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LAURENCIANOL,A NEW HALOGENATED DITERPENOID FROM THE MARINE ALGA LAURENCIA OBTUSA

Salvatore Caccamese [x] ,Rosa Maria Toscano
Dipartimento di Chimica,Università di Catania,viale Doria 8,Catania,Italy

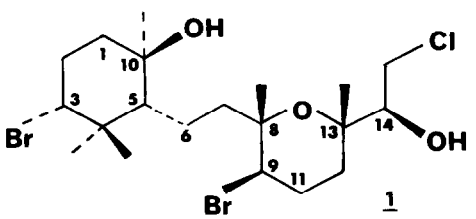
Silvio Cerrini [x] ,Enrico Gavuzzo
Istituto di Strutturistica Chimica "G. Giacomello" C.N.R.
C.P. 10, 00016 Monterotondo Stazione,Roma,Italy

Abstract: The structure of an antimicrobial dibromomonochloroditerpene alcohol, laurencianol,has been determined by spectral and X-ray crystallographic techniques. The compound has been isolated from the Mediterranean red alga Laurencia obtusa.

Red algae of the genus Laurencia (Rhodomelaceae,Rhodophyta) are prolific producers of interesting secondary metabolites consisting of sesquiterpenoids [1a],diterpenoids [1b] and nonisoprenoids [1c]. Among them,most marine bromine-containing diterpenoids have been isolated from this genus [2-6] or from sea-hares which feed preferencially on it [7-9]. We now wish to report the isolation and identification of a novel dibromomonochloroditerpenoid from Laurencia obtusa (Huds) Lamour.

Our interest in this species stems from the antimicrobial activity shown by its extract in the course of a screening program in marine algae from Eastern Sicily [10,11] and from the marked variation in the halogenated metabolites in collections from different sites [12].

Laurencia obtusa was collected at Capo Murro di Porco (Syracuse) in June 1979. The alga is orange-brown in color and grows in scattered patches on the reef where the wave action is substantial [13]. Extraction of the air-dried alga (47 g) with methanol-toluene (3:1) gave a residue which was partitioned between sodium nitrate (1 M) and diethyl ether. The ether extract (3 g) was column chromatographed on silica gel using increasing concentration of diethyl ether in petroleum ether. Fractions were screened for antimicrobial activity against Bacillus subtilis. A series of fractions eluted with 50 % Et_2O showed strong antimicrobial activity. They were combined and further purified by two silica gel HPLC (methylene chloride: 2-propanol 99:1) to yield laurencianol (1)(30 mg),m.p. 114-116 dec. (from n-hexane-benzene).



1

High resolution electron-impact mass spectrometry established,by peak matching, the elemental composition $C_{20}H_{33}{}^{79}Br_2{}^{35}ClO_2$ (obs. m/z 498.0536,Δ= -0.8 mmu) for the M-H_2O]+ fragment. The molecular ion was too weak to measure,but it was clearly detected at m/z 516 ($C_{20}H_{35}{}^{79}Br_2{}^{35}ClO_3$) by field desorption mass spectrometry.

The infrared spectrum (CCl_4) showed significant absorptions(cm^{-1}) for hydroxyl (3590 free,3480 broad and 1155 tertiary),gem-dimethyl (1385 and 1395),cyclic ether function (1110,1090,1080) and aliphatic C-Hal (760 and 710) and further illustrated the absence of carbonyl functionality. Only end absorption was noted in the UV spectrum.

The ^{1}H NMR spectrum (CD_2Cl_2) revealed signals for five tertiary methyl groups at δ 0.96,1.09,1.19;1.24,1.42 (the last three attached to carbons bearing oxygen) ,a proton α to an equatorial bromine in a cyclohexane ring adjacent to a fully substituted carbon atom (3.82 dd,J=13 and 8 Hz),an unresolved ABC pattern at δ 3.47,3.92 and 4.05 (m) and two multiplets centered at 2.11 and 1.71 δ for four and eight methylene protons,respectively. Furthermore,the ^{1}H NMR spectrum showed the absence of olefinic or exocyclic methylene protons.

Hence it was evident that the two elements of unsaturation required by the molecular formula of 1 were to be a substituted cyclohexane ring and a cyclic ether.

Further support was obtained from the ^{13}C NMR spectrum (CD_2Cl_2) which established the absence of olefinic carbons resonances and the presence of three oxygen-bearing quaternary carbons at 78.2,76.2,74.0 ppm (all singlets in the off-resonance spectrum),one more quaternary carbon attached to gem-dimethyl groups at 41.7 (s),a secondary alcohol carbon at 80.5 (d),three downfield shifted methylene carbons at 46.9,46.4 and 44.2 (one of them attributable to -$\underline{C}H_2Cl$). The remainder of the spectrum showed methylene and methyl resonances at 34.3,33.0,30.7,27.8, 23.4,23.0,21.4,20.6 and 17.8 ppm .

Consideration of these combined data gave no correlation with known diterpene systems. Hence the structure and the assignment of stereochemistry was determined unequivocally by single-crystal X-ray diffraction analysis. A stereoscopic view of the molecule is shown in Figure 1. Atom numbering in formula 1 and Figure 1 is based upon labdane skeleton [14]. The absolute configurations at the chiral centers are 3(R),5(R),8(S),9(R),10(R),13(S),14(R).

