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CHEMICAL STUDIES OF MARINE INVERTEBRATES—XI[1]

SINULARIOLIDE, A NEW CEMBRANOLIDE DITERPENE FROM THE SOFT CORAL *SINULARIA FLEXIBILIS* (COELENTERATA, OCTOCORALLIA, ALCYONACEA)

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Abstract—Sinulariolide, a novel diterpene lactone has been isolated from the alcyonarian *Sinularia flexibilis*. Its structure [**1**] has been determined by using spectroscopic and chemical methods. Its relative and absolute configuration was established by X-ray diffraction as depicted in **10**.

The presence of terpenoids in some Anthozoan Coelenterates is well documented.[2] Gorgonians (sea fans), which have a world wide distribution, but are particularly abundant in the shallow water of the Caribbean region, have yielded a large number of sesquiterpenes and a variety of diterpenes[2] which are all lactones derived from the cembrane skeleton, with the exception of eunicellin,[3] which however could be considered as a cembrane having undergone further cyclisation.

Little attention has until now been paid to the chemical examination of another very important group of Anthozoans: the Alcyonarians (soft corals), which are most abundant in the Indo-Pacific region, where they often constitute a dominant part of the reef biomass. The isolation of novel sesquiterpenes from the Alcyonarians *Lemnalia africana*[4] and *Capnella imbricata*[5] and of cembrane diterpenes from *Lobophytum cristagalli*[1] and *Nephtea sp*[6] has recently been reported. We wish to report here on the structure of a new cembranolide isolated from the soft coral *Sinularia flexibilis* (Quoy and Gaimard, 1833).

Sun-dried specimens of *S. flexibilis*, collected off Serwaru (Leti Island) and Pantai Wonreli (Kissar Island), Province of Maluku, Indonesia,[7] were defatted with hexane, then extracted with methylene chloride. Direct crystallization of the evaporated extract from ether, followed by silicagel column chromatography and final recrystallization from benzene afforded sinulariolide (**1**, $C_{20}H_{30}O_4$).

The spectral properties of **1** suggest the presence of a Me group on a trisubstituted double bond (broad singlet of 3H at 1·63 ppm, and multiplet of 1H at 5·20 ppm), two Me groups attached to quaternary C atoms, probably vicinal to O atoms (two singlets of 3H each, at 1·35 and 1·22 ppm), an exomethylene double bond conjugated to a lactone group (λ_{max} 212 nm (ϵ 6000), $\nu_{C=C}$ at 1630 cm^{-1} and $\nu_{C=O}$ at 1720 cm^{-1}, two singlets of 1H each at 6·3 and 5·5 ppm, respectively)[8] and only one OH function which is secondary (ν_{OH} at 3460 cm^{-1}; multiplet of 1H at 4·15 ppm shifted to 5·3 ppm in the spectrum of the monoacetate **2**, $C_{22}H_{32}O_5$).

The ^{13}C NMR spectrum[*] of **1** confirms the proposed double bond pattern (two C=, respectively at 67·1 and 57·6 ppm; one HC= at 49·2 ppm and one H_2C= at 46·7 ppm) and indicated the presence of only one CO group (91·6 ppm) which must belong to the lactone. Elimination of alternative possibilities leads to the attribution of the fourth O atom to an ether function.

Compound **1** is sensitive to acids, for instance hydroiodic acid, affording an isomer **3** ($C_{20}H_{30}O_4$), containing two Me groups on trisubstituted double bonds (two broad singlets of 3H each, at 1·67 and 1·53 ppm, and a multiplet of 2H at 5·3 ppm), and two secondary alcohols (two signals of 1H each, at 4·20 and 3·80 ppm respectively) as confirmed by acetylation to a diacetate (**4**, $C_{24}H_{34}O_6$). In contrast to **1**, **3** could be oxidized by MnO_2 into an α,β-unsaturated ketone (**5**, $C_{20}H_{28}O_4$), indicating that **3** possesses an allylic alcohol generated by the opening of an epoxide ring. It follows that sinulariolide is necessarily mono-carbocyclic, since the ethereal O atom of **1** is part of an epoxide.

