

MARINE ANTICANCER AGENTS: ASPERDIOL, A
CEMBRANOID FROM THE GORGONIANS, *EUNICEA ASPERULA* AND *E. TOURNEFORTI*

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In our earlier survey¹ of marine organisms for antineoplastic activity, aqueous alcohol extracts of two Caribbean gorgonians of the *Eunicea* genus, *E. asperula* and *E. tourneforti* were shown to possess *in vivo* activity against the National Cancer Institute's P-388 lymphocytic leukemia (PS). Fractionation of these extracts was undertaken, using the *in vitro* PS and KB (cytotoxicity) bioassays for guidance. The same active principle, asperdiol (Fig. 1), has been isolated from each gorgonian.

The isolation of asperdiol was accomplished by a series of solvent partitions and column chromatographies monitored at each step by *in vitro* bioassay. Bioactivity was first concentrated into the chloroform phase of a water-chloroform partition. This fraction was further refined by three successive partitions using hexane, carbon tetrachloride and chloroform *vs.* 10, 25 and 35% water in methanol, respectively. The bioactivity was localized in the chloroform phase. Chromatography of this material through Sephadex LH-20 with methanol yielded an orange colored biologically active fraction. Rechromatography of this colored material through silica gel using acetone-hexane mixtures as eluant yielded crystalline asperdiol, mp 109-110° (acetone-hexane), $[\alpha]_D^{20} -87^\circ$, chf., $C_{20}H_{32}O_3$ (Calc. 74.96% C, 10.06% H; found 74.79% C, 9.95% H), m/e 320 (M^+), ir (KBr): 3450 (OH) and 1645 cm^{-1} (C=C). Its nmr ($CDCl_3$) showed signals for three methyl groups (δ 1.20 s; 1.62, bs; 1.77, bs), an epoxide proton (δ 2.70, dd, $J=4,6$ Hz) methylene and methine protons of alcohol functions (δ 4.05, 2H, bs and 4.50, 1H, dd, $J=5,8$ Hz), and four vinyl protons (δ 4.75, bs; 4.94, bs; 5.14 bt, $J=7$ Hz; 5.45 bd, $J=8$ Hz).

Double resonance experiments established an isopropenyl residue (coupling between the two vinyl proton singlets and the vinyl methyl at δ 1.77), a substituted 2-butene-1,4-diol function (coupling between the vinyl proton doublet and each of the alcohol functions) and a trisubstituted double bond (coupling between the vinyl methyl at δ 1.60 and the vinyl proton at δ 5.14). The remaining signals are typical for a trisubstituted epoxide (Me at δ 1.20, H at δ 2.70).

The preceding structural features account for six carbon atoms which are not part of the one carbocycle required, suggesting the nucleus consists of a 14-membered cembrane ring.

The compound formed clear prismatic needles on slow evaporation from benzene. The space group is monoclinic, $P2_1$, with cell dimensions (at -160°C) $a=8.2181(4)$, $b=8.8503(5)$, $c=12.6649(11)\text{\AA}$, $\beta=90.116(5)^\circ$ as determined from the $+2\theta$ and -2θ values of 39 reflections spaced through all octants of reciprocal space using $\text{CuK}\alpha_1$ radiation ($\lambda=1.54051\text{\AA}$). $D_c(-160^\circ\text{C}) = 1.155\text{ g/cm}^3$, $D_c(26^\circ\text{C}) = 1.113\text{ g/cm}^3$, $D_m(26^\circ\text{C})=1.120\text{ g/cm}^3$ (floatation in $\text{H}_2\text{O/KBr}$), F.W. = 320.5, $Z=2$. A crystal with dimensions $0.088 \times 0.264 \times 0.572\text{ mm}$ was used for all measurements. The intensities of all 2021 unique reflections with $2\theta_{\text{max}} \leq 150^\circ$ were measured as -160°C using Ni-filtered $\text{CuK}\alpha$ radiation ($\lambda=1.5418\text{\AA}$) with θ - 2θ scans on a Nonius CAD-4 diffractometer. For 1983 reflections $I > 2\sigma(I)$, where $\sigma(I)$ is determined from counting statistics.² Lorentz and polarization corrections were applied, but no absorption corrections were made.

