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MARINE ANTICANCER AGENTS: SINULARIN AND DIHYDROSINULARIN, NEW CEMBRANOLIDES FROM THE SOFT CORAL, *SINULARIA FLEXIBILIS*

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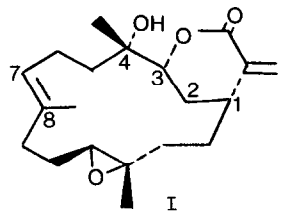
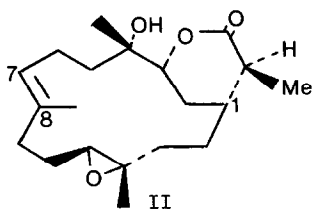
The aqueous alcohol extract of the soft coral *Sinularia flexibilis* from Hayman Island on the Great Barrier Reef of Australia has shown[1] confirmed antineoplastic activity in the National Cancer Institute's *in vivo* bioassay against P-388 lymphocytic leukemia (PS test). We have fractionated this extract using the guidance of bioassays in NCI's *in vitro* PS and KB (cytotoxicity) test systems, and have isolated three active principles, all cembranolides. Two of them, sinularin (I) and dihydrosinularin (II), are new compounds. The third, sinulariolide (III), has been reported previously by Tursch *et al.*[2]



I



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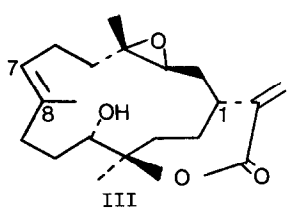


III

The isolation of this group of compounds entailed 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 carbon tetrachloride and chloroform phases.

Chromatography of these materials through Sephadex LH-20 with methanol yielded in each case an orange colored biologically active fraction. Rechromatography of this colored material through silica gel using acetone-hexane mixtures as eluant yielded, in order of elution, crystalline sinulariolide, dihydrosinularin and sinularin. Sinularin (I) melted at 150-152° (acetone-hexane),

$[\alpha]_D^{20}$-127° (chf.), $C_{20}H_{30}O_4$ (Calc'd: C, 71.82; H, 9.04%. Found: C, 71.83; H, 8.59%), m/e 334 (M^+), ir (KBr): 3600 (OH), 1735 (C=O) and 1668 cm^{-1} (C=C). Its nmr ($CDCl_3$) showed methyl signals at δ 1.32, s and 1.44, s (MeC=O), 1.66, bs (MeC=C) and one proton absorptions at δ 2.82, dd, J=8, 1.5 Hz (epoxide); 4.00, d, J=10 Hz (lactone); 5.27, bt, J=7 Hz (HC=C); 5.71, d, J=1.5 Hz and 6.28, d, J=1.5 Hz (H_2C=C).

These spectral data are indicative of an α-methylenic lactone, a tertiary methyl carbinol, a methyl substituted epoxide and a methyl substituted olefin. Together these features account for six carbon atoms which are not part of the one carbocycle required, suggesting that sinularin is a new cembranolide.

Dihydrosinularin (II) crystallized from acetone-hexane, mp 110-112°, $[\alpha]_D^{20}$-45° (chf.), $C_{20}H_{32}O_4$ (Calc'd: C, 71.39; H, 9.59%. Found: C, 71.25; H, 9.41%), m/e 336 (M^+), ir (KBr): 3500 (OH), 1733 (C=O) cm^{-1}. Its nmr spectrum ($CDCl_3$) showed methyl signals at δ 1.61, s, and 1.48, s (Me-C-O), 1.38, d, J=7 Hz (α to C=O), and 1.80, bs (MeC=C), and one proton absorptions at 2.90, t, J=6 Hz (epoxide), 4.04, bd, J=8 Hz (lactone) and 5.30, bt, J=7 Hz (HC=C).

These spectral features suggested a close similarity to sinularin (I). The location of the two additional hydrogens in II were indicated in the nmr by the replacement of the *exo*-methylene signals of I by a secondary methyl doublet, and by the absence of the characteristic 1660 cm^{-1} conjugated absorption in the ir.

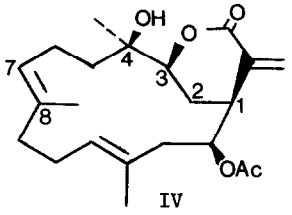
The third antineoplastic compound isolated from this organism was recognized as the known compound sinulariolide (III),[2] mp 176.5-177° (acetone-hexane), $[\alpha]_D^{20}$+134° (chf.), m/e 334 (M^+), based on the similarity of its nmr spectrum (except for the 1H doublet at δ 4.04, J=10 Hz, reported as a multiplet). This identity was confirmed by the unit cell dimensions and space group determined by X-ray diffraction.

