

X-RAY STRUCTURE DETERMINATION OF NEW BROMINATED METABOLITES

ISOLATED FROM THE RED SEAWEED *LAURENCIA OBTUSA*¹⁾

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Summary. The structure and absolute configuration of the new sesquiterpene 1 and the bromine containing C₁₅ nonterpenoid obtusin (2), isolated from a variety of the Canary Island red alga *Laurencia obtusa* were secured by X-ray crystallographic techniques.

In the course of our continuous research into the chemistry of bioactive metabolites from marine organisms of the Canary Islands, we have studied a new variety of the halogen-metabolizing red alga *Laurencia obtusa* (Rhodomelaceae, Rhodophyta)²⁾. Ether extraction of the seaweed collected during September 1981 at Graciosa Island afforded the unique dibrominated sesquiterpene 1 and the highly crystalline tribrominated C₁₅ nonterpenoid compound 2, along with several minor and previously unknown sesquiterpenoids having a new carbon skeleton³⁾.

Freshly-gathered algae were extracted with cold ether in inert atmosphere and subjected to a quick succession of chromatographies on silica gel at low temperatures in dry conditions. Fractions eluted with petroleum ether-ether (98:2) yielded compound 1 (112 mg, 0.42% of lipid weight), mp 100-102°C (from n-hexane), $[\alpha]_D^{25} -8.7^\circ$ (c, 2.86, CHCl₃); the compound was analyzed as C₁₅H₂₄Br₂O, *m/z* M⁺ 382, 380, 378; high resolution *m/z* 364.0050 (C₁₅H₂₂⁸¹Br₂; Δ -0.3 mmu), 323.9729 (C₁₂H₁₈⁸¹Br₂; Δ +0.5), 202.0184 (C₉H₁₃⁸¹Br; Δ -0.4). IR ν_{\max}^{KBr} 1450, 1380, 1220, 1105, 860 and 760 cm⁻¹. ¹H-NMR (CDCl₃, 360 MHz) 3.92 (1H, *dd*, *J*= 12.5, 4.3 Hz), 3.83 (1H, *d* of *dd*, *J*= 10.8, 8.4, 2.6), 2.54 (1H, *d* of *dd*, *J*= 5.4, 3.4, 1.2), 2.30 (1H, *bt*, *J*= 12.5), 2.16 (1H, *dq*, *J*= 11.0, 3.4), 2.0 (2H, *m*), 1.5-1.8 (6H, *m*), 1.43 (3H, *s*), 1.1-1.2 (2H, *m*), and 1.06 (6H, *s*). The ¹³C-NMR (CDCl₃) data for 1 assisted by off-resonance and selective proton-noise decoupling techniques showed the presence of three methyls (31.8, 20.5, 18.8), six methylenes (44.4, 41.6, 33.9, 33.7, 32.0, 29.3), three methines (65.8, 52.2, 37.9), and three fully substituted carbon atoms (89.0, 86.1, 36.5). The structure of 1 was solved by X-ray diffraction methods. A crystal of .2x.2x.2 mm was used for the crystallographic study; it was orthorhombic, space group P2₁2₁2₁, with a = 19.558(14), b = 13.274(8), c = 6.175(4) Å, V = 1603(2) Å³, D_c = 1.575 g.cm⁻³, and Z = 4. 1604 independent Friedel pairs were collected in the range 2° < θ < 65° using monochromated CuKα radiation. No crystal decomposition was observed during the data collection. 1444 Friedel pairs were considered observed by the criterion I > 2 σ (I), and were used for the refinement.

The structure was solved using MULTAN program⁴⁾. Atomic parameters were refined by full-matrix least-squares analysis using anisotropic thermal coefficients for non-hydrogen atoms. The

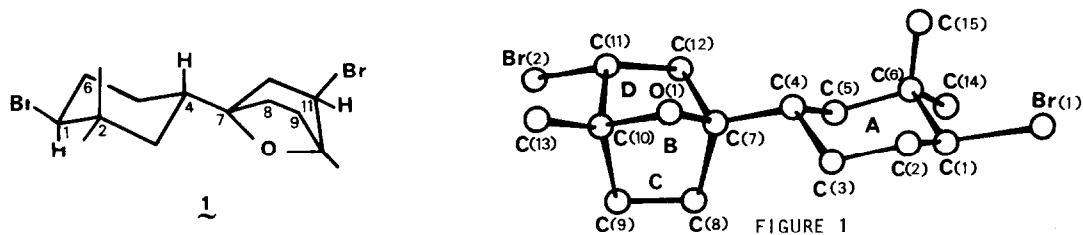


FIGURE 1

H-atoms were located at their expected positions, although confirmed in a difference map. Including the H-atoms contribution, a weighting scheme to normalize $\langle \omega \Delta^2 F \rangle$ vs. $\langle Fo \rangle$ and $\langle \sin \theta / \lambda \rangle$ was applied. A final weighted refinement on hkl and $\bar{h}\bar{k}\bar{l}$ reflections, excluding four Friedel pairs affected by secondary extinction, gave the unweighted and weighted factors: $R = 4.6\%$ and $R_w = 6.2\%$ ⁵⁾. The absolute configuration of the molecule is shown in Figure 1; it was calculated from the 57 more relevant Bijvoet pairs, those with $F_o > 8$, which are in the ranges of $3.5 < F_o < 40.0$ and $.3 < \sin \theta < .60$. The averaged Bijvoet difference was of 0.40 for the correct enantiomer vs. 2.180 for the wrong one.

The relative position of the two six-membered rings of the molecule is staggered with H_4-C_8 *trans*. Conformational Cremer's parameters⁶⁾, shown in Table 1, have been calculated for the two six-membered rings A and B, beginning at atoms C_1 and C_7 , and for the two five-membered rings C and D, beginning at O_1 . The sense in both cases was anticlockwise.