The crystal structure was determined by direct methods with the MULTAN program.³ The structure was refined using a block-diagonal least-squares program⁴ with isotropic temperature factors for all carbon and hydrogen atoms, and anisotropic temperature factors for all oxygen atoms. In the final stages of refinement the observed structure factors were corrected for the anomalous dispersion of the oxygen atoms⁵ (*vide infra*). The refinement was terminated when all shifts for the non-hydrogen atoms were less than 0.37 of the corresponding estimated standard deviation. Scattering factors for C and O atoms were taken from International Tables for X-ray Crystallography⁶ and those for H atoms from Stewart, Davidson, and Simpson.⁷ The R value for all data (2021) based on the final parameters (Table 1, standard deviations for last digit in parentheses. Isotropic thermal parameters in \AA^2 ; anisotropic parameters are of the form: $\exp-2\pi^2(U_{11}h^2a^{*2}+U_{22}k^2b^{*2}+U_{33}l^2c^{*2}+2U_{12}hka^*b^{*}+2U_{13}hla^*c^{*}+2U_{23}klb^*c^*)$), $(R = \frac{\sum ||kF_o| - |F_c||}{\sum |kF_o|})$ was .046. The weights of F in the least-squares calculation were calculated from $\sigma(I)$.

The ORTEP drawing⁸ of a single molecule with thermal ellipsoids is given in Figure 2, bond lengths in Figure 1, and bond angles in Figure 3. The range of C-H bonds is .75 to 1.07 \AA with an average value of .98 \AA .

The shortening of C(8)-C(9) single bond distance in the epoxide ring (1.474 \AA) is a normal observation. On the other hand the C(15)-C(16) bond is unusually short. The difference Fourier map based on the final parameters shows anisotropy for C(15) and C(16), which can cause the apparent shortening of the bond.

All but one of the endocyclic bond angles (C(9), C(10), C(11)) are larger than expected (109.5° or 120°) and this is the common observation for these 14-membered ring systems. The conformational angles are shown in Table 2, which show, as can also be seen in Figure 1, that the C(4) = C(5) double bond is *trans* (-175°) and the C(12) = C(13) double bond is *cis* (-4°), while the epoxide ring is *trans*-fused to cembrane ring (C(7), C(8), C(9), C(10): 154°).

The absolute configuration was determined with the Bijvoet method⁹ using the anomalous dispersion of the oxygen atoms for Cu - radiation. After the refinement with nonanomalous scattering factors the F^+ ² and F^- ² were calculated for all reflections using f' and f'' of the oxygen atom.¹⁰ A set of 19 reflections were chosen which had large values of $|F^+|^2 - |F^-|^2|/\sigma(I)$. For each reflection the intensities of hkl , $\bar{h}\bar{k}\bar{l}$, $h\bar{k}l$, and $\bar{h}k\bar{l}$ were measured, 10 times each,