The presence of a lactone ring was further substantiated by treatment of **3** with NaOH/MeOH, affording the methyl ester **6** ($C_{21}H_{34}O_5$). Furthermore, treatment of **3** by $NaBH_4$ yielded compound **7** ($C_{20}H_{32}O_4$), by selective reduction of the conjugated exomethylene function, as evidenced by the disappearance of the singlets at 6·22 ppm and 5·48 ppm, in the NMR spectrum, and of the

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[*] 22·63 MHz, $CDCl_3$, positive values in ppm are downfield from internal $CDCl_3$.

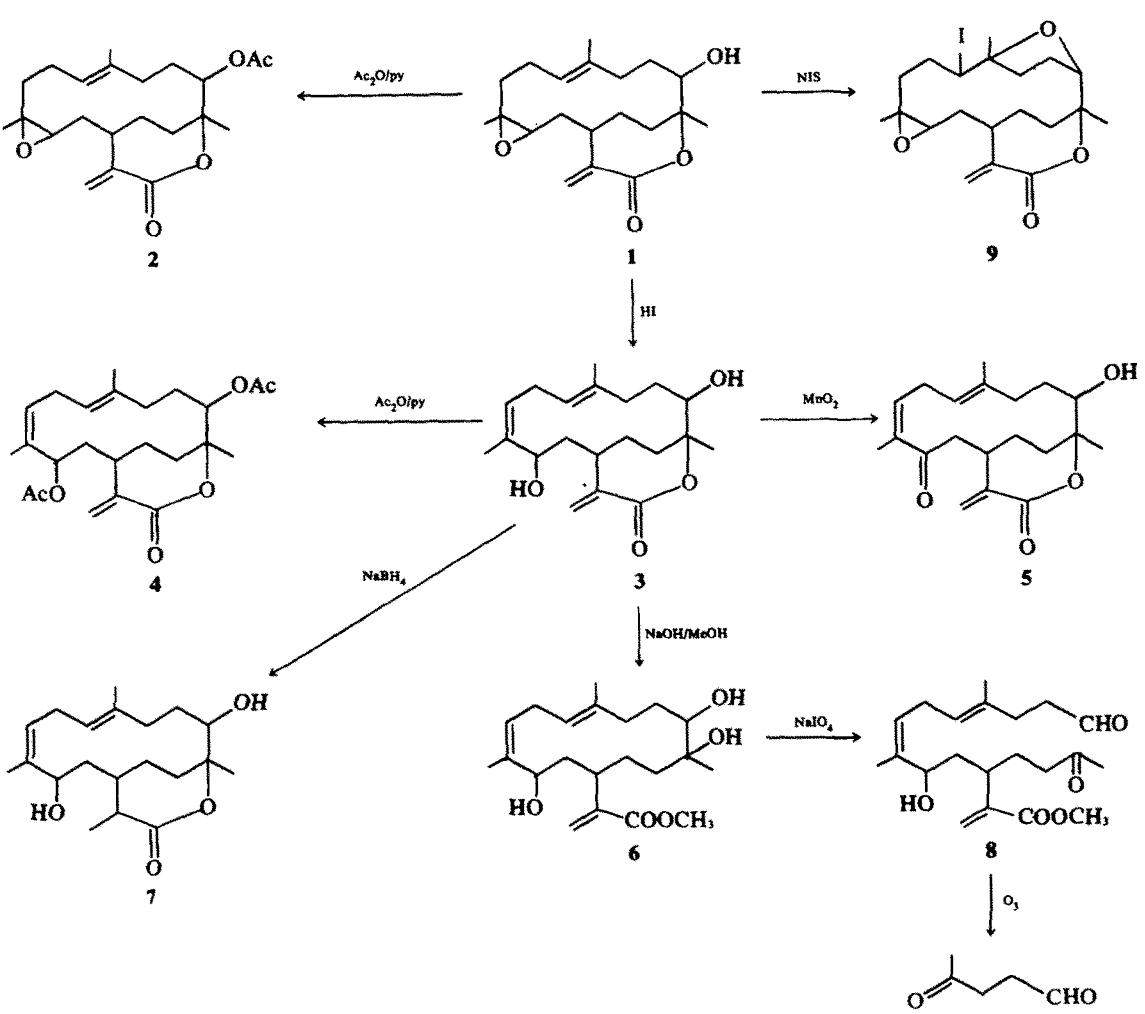


Fig. 1.