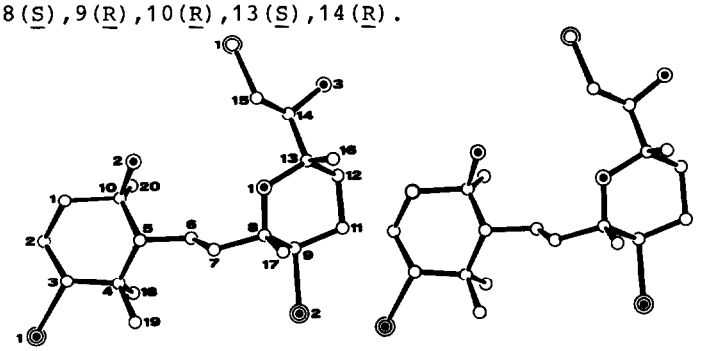


FIGURE 1 Br Cl O C

Laurencianol crystallises in colorless prisms belonging to the orthorhombic class; $C_{20}H_{35}Br_2ClO_3$, M.W.=518.768,S.G. $P2_12_12_1$,a=26.94(2),b=11.97(1),c=7.116(8) Å,D_c=1.50 gcm^{-3} for Z=4, μ(Mo-Kα)=38.7 cm^{-1}.

A total of 2224 unique reflections (with $2\theta \leq 49^\circ$) were collected with ω-scan mode on a Syntex four-circle diffractometer,1680 out of them were considered observed. Lorentz and polarization factors,but no absorption corrections,were applied. The crystal structure was determined by conventional heavy-atom methods and refined using block-diagonal least-squares calculations with anisotropic temperature factors for all non-hydrogen atoms. During the first stages of the structure determination,all the atoms were assigned carbon form factors,except bromine and chlorine. The final recognition of the atomic species of three oxygen atoms was based on the local analysis of the variation of the thermal parameters of these atomic positions,depending upon the choice of either carbon or oxygen scattering curves,and on the presence of hydrogen bonds. This model was refined to the conventional factors R=0.093 and R_w=0.134,taking into account the anomalous dispersion effects of the bromine and chlorine atoms [15]. The ratio between $R_w(+)$ and $R_w(-)$ was 1.083,allowing the opposite configuration to be rejected at high confidence level [16].

The Figure 1 shows a stereoscopic view of the molecule in the absolute configuration.

Both rings exist in the chair conformation,however a higher degree of symmetry is observed for the cyclohexane ring,in which the mean absolute value (and standard deviation) of the torsion angles is 52.4 (1.4)°,while the same value is 54.6 (5.1)°,for the saturated pyran ring. The Br(1),C(19),C(6),O(2) substituents of the cyclohexane ring are all in equatorial positions as well as Br(2) and the unusual 1-chloro,2-hydroxy ethane side chain of the heterocyclic ring.

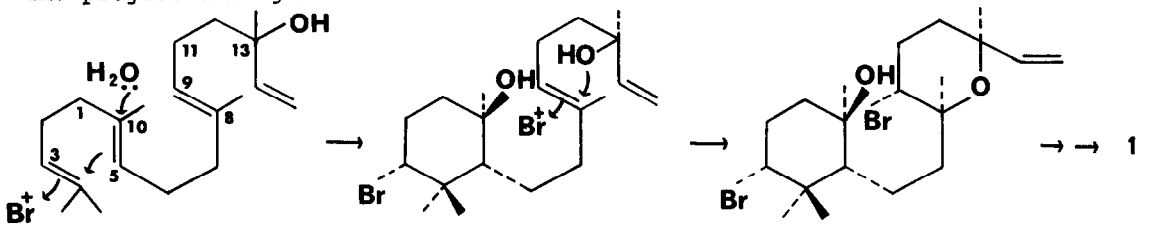
Both rings have two methyl groups in 1,3 diaxial position,namely the pairs C(16),C(17) and C(18),C(20) respectively. The non bonded distances are respectively 3.12 for the C(16)--C(17) and 3.22 Å for C(18)--C(20). The diaxial methyls pair in the ether ring stands on the opposite side with respect to the pair C(18),C(20) of the cyclohexane ring.

The diedral angle C(5)-C(6)-C(7)-C(8) is 163°,thus C(5)-C(6) and C(7)-C(8) bonds are arranged in an almost trans conformation.

The two hydroxyl groups O(2)H and O(3)H are engaged in an intermolecular hydrogen bond (2.92 Å) with screw-related molecules along the c axis.

All the calculations were performed on the HP 1000 minicomputer of the Research Area of Rome C.N.R. with programmes of references 17.

The projected biogenesis of laurencianol is drawn below:



The bromonium addition upon the terminal double bond of geranyllinalool, followed by water attack in C-10 leads to ring A. This mechanism of cyclization is well recognized in marine bromoterpene biosynthesis [18]. Thus, the subsequent closure at C-9 -- C-10 to give ring B of labdane, observed in other bromoterpenoids from Laurencia species, cannot occur in this case. A second bromonium attack at C-9 and subsequent hydroxilic C-13 oxygen trapping yields the ether ring.

Laurencianol has strong antibacterial activity vs. Bacillus subtilis and Escherichia coli, giving 17 and 16 mm zones of inhibition respectively, at 8 µg/13 mm disk. Moreover by bioautography spots of 3 µg eluted by TLC are still detectables.

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