0.1°. Out of the total, 117 reflections with $I < 2\sigma(I)$ were considered unobserved and were assigned an intensity equal to T, where $T = P + 2(LB + RB)$, for the purpose of least-squares refinements. Intensities were corrected for Lorentz and polarization factors. A Gaussian method[11] was employed to make the absorption correction. Each structure amplitude was assigned an experimental weight, w_F, based on counting statistics.[12]

The position of the Br atom was obtained from a three-dimensional sharpened Patterson map. All nonhydrogen atoms were located from successive difference Fourier maps. The atom positions were refined by least-squares methods, first isotropically and later anisotropically. Hydrogen atoms were located from a difference Fourier map, and their parameters were refined isotropically. The observed structure factors were corrected for the anomalous scattering of Cu radiation by the Br atom. All least-squares refinements were carried out by using a block-diagonal least-squares program[13] in which the quantity $\sum w_F(|KF_o| - F_c|)^2$ was minimized. The scattering factors for Br, O, and C atoms and $\Delta f'$ and $\Delta f''$ for the Br atom were taken from ref 14. Hydrogen scattering factors were taken from Stewart, Davidson, and Simpson.[15] The refinement was terminated when the maximum parameter shifts for nonhydrogen atoms were less than half of their corresponding standard deviations. The final R factor is 0.0524 for 2145 reflections (included in the least-squares calculations) and 0.0545 for all 2254 reflections. The final positional parameters of the nonhydrogen atoms are given in Table I.

The absolute configuration of the molecule was ascertained by the R method of Hamilton.[16] The R factors for the two enantiomers are 0.0524 and 0.0551. This indicated that one of the enantiomers can be rejected at better than the 0.005 level. The correct absolute configuration (3S,6R,7S,10R,11S,14R) is shown in Figure 1 and formula 1.

Acknowledgments. The mass spectrometry facility at the Massachusetts Institute of Technology supported by a grant (Principal Investigator, Professor K. Biemann) from the Biotechnology Research Branch, Division of Research Resources, provided the high-resolution mass spectrum. We are grateful for the use of the former Lerner Marine Laboratory, Bimini, Bahamas, for specimen collection and acknowledge with thanks grants from the Phillips Petroleum Co., Bartlesville, Okla., and the NSF (Grant GP 38410) which aided in the purchase of NMR spectrometers. We thank Dr. W. H. Fenical, Scripps Institute of Oceanography, La Jolla, Calif., for providing a 220-MHz NMR spectrum.

Registry No. 1, 70145-28-7.

Supplementary Material Available: Anisotropic thermal parameters of nonhydrogen atoms (Table A), all hydrogen parameters (Table B), and a structure factor table (Table C) (15 pages). Ordering information is given on any current masthead.

(11) P. Coppens, L. Leiserowitz, and D. Rabinovich, *Acta Crystallogr.*, **18**, 1035 (1965).

(12) D. van der Helm, S. E. Ealick, and J. E. Burks, *Acta Crystallogr., Sect. B*, **31**, 1013 (1975).

(13) F. R. Ahmed, SFLS program, NRC-10, National Research Council, Ottawa, 1966.

(14) "International Tables for X-ray Crystallography", Vol. IV, Kynoch Press, Birmingham, 1974, pp 73, 75, 80, 149.

(15) R. F. Stewart, E. R. Davidson, and W. T. Simpson, *J. Chem. Phys.*, **42**, 3175 (1965).

(16) W. C. Hamilton, *Acta Crystallogr.*, **18**, 502 (1965).