C═C stretching band at 1630 cm^{-1}, in the IR spectrum. This type of reduction happens when a double bond is conjugated to a CO group and proceeds through a cyclic intermediate which is favored by a cisoid unsaturated carbonyl system.[9] The lactone must terminate on a quaternary C atom (thus bearing a Me group), since any other situation would imply the presence of a supplementary deshielded proton in the NMR spectrum of sinulariolide (1).

Methyl ester 6 reacted with sodium metaperiodate to give ketoaldehyde 8 ($C_{21}H_{32}O_5$). This strongly suggests that the tertiary alcohol, generated by the opening of the

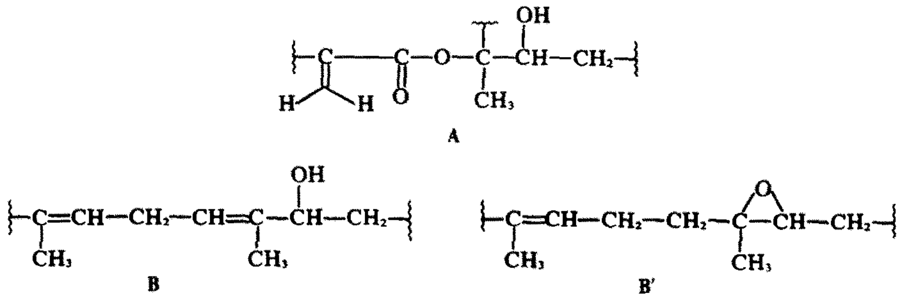


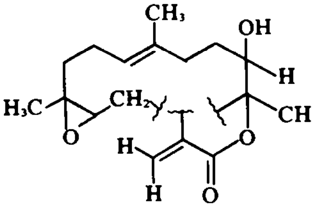
Fig 2.

lactone, is adjacent to the secondary alcohol initially present in sinulariolide. Indeed, if the allylic alcohol was implied in the reaction, one would obtain an unsaturated aldehyde.

The relationship between the epoxide and the trisubstituted double bond of sinulariolide appears in the 270 MHz spectrum of compound **3** which clearly presents two deshielded protons at 3·15 ppm (1H, m) and 2·40 ppm (1H, m) respectively, attributed to a methylene group located between two double bonds. Irradiation at 3·15 ppm caused a simultaneous collapse of the multiplets at 5·35 ppm (two vinylic protons) and 2·40 ppm, and inversely, irradiation at 5·35 ppm changed the multiplicity of the signals at 3·15 ppm and 2·40 ppm.

All these arguments indicate the partial sequences A and B in **3** and A and B′ in sinulariolide.

Ozonolysis of **8** yielded laevulinaldehyde, detected in GC by comparison with an authentic sample, thus extending sequences A and B′ to C, compatible with a cembrane skeleton.

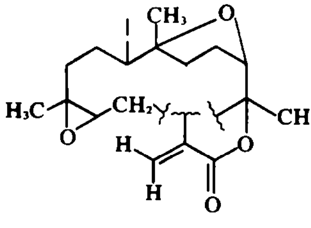


C

Fig 3.

Treatment of **1** by N-iodosuccinimide afforded compound **9** ($C_{20}H_{29}O_4I$) devoid of trisubstituted double bond and of ν_{OH} absorption, whose spectral properties suggest the partial structure D.

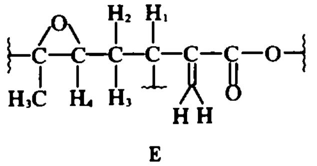
An analogous bromo-compound was also formed by treatment of **1** with N-bromosuccinimide, such cyclisation with electrophilic agents being well documented.[10] Compound **9** is remarkable because the introduction of the I atom causes the signal of the hydrogen H_1 (Fig 5), presumably vicinal to the exomethylene, to appear clearly at 2·41 ppm in the 270 MHz NMR spectrum and induces a downfield shift of the absorption of one additional proton H_2, to a value of 3·18 ppm. Double irradiation experiments have permitted to locate a proton H_3, geminal to H_2, at 2·02 ppm and have proved that H_1 and the hydrogen of the epoxide ring H_4 are both vicinal to H_2—C—H_3. Another partial structure would thus be E.



