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NORCEMBRENE DITERPENOIDS FROM PACIFIC SOFT-CORALS OF THE GENUS SINULARIA (ALCYONACEA; OCTOCORALLIA)

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Abstract: Four new norcembrene diterpenoids, 2-5, have been isolated from several species of the soft-coral Sinularia collected in Palau, Western Caroline Islands. These new compounds are closely related to a unique norcembrenolide, 1, previously reported from the Australian soft-coral Sinularia leptoclados. The structure assignments of these new compounds were provided by interpretation of spectral data, and the structures of norcembrenes 2 and 3 were confirmed by single crystal X-ray analysis.

Soft-corals (Octocorallia, Cnidaria) in general, but in particular those true soft-corals (Alcyonaceae) of the genera Sarcophyton, Lobophytum and Sinularia, have been found to be a rich source of diterpenoids of the cembrene class.[1,2] In 1978 Bowden et al. reported[3] the structure of a unique norcembrenolide derivative, 1, isolated from the Australian soft-coral Sinularia leptoclados. Since this first report, no other examples of this interesting C-4 nor ring system have been reported. In our studies of the alcyonacean corals from Palau, we have encountered several Sinularia species which produce 1, but also several closely related derivatives. We report here the isolation and structure elucidation of four new modifications of this interesting norcembrene class.

Four different Sinularia species, S. querciformis (Pratt), S. inelegans Tixier-Durivault, S. numerosa Tixier-Durivault, and an apparently new Sinularia species (PSC-159), were collected in numerous habitats within the Archipelago of Palau. Conventional chloroform extraction, rapid elution silica chromatography, and finally HPLC purification, showed S. querciformis to contain norcembrenes 1 and 5. Similarly, S. inelegans was found to contain compounds 3 and 4, S. numerosa 1 and 2, and the unknown Sinularia species contained only norcembrene 2.

The structure elucidations of the new compounds 2-5 relied heavily upon comparisons of their ^1H NMR characteristics with those of norcembrenolide 1. The structure of 1 had been confidently assigned earlier using X-ray crystallographic methods (relative stereochemistry only). However, for positive identification and for an effective comparison with 2-5, we performed extensive ^1H NMR experiments (360 MHz) with compound 1, which resulted in the complete assignment of all proton resonances (see NMR Table).

A new norcembrenolide, 2, mp 138-140° was isolated from S. numerosa. This compound analyzed for $C_{19}H_{24}O_5$ by combined spectral methods, and was thus recognized as a deoxy derivative of 1. As in the case of 1, the infrared spectrum of this metabolite contained three carbonyl absorptions at 1767, 1740 and 1700 cm^{-1}. These latter bands were assigned to the lactone carbonyl (C-19), the oxycyclopentanone carbonyl (C-6) and the C-3 ketone constellation, respectively. The conspicuous hydroxyl absorptions of 1 were lacking in this compound. As in 1, the ^1H NMR bands for 2 could be readily interpreted by decoupling methods and all protons were subsequently assigned. The differences between 1 and 2 were established by these methods to be in the lactone portion of the molecule. The low-field polarized olefin proton (assigned as C-11) at δ 6.68 was coupled by the expected small J value of 1.3 Hz with the C-10 lactone proton. Since all other proton resonances were highly analogous to 1, this compound was assigned as the C-11 deoxy analog. While it was tempting to assign the same relative stereochemistry to 2 as observed in 1, it was felt that this

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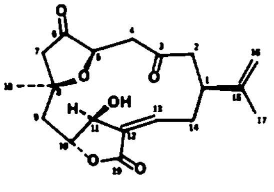

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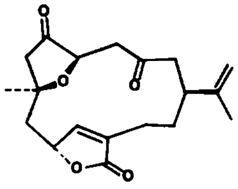

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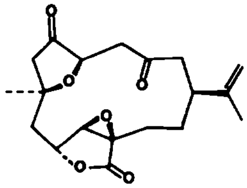

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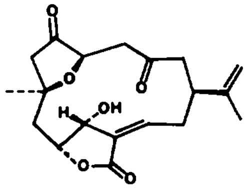

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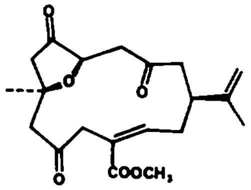

5

ring system was not sufficiently well known to warrant such extrapolations. Hence, confirmation of the gross structure and of the relative stereochemical features of 2 was sought by X-ray crystallographic methods.

Figure 1 shows the computer generated perspective drawing, derived from the successful X-ray experiment, which fully confirmed the structure of 2 as proposed. The X-ray data confirmed the relative stereochemistry but did not define the absolute configuration. The unsaturated γ-lactone was found to be totally planar and the oxycyclopentanone ring can be described as an envelope with the oxygen atom as the flap.