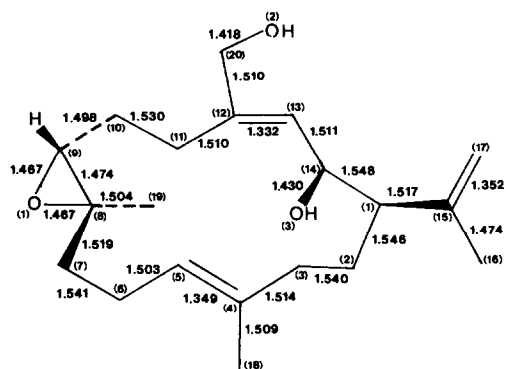


Figure 1. Bond Distances

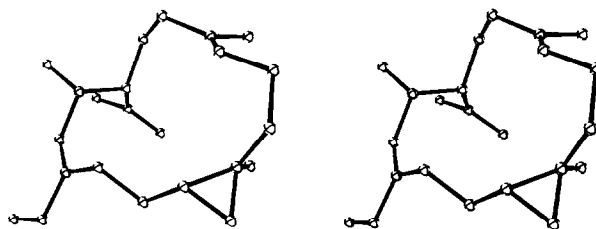


Figure 2. Single Molecule

Figure 3. Bond Angles

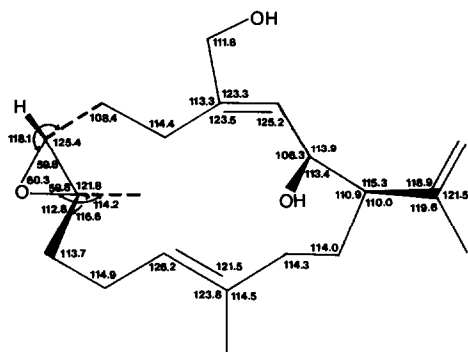


Table 1. Final Parameters

Atom	x	y	z	B	Atom	x	y	z	B
C(1)	0.1448(3)	0.6905(3)	0.2194(2)	1.23(3)	H(61)	-0.301(4)	1.019(4)	0.226(2)	1.8(6)
C(2)	0.0096(3)	0.5918(3)	0.1697(2)	1.27(3)	H(62)	-0.368(4)	1.052(5)	0.129(3)	3.1(7)
C(3)	-0.1629(3)	0.6288(3)	0.2088(2)	1.38(3)	H(71)	-0.347(4)	1.156(4)	0.342(3)	2.1(6)
C(4)	-0.2273(3)	0.7806(3)	0.1708(2)	1.37(3)	H(72)	-0.353(4)	1.257(4)	0.231(2)	1.6(6)
C(5)	-0.2944(3)	0.8810(3)	0.2380(2)	1.48(3)	H(91)	-0.111(3)	1.141(4)	0.429(2)	1.4(6)
C(6)	-0.3746(3)	1.0277(3)	0.2083(2)	1.86(3)	H(101)	0.192(4)	1.170(5)	0.425(3)	2.5(7)
C(7)	-0.3112(3)	1.1674(3)	0.2684(2)	1.63(4)	H(102)	0.180(5)	1.092(5)	0.311(3)	3.3(9)
C(8)	-0.1270(3)	1.1810(3)	0.2691(2)	1.33(3)	H(111)	0.040(4)	0.895(5)	0.406(3)	3.1(8)
C(9)	-0.0415(3)	1.1550(3)	0.3700(2)	1.56(3)	H(112)	0.104(5)	0.972(5)	0.515(3)	3.4(9)
C(10)	0.1301(3)	1.1014(3)	0.3815(2)	1.79(4)	H(131)	0.421(3)	0.687(4)	0.388(2)	0.9(5)
C(11)	0.1286(3)	0.9506(3)	0.4406(2)	1.46(3)	H(141)	0.077(4)	0.662(4)	0.370(2)	1.9(6)
C(12)	0.2084(3)	0.8664(3)	0.4381(2)	1.39(3)	H(161)	0.339(5)	0.444(6)	0.150(3)	4.1(10)
C(13)	0.3088(3)	0.7347(3)	0.3991(2)	1.23(3)	H(162)	0.486(4)	0.571(6)	0.084(3)	3.4(8)
C(14)	0.1754(3)	0.6450(3)	0.3358(2)	1.35(3)	H(163)	0.479(6)	0.550(9)	0.227(4)	6.1(13)
C(15)	0.2943(3)	0.6902(3)	0.1493(2)	1.52(3)	H(171)	0.273(4)	0.880(5)	0.087(3)	2.7(7)
C(16)	0.4050(3)	0.5592(4)	0.1510(2)	2.20(3)	H(172)	0.413(4)	0.803(5)	0.023(3)	2.9(8)
C(17)	0.3182(3)	0.8074(4)	0.0827(2)	2.47(4)	H(181)	-0.108(4)	0.829(5)	0.035(3)	2.3(7)
C(18)	-0.2186(3)	0.8033(3)	0.0529(2)	1.87(4)	H(182)	-0.250(5)	0.712(5)	0.023(3)	3.8(9)
C(19)	-0.0425(3)	1.1629(3)	0.1646(2)	1.78(3)	H(183)	-0.294(4)	0.892(5)	0.023(3)	3.0(8)
C(20)	0.4230(3)	0.9463(3)	0.4871(2)	1.44(3)	H(191)	0.067(4)	1.194(4)	0.187(3)	2.8(7)
H(1)	0.104(3)	0.801(4)	0.218(2)	0.9(5)	H(192)	-0.053(5)	1.055(6)	0.138(3)	3.7(9)
H(2)	0.032(3)	0.486(3)	0.183(2)	0.8(5)	H(193)	-0.088(4)	1.229(5)	0.108(3)	2.6(7)
H(22)	0.011(3)	0.604(4)	0.298(2)	1.0(5)	H(201)	0.449(4)	1.050(4)	0.467(2)	1.9(6)
H(3)	-0.168(4)	0.617(5)	0.286(3)	2.5(7)	H(202)	0.383(4)	0.970(4)	0.564(3)	2.4(7)
H(32)	-0.233(4)	0.554(5)	0.179(2)	2.1(6)	H(200)	0.627(5)	0.906(6)	0.543(3)	3.7(9)
H(5)	-0.295(4)	0.857(4)	0.316(2)	1.9(6)	H(300)	0.132(4)	0.640(4)	0.340(2)	1.5(6)
Atom	x	y	z	U ₁₁	U ₂₂	U ₃₃	U ₁₂	U ₁₃	U ₂₃
O(1)	-0.0691(2)	1.3095(2)	0.3320(1)	0.0204(8)	0.0131(7)	0.0193(7)	0.0006(6)	-0.0034(6)	0.0009(7)
O(2)	0.5676(2)	0.8589(2)	0.9003(1)	0.0195(8)	0.0224(8)	0.0257(8)	0.0019(7)	-0.0107(6)	-0.0067(7)
O(3)	0.2173(2)	0.4893(2)	0.3486(1)	0.0200(8)	0.0145(8)	0.0254(8)	-0.0020(6)	-0.0118(7)	0.0032(7)