D

Fig 4.

Combination of the latter with partial structure C leads to the complete formulation of sinulariolide as the cembranolide **1**, since there remains no alternative for the positioning of the still missing C_2H_4 fragment.



E

Fig 5.

Confirmation of the proposed planar structure as well as determination of the relative and absolute configuration was achieved independently by X-ray diffraction analysis* (sinulariolide crystallised in the orthorhombic system, space group $P2_12_12_1$ with a = 19·84 Å, b = 11·33 Å, c = 8·94 Å; Z = 4) leading to complete structure **10**.

In addition to sinulariolide, *Sinularia flexibilis* contains minor related companion diterpenes, whose structures are presently being investigated.

EXPERIMENTAL

The UV spectra (in 99·7% MeOH) were recorded with UNICAM SP 800 spectrophotometer and the IR spectra with UNICAM SP 1000 spectrophotometer. ^1H NMR spectra were recorded at 60 MHz with a Varian T 60 or at 270 MHz with a Bruker HFX-270 instrument, chemical shifts are in ppm relative to internal TMS. Spin decoupling experiments were performed at 270 MHz. The ^{13}C NMR spectrum has been recorded with a Bruker HX-90 instrument. Rotations were determined with a PERKIN-ELMER 141 automatic polarimeter. Mass spectra were measured on a HITACHI PERKIN-ELMER RMU-6D spectrometer. GC analyses were effected on a HEWLETT PACKARD 402 instrument (FID).

Isolation of sinulariolide (**1**). Sun-dried specimens of *Sinularia flexibilis* (7 Kg) were defatted with hexane, then extracted with methylene chloride. Direct crystallization from ether of the methylene chloride extract afforded crude **1**, which was then separated from dyes and from a remaining minor diterpene (500 mg) by silicagel column chromatography (elution with hexane-acetone 9:1). Recrystallization from benzene gave **1** (80 g); 1·1% on the basis of dry starting material; $C_{20}H_{30}O_4$; M^+ 334; m.p. 170–173°; $[\alpha]_D^{MeOH}$: +76° (c = 0·7); IR (film): ν_{OH} 3460 cm^{-1}, $\nu_{C=O}$ 1720 cm^{-1}, $\nu_{C=C}$ 1630 cm^{-1}; UV (MeOH); λ_{max} 212 nm (ϵ 6000); NMR: 6·30 ppm (1H, s), 5·50 ppm (1H, s) 5·20 ppm (1H, m), 4·15 ppm (1H, m), 3·00 ppm (1H, dd), 1·63 ppm (3H, broad s), 1·35 ppm (3H, s), 1·22 ppm (3H, s). Found: C, 71·58; H, 9·24. Calcd. for $C_{20}H_{30}O_4$: C, 71·82; H, 9·04.

Acetylation of **1**. A solution of **1** (50 mg) in pyridine (3 ml) and Ac_2O (3 ml) was kept at room temp for 20 hr and the excess anhydride then destroyed by addition of water. Extraction with chloroform and evaporation gave 53 mg (yield: 95%) of **2** ($C_{22}H_{32}O_5$; MS: *m/e* 376 (M^+), 361 (M—CH_3), 334 (M—CH_2CO),

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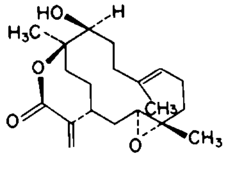
*A full paper on the X-ray work will appear shortly.



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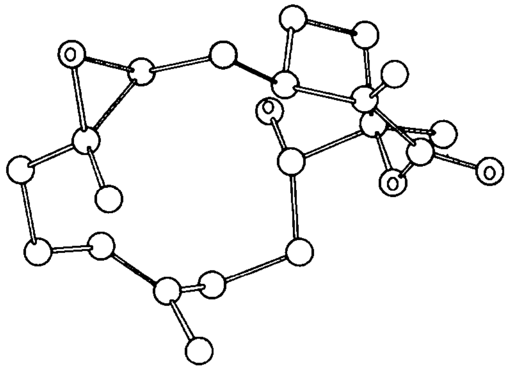

Fig 6.