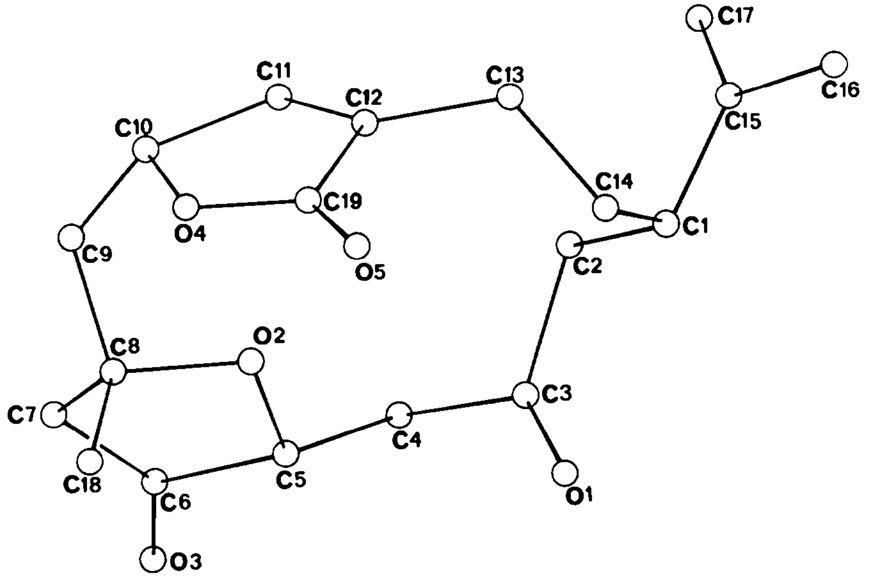

Figure 1. A computer generated perspective drawing of *Sinularia* norcembrenolide 2. Absolute stereochemistry was not defined in the x-ray experiment and the enantiomer shown is an arbitrary choice.

The soft coral S. inelegans was found to contain two new crystalline cembrenolides, 3 and 4, both of which were recognized as being closely related to 1. The epoxylactone 3, a minor metabolite, analyzed for $C_{19}H_{24}O_6$ by high resolution mass spectrometry, and showed infrared absorptions characteristic of the three carbonyl groups consistently encountered in this ring system (1770, 1760 and 1710 cm^{-1}). Although 3 was isomeric with 1, infrared and NMR features clearly showed it to lack the hydroxyl group at C-11 and the unsaturation at C-12-C-13. Instead, spectral features for the uncommon α,β-epoxy-γ-lactone[4] were apparent. The proton NMR features of 3 were highly comparable with 1, and all protons were readily assigned via decoupling methods (NMR Table). Although the structure assignment of 3 appeared straightforward, with 1 as a convincing comparison, ^{13}C NMR data could not be obtained (due to insolubility and decomposition) to confirm this assignment. Hence, the structure of norcembrenolide 3 was also confirmed by X-ray crystallography.

Figure 2 illustrates the computer generated drawing for the final model of 3 with relative stereochemistry only. The relative stereochemistry at all comparable centers was found to be identical to norcembrene 1.

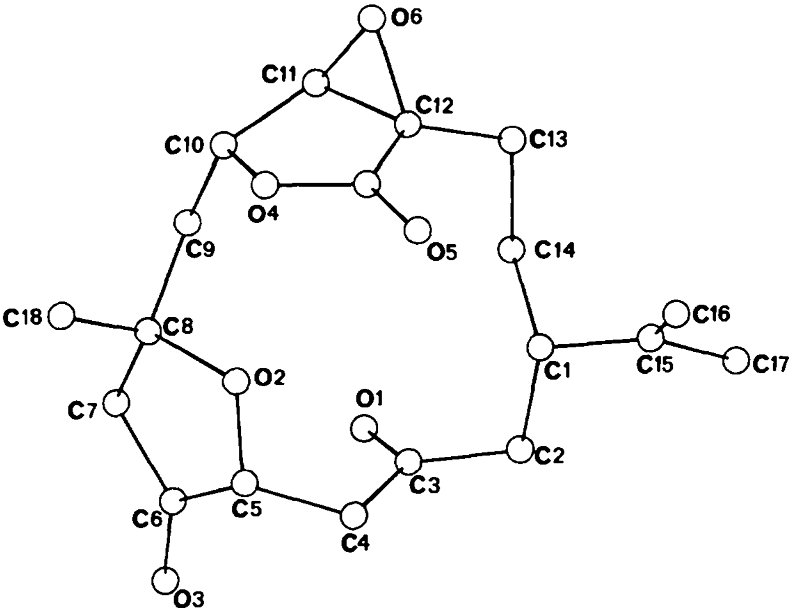

Figure 2. A computer generated perspective drawing of Sinularia norcembrenolide 3. Absolute stereochemistry was not defined in the x-ray experiment and the enantiomer shown is an arbitrary choice.

Norcembrenolide 4, mp. 196-7°, analyzed for $C_{19}H_{24}O_6$ by high resolution mass spectrometry and was, therefore, also isomeric with norcembrene 1. The infrared features of 4 were identical to those of 1 showing the presence of three carbonyl groups as well as a hydroxyl functionality (see experimental). The ^{1}H NMR features of 4 were virtually superimposable with those of 1 except for one minor but highly significant variation. Instead of the 2 Hz coupling between the alcohol and lactone methine protons (C-10-C-11) as observed in 1, this metabolite illustrated virtually no coupling. Since all other couplings and chemical shifts in the spectrum of 4 were virtually identical with those of 1, this was interpreted to suggest that 4 possessed the C-11 epi configuration.