Figure 4. Hydrogen Bonding

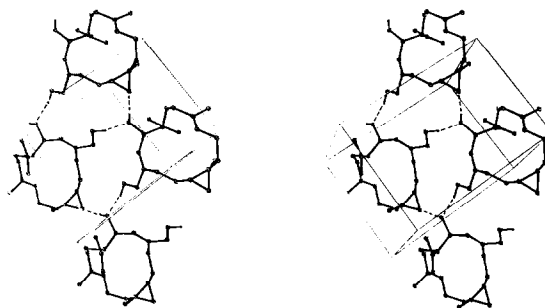


Table 2. Conformational Angles

C(1)	C(2)	C(3)	C(4)	70°	C(13)	C(14)	C(1)	C(2)	179°
C(2)	C(3)	C(4)	C(5)	-131	C(14)	C(1)	C(2)	C(3)	73
C(3)	C(4)	C(5)	C(6)	-175	C(1)	C(3)	C(4)	C(18)	52
C(4)	C(5)	C(6)	C(7)	-128	C(6)	C(7)	C(8)	C(18)	48
C(5)	C(6)	C(7)	C(8)	50	C(6)	C(7)	C(8)	C(1)	-177
C(6)	C(7)	C(8)	C(9)	-110	C(10)	C(11)	C(12)	C(20)	66
C(7)	C(8)	C(9)	C(10)	154	C(11)	C(12)	C(20)	C(1)	175
C(8)	C(9)	C(10)	C(11)	-119	C(12)	C(13)	C(14)	C(5)	-47
C(9)	C(10)	C(11)	C(12)	168	C(13)	C(14)	C(1)	C(15)	53
C(10)	C(11)	C(12)	C(13)	-114	C(14)	C(1)	C(15)	C(16)	48
C(11)	C(12)	C(13)	C(14)	-4	C(2)	C(1)	C(15)	C(17)	88
C(12)	C(13)	C(14)	C(1)	87					

at -160°C , and the results averaged for hkl and $\bar{h}\bar{k}\bar{l}$ on one hand, and for $h\bar{k}l$ and $\bar{h}k\bar{l}$ on the other hand. The results for all 119 reflections indicated the same absolute configuration which is the one shown in the figures. Another set of 9 reflections was chosen which had lower values for $|F^{+2} - F^{-2}|/\sigma(I)$, but much larger intensities. A similar series of intensities was measured for those reflections. The results of 8 reflections showed the absolute configuration shown, while 1 showed the opposite configuration. It is certain therefore that the absolute configuration which is reported is the correct one, and that the method used for this determination, especially the use of the discriminator $|F^{+2} - F^{-2}|/\sigma(I)$, appears to be reliable.

The molecules are hydrogen bonded as evidenced by the $O(2) - O(3)$ distance, 2.85 \AA , and $O(1) - O(3)$ distance, 2.85 \AA . The packing and hydrogen bonding is illustrated in Figure 4, in which the hydrogen atoms involved in the hydrogen bonding are included.

The numbering of the ring members of asperdiol (Fig. 1) is chosen to parallel that of the lactonic cembranes previously isolated¹¹ from related gorgonians, in which the substituent of position 1 (lactone) is β -oriented and positions 8 and 9 comprise a transoid double bond. Asperdiol is the first non-lactonic cembrane from gorgonians to display anticancer activity. Its effective doses for 50% inhibition (ED_{50}) of the *in vitro* KB, PS and LE cell lines are 24, 6 and 6 $\mu\text{g/ml}$, respectively.

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