316 (M—CH_3COOH); IR (film: no ν_{OH}, $\nu_{C=O}$ 1740 and 1720 cm^{-1}, $\nu_{C=C}$ 1630 cm^{-1}; NMR: 6·35 ppm (1H, s), 5·60 ppm (1H, m), 5·57 ppm (1H, s), 5·30 ppm (1H, m), 3·10 ppm (1H, dd), 2·12 ppm (3H, s), 1·62 ppm (3H, broad s), 1·40 ppm (3H, s), 1·20 ppm (3H, s).

Treatment of **1** *with hydroiodic acid.* A 6% aqueous solution of HI (1·5 ml) was added to a soln of **1** (200 mg) in chloroform (10 ml). The mixture was stirred and warmed at 50° for 3 hrs. The chloroform layer was then decanted, washed first with a 10% $NaHCO_3$, then with a soln of sodium metabisulfite and finally with water. The organic layer was dried on $MgSO_4$, filtered and evaporated. After silicagel column chromatography of the residue, crystallization from hexane-acetone gave 140 mg (yield: 70%) of **3** ($C_{20}H_{30}O_4$; M$^+$ 334; m.p. 191–195°; IR (KBr): ν_{OH} 3440 cm^{-1}, $\nu_{C=O}$ 1692 cm^{-1}, $\nu_{C=C}$ 1630 cm^{-1}; UV (MeOH): λ_{max} 213 nm (ϵ 6300); NMR (270 MHz): 6·22 ppm (1H, s), 5·48 ppm (1H, s), 5·35 ppm (2H, m), 4·20 ppm (1H, dd), 3·80 ppm (1H, d), 3·15 ppm (1H, m), 2·40 ppm (1H, m), 1·67 ppm (3H, broad s), 1·53 ppm (3H, broad s), 1·27 ppm (3H, s).

Acetylation of **3**. **3** (2 mg) dissolved in pyridine (0·5 ml) and Ac_2O (0·5 ml) was kept at room temp for 15 hr and the excess anhydride destroyed by addition of water. Extraction with chloroform gave **4** ($C_{24}H_{34}O_6$; MS: *m/e* 418 (M$^+$), 376 (M—CH_2-CO), 358 (M—CH_3COOH), 316 (M—CH_3COOH—CH_2CO), 298 (M—2CH_3COOH).

MnO_2 *oxidation of* **3**. A soln of **3** (40 mg) in chloroform (25 ml) was stirred at room temp in the presence of 4 g of MnO_2. After 3 days, the mixture was filtered and the products separated by silicagel column chromatography. Elution with hexane-acetone 8:2 gave 7 mg (yield: 18%) of **5** ($C_{20}H_{28}O_4$; M$^+$ 332; IR (film): ν_{OH} 3500 cm^{-1}, $\nu_{C=O}$ 1710 and 1692 cm^{-1}, $\nu_{C=C}$ 1665 and 1632 cm^{-1}; UV (MeOH): λ_{max} 218 and 236 nm; NMR: 6·45 ppm (1H, m), 6·40 ppm (1H, s), 5·60 ppm (1H, s), 5·40 ppm (1H, m), 3·95 ppm (1H, m), from 3·15 to 2·75 ppm (2H, m), 1·80 ppm (3H, broad s), 1·60 ppm (3H, broad s), 1·30 ppm (3H, s).