Table 1. Cremer's parameters⁶⁾ for rings: A, B, C and D (Figure 1)

Parameters	A	B	C	D
θ_2 ($^\circ$)	1	91		
ϕ_2 ($^\circ$)	42	184	177	173
Q (Å)	.59	.96	.54	.57
Conform.	4C_1	${}^{7,10}B$	0_1E	0_1E

Fractions eluted with petroleum ether-ether (90:10) yielded compound 2 (830 mg 2.9% of lipid weight), mp 93-95°C (from n-hexane), $\{\alpha\}_D -107^\circ$ (c, 0.84, CHCl_3). High resolution electron impact (HREI) mass spectrometry established by peak matching the elemental composition $C_{15}H_{19}^{79}Br_2^{81}BrO_3$ (observed 485.8861; $\Delta -0.3$ mmu), as well as fragment peaks at m/z 348.9430 ($C_{12}H_{15}^{79}Br_2O_2$; $\Delta +0.8$), 287.0238 ($C_{12}H_{15}^{79}BrO_3$; $\Delta -0.1$), and 247.1296 ($C_{15}H_{18}O_3$; $\Delta -0.7$). The 1H - and ^{13}C -NMR data of compound 2 are almost identical with those reported for obtusin: oil, $\{\alpha\}_D -65.8^\circ$, isolated by B.M. Howard *et al.* from a Mediterranean variety of *Laurencia obtusa*²ⁱ⁾,

for which structure 2 was proposed on the basis of X-ray crystallographic studies of the crystalline obtusinol (3), compound obtained by treatment of obtusin with excess of silver acetate in glacial acetic acid at 25° for 2h, followed by mild saponification of the resulting acetyl derivative with KOH in methanol.



Compound 2 crystallizes in the orthorhombic system $P2_12_12_1$ with four molecules in the unit cell: $a = 20.440(1)$, $b = 9.9925(8)$, and $c = 8.8503(5)$ Å, $D_c = 1.790$ gcm⁻³, $\mu = 84.3$ cm⁻¹. The intensity of the 1888 independent Friedel pairs up to $\theta = 67^\circ$ was measured. Data were collected on a computer-controlled four-circle diffractometer, using graphite-monochromated CuK α radiation ($\lambda = 1.5418$ Å) and $\omega/2\theta$ scan at $-1.2^\circ/\text{min}$. Two reference reflections were monitored every two hours showing an intensity decay of 50% along the experiment. No absorption correction was done. The structure was solved by heavy atom method and refined using the 1221 observed independent reflections with $I > 2\sigma(I)$. The non-hydrogen atoms were isotropically refined. Assuming reasonable isotropic thermal parameters for Br(16), Br(20), and Br(21), their population parameters were refined to 73%, 88%, and 85% respectively. The C(15) methyl is statistically disordered about 50% between two positions, although only one is described in Figure 2.

The hydrogen atoms, except those of C(14) and C(15) were located at their expected positions and included in the structure factors calculation. A weighting scheme to normalize $\langle \Delta F \rangle$ vs. $\langle \sin \theta / \lambda \rangle$ was applied in the last refinement, using anisotropic thermal parameters for Br atoms, isotropic for C and O atoms, and fixed isotropic contribution for H atoms. Refinement of both observed h, k, l and $\bar{h}, \bar{k}, \bar{l}$ reflections converged to $R = 8.6\%$ and $R_w = 11.5\%$ ⁵⁾. The absolute configuration was determined by comparison of the more significant 32 Bijvoet pairs with $\Delta F_c > 1$. The averaged Bijvoet differences are 0.756 for the right enantiomer vs. 2.809 for the wrong one. The absolute configuration of the chiral centres are 3(R), 4(R), 6(R), 7(R), 9(R), 10(S), 12(S), and 13(R).

Figure 2 shows a computer-generated perspective drawing of the X-ray absolute molecular model of compound 2, which is essentially the same as that reported for obtusinol (3)²ⁱ⁾. In compound 2 and in both independent molecules of obtusinol (3), the five-membered rings A and B have about the same conformation which is almost C_s⁷⁾. The conformation of ring C changes from C_s in obtusinol (3) to the next C₂ conformation in compound 2, which is probably due to the different substitution at C(3). The Altona⁸⁾ conformational parameters, taking τ_0 opposite to the O atoms and in the senses 10 \rightarrow 11, 7 \rightarrow 8, and 6 \rightarrow 5, are indicated in Table 2.

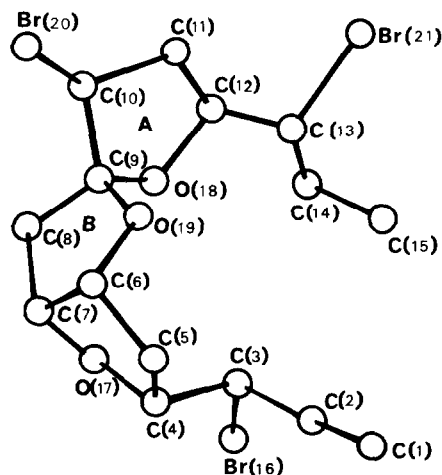


FIGURE 2

	Δ_A	Δ_B	Δ_C	e_A^m	e_B^m	e_C^m (degrees)
Compound <u>2</u>	678	669	637	45	30	33
Obtusinol (<u>3</u>) ²ⁱ	672	678	700	45	30	30
	680	684	679	39	28	35

Table 2: Altona⁸⁾ conformational parameters for compound 2 and both independent molecules of obtusinol (3).

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