Crystal and molecular structures of the two new cembranolides have been determined by using three-dimensional X-ray diffraction data collected at -135°C. Sinularin crystallizes in the orthorhombic system with a = 5.751, b = 10.591, c = 30.160, space group $P2_12_12_1$, and dihydrosinularin crystallizes in the monoclinic system with a = 14.486, b= 5.799, c = 10.950, β = 93.09°, space group $P2_1$. The absolute configurations of these compounds were determined using anomalous dispersion of Cu-radiation by oxygen atoms and are shown in I and II. Stereoviews of I and II are shown in Figures 1 and 2, respectively.[3]

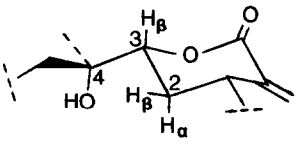
Consideration of the location and stereochemistry of the oxirane functions remaining in sinularin (I) and sinulariolide (the absolute configuration [2] of which is shown in III) suggests that each is derived from a single biogenetic precursor which possesses a nucleophilic carboxylate ion in the substituent at position 1. Intramolecular displacement with inversion at the precursor's trans 3,4-epoxide would generate sinularin I, whereas the same mode of reaction at its trans 11, 12-epoxide would generate sinulariolide (III).

Sinularin (I) shows a striking structural and stereochemical similarity to crassin acetate (IV, absolute configuration[4]), an antineoplastic[5] cembranolide present in Caribbean gorgonians. Each possesses the infrequently encountered δ-lactone, each possesses the all-*cis* substitution pattern at positions 1, 3, and 4, and each possesses *trans*oid backbone geometry about positions 7, 8 and 11, 12. However, their configurational relationship is fundamentally enantiomeric. In accord with the convention proposed here[6], all of the Caribbean cembranes for which absolute configurations have been reported [4, 7] belong to the same configurational series as IV, i.e., the

β-series. On the other hand, the Pacific cembranes of known absolute configuration, i.e., the *Sinularia* lactones (I, II, III), nephthenol[8] and epoxynephthenol acetate,[9] all belong to the α-series.



IV



V

A peculiarity of the nmr spectrum of sinularin (I) is the simple doublet character of the δ 4.00 absorption for H_3 which is vicinal to the 2-methylene. The rather large coupling constant (10 Hz) is attributable to coupling between axial $H_{3\beta}$ and axial $H_{2\alpha}$ (V). The absence of the second coupling (J<1 Hz) may be attributed to the combined electronegativity effects[10] of vicinal *trans*-coplanar oxygen atoms on the coupling strengths of the 3β and 2β protons. The dihedral angles between these protons and their respective vicinal oxygens (O_4-C_4-C_3-$H_{3\beta}$ and O_3-C_3-C_2-$H_{2\beta}$) in the crystal are 180 and 171°, respectively. The diminished coupling strength of each proton reduces their mutual coupling to less than 1 Hz. The corresponding dihedral angles in the crystal (H locations inferred) of crassin acetate (IV) are 178 and 172°, and the corresponding coupling constant is also small (J=2 Hz).

The effective doses for 50% inhibition (ED_{50}) of the *in vitro* KB and PS cell lines by sinularin (NSC 285706), dihydrosinularin (NSC 285707) and sinulariolide (NSC 285705) are 0.3 and 0.3, 16 and 1.1, and 20 and 7.0 μg/ml, respectively.

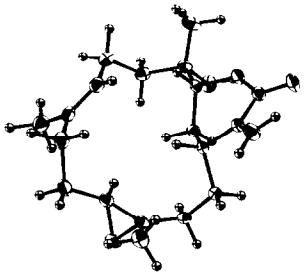


Figure I

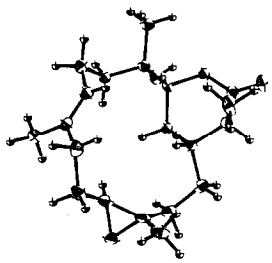


Figure 2

Acknowledgements: This investigation was supported by Grant Nos. CA 17562 and CA 11055, and Contract No. CM-67108, awarded by the National Cancer Institute, DHEW, and by Grant No. 04-158-44062 awarded by the Office of Sea Grant, NOAA. We thank Dr. Robert E. Schroeder for collecting the specimens, Dr. J. Verseveldt for their identification, Dr. Tom Karns for supervising preparation of extracts, and the University of Oklahoma for providing computer time.

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3. Details of the X-ray studies will be reported separately.

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6. Any invariant unsymmetrical marker within the skeletally symmetrical cembrane structure would serve as a suitable reference for comparisons of configuration amongst the natural cembranoids. It is suggested that the Δ^7 nuclear unsaturation be adopted as this internal point of reference. This unsaturation, or some vestigal indication of its original location, such as an epoxide, is present in all known natural cembranes, and is the only asymmetric marker which occurs universally in the cembranes known to date.

   We suggest that the skeleton be numbered in the direction which locates this key unsaturation at position 7 (rather than 8*), thereby according it priority over "accidental" oxygen substituents. Next, we propose the designation of absolute configurations as α or β, depending upon the downward or upward disposition, respectively, of the three carbon substituent at position 1, when the structures (as drawn herein) are oriented 1) with position 1 at the viewer's right, and 2) with the reference unsaturation, Δ^7, situated above the 1,8-axis of skeletal symmetry.

   *This alternate numbering, employed in our earlier publications (cf. references 4 and 7), is herewith abandoned.

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