Methyl ester **6**. **3** (60 mg) was dissolved in 5 ml of 1% methanolic NaOH, and kept at 0° for 5 hrs. After addition of 20 ml water, evaporation of the MeOH, and neutralisation with HBr, the aqueous layer was extracted with chloroform. The organic layer was washed with water, dried on $MgSO_4$ and evaporated. Purification by silicagel column chromatography gave 57 mg (yield: 87%) of **6** ($C_{21}H_{34}O_5$; MS: *m/e* 348 (MH_2O); IR (film): ν_OH 3420 cm^{-1}, $\nu_{C=O}$ 1720 cm^{-1}, $\nu_{C=C}$ 1630 cm^{-1}, ν_{C-O} 1260 cm^{-1}; UV (MeOH): λ_{max} 213 nm (ϵ 6300); NMR: 6·22 ppm (1H, s), 5·50 ppm (1H, s), from 5·50 to 5·00 ppm (2H, m), 4·10 ppm (1H, m), 3·70 ppm (3H, s), 3·35 ppm (1H, m), 1·67 ppm (3H, broad s), 1·63 ppm (3H, broad s), 1·10 ppm (3H, s).

$NaBH_4$ *reduction of* **3**. A soln of **3** (60 mg) in THF (5 ml) was treated with $NaBH_4$ (1 g) this reagent being dissolved by dropwise addition of water. The mixture was kept at room temp for 30 min, and the excess $NaBH_4$ destroyed by addition of acetone. After evaporation, the product was extracted with chloroform and the organic layer washed with 5% HCl and finally with water. Purification by silicagel column chromatography and crystallization from hexane-acetone afforded 45 mg (yield: 74·5%) of **7** ($C_{20}H_{32}O_4$); M$^+$ 336; m.p. 184–187°; IR (film): ν_{OH} 3380 cm^{-1}, $\nu_{C=O}$ 1740 cm^{-1}; NMR: from 5·70 to 5·20 ppm (2H, m), 4·15 ppm (1H, m), 3·85 ppm (1H, m), 1·68 ppm (3H, broad s), 1·60 ppm (3H, broad s), 1·27 ppm (3H, s), 1·25 ppm (3H, d, J = 7 cs).

Metaperiodate cleavage of methyl ester **6**. $NaIO_4$ (50 mg) were added to a soln of **6** (40 mg) in 40 ml MeOH—H_2O (1:1). The reaction was followed by TLC. The reaction was stopped after 2 hr because of the appearance of undesirable side-products. The MeOH was then evaporated, and the aqueous soln extracted with chloroform. Separation of the excess of starting product **6** was effected by silicagel column chromatography. This afforded 4 mg (yield: 10%) of **8** ($C_{21}H_{32}O_5$; M$^+$ 364; IR (film): ν_{OH} 3480 cm^{-1}, ν_{CH} 2720 cm^{-1}, $\nu_{C=O}$ 1725 and 1715 cm^{-1}, $\nu_{C=C}$ 1630 cm^{-1}; UV (MeOH): λ_{max} 212 nm.

Ozonolysis of keto-aldehyde **8**.[11] **8** was treated with O_3 at −70° in 5 ml anhydrous EtOAc for 45 min. After treatment with a little triphenylphosphine, the ozonolysis mixture was analysed by GC (10% Carbowax 20 M, 6 feet, 108°). The most intense peak was assigned to laevulinaldehyde, by comparison with an authentic sample, obtained by ozonolysis of farnesol, in the same condition.

Reaction of **1** *with* N-*iodosuccinimide*. **1** (50 mg) dissolved in 10 ml chloroform, was treated with 34 mg (1 eq) of freshly prepared N-iodosuccinimide[12] for 30 min. The chloroform layer was then washed with water, and the product separated from the remaining succinimide by filtration on silicagel. Evaporation of the chloroform and recrystallization from hexane-acetone gave 63 mg (yield: 91·5%) of **9** ($C_{20}H_{29}O_4I$; m.p. 143–145°; the mass spectrum showed the molecular ion at *m/e* 460 and three intense peaks at *m/e* 127 (I), 128 (HI) and 254 (I_2); IR (film): no ν_{OH}, $\nu_{C=O}$ 1720 cm^{-1}, $\nu_{C=C}$ 1635 cm^{-1}; NMR (270 MHz): 6·23 ppm (1H, s), 5·40 ppm (1H, s), 4·46 ppm (1H, dd), 4·00 ppm (1H, dd), 3·18 ppm (1H, m), 3·08 ppm (1H, dd), 2·41 ppm (1H, eight lines signal), 1·37 ppm (3H, s), 1·23 ppm (6H, s).

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