The norcembrene derivative 5, mp. 183-5°, was isolated as a minor metabolite, along with 1, from S. queroiformis. This metabolite analyzed for $C_{20}H_{26}O_6$ by combined spectral methods (HMRS + ^{13}C NMR) and showed a complex infrared spectrum which possessed multiple overlapping carbonyl absorptions (ca. 1675-1730 cm^{-1}, very broad) but which lacked absorptions characteristic of hydroxyl. ^{13}C NMR data greatly aided the interpretation of these latter bands by showing the presence of 4 discrete carbonyl resonances. Three ketone bands were observed as off-resonance singlets at 206.1, 206.6 and 214.8 ppm, along with a methyl ester carbonyl at 167.4 ppm (accompanied by an O-methyl quartet at 51.9 ppm). The presence of a polarized double bond [128.1 (s) and 140.2 (d)], and intense UV end absorption, placed the ester at C-19 and the unsaturation at C-12, C-13. Proton NMR data were useful in confirming this latter assignment. The polarized (low field) proton at

C-13 was resolved at δ 6.76 (dd) and found to be coupled, as in 1, to a methylene pair at C-14. In consistent fashion, the C-14 protons were easily correlated, by decoupling experiments, to the remaining protons on the C-1-C-2 and C-1-C-16-C-17 spin systems (C-1 to C-16 and C-16 to C-17 by unmeasurable but very reliable allylic coupling).

The third carbonyl functionality in 5 could be placed at C-10 by simple evaluation of the remaining ^{1}H NMR features of this metabolite. In addition to the AB double doublet generated from the isolated C-7 protons in these five molecules, metabolite 5 possessed two additional AB double doublets at δ 2.50 and 3.02 (J = 13 Hz), and δ 3.52 and 3.67 (J = 18.6 Hz), respectively. Since all of the remaining carbons in 5 had already been assigned based upon their similarities to 1, the remaining carbonyl must be positioned at C-10. Although on somewhat tenuous grounds (favorable carbon and proton NMR comparison) the relative stereochemistries at C-1, C-5 and C-8 were assigned as identical to metabolites 1-4.

Experimental

General

Infrared spectra were recorded on a Perkin-Elmer model 137 spectrophotometer and ultraviolet spectra were obtained on a Beckman Acta XIV spectrophotometer. Proton NMR spectra were obtained, in the indicated solvents, on a 360 MHz FT-NMR system consisting of an Oxford narrow-bore magnet interfaced with a Nicolet 1180E Fourier transform data system. Carbon NMR data were obtained at 50 MHz on a Nicolet NT-200 wide-bore spectrometer. High resolution mass measurements were provided by the University of California Mass Spectrometry Resource Center, University of California, San Francisco. Optical rotations were measured on a Perkin-Elmer model 141 polarimeter using a 10 cm microcell. Melting points were determined using a Fisher-Johns apparatus and are reported uncorrected.

Collection and Extraction of Sinularia species.

Collection of *Sinularia querciformis* (collection code number PSC-30), a new *Sinularia* sp. (PSC-159), *S. minima* (PSC-171), *S. numerosa* (PSC-187), and *S. inelegans* (PSC-217) were made using SCUBA in September 1979 within various shallow water habitats (~ -10m) of the Palau Archipelago (Western Caroline Islands). Voucher specimens have been placed on deposit at the Rijksmuseum Van Naturrlijke Historie, Lieden, The Netherlands, under the curation of Dr. J. Verseveldt. The freshly collected specimens (large individuals) were immediately frozen and stored at ca. -20°C until extraction. The samples were next chopped into small pieces, freeze-dried, and the dry animals were blended in $CHCl_3$. The filtered residues were re-extracted twice with the same solvent, all extracts were combined and the solvents were reduced *in vacuo* to leave dark gummy residues. In each case the $CHCl_3$ extractable materials represented between 7 and 10% of the dry weight of the animal. The crude extracts were next fractionated by conventional rapid elution silica column chromatography methods eluting with EtOAc/isooctane mixtures (0-100% EtOAc). Compounds 1-5 generally crystallized from the more polar column fractions (ca. 40-60% EtOAc/isooctane), or were purified by repeated silica column chromatography or by HPLC until sufficiently pure for further study.

Norcembrenolide 2

Norcembrenolide 2, as isolated from extracts of *S. numerosa* (PSC-187) and *Sinularia* (PSC-159), showed mp. 138-140° and $[\alpha]_D$ -47° (c 0.01, $CHCl_3$) and had the following spectral features; LRMS: M^+ = 332 ($C_{19}C_{24}O_5$) IR ($CHCl_3$): 1767, 1740, 1700 cm^{-1}; UV (EtOH); = only end absorption; ^{13}C NMR ($CDCl_3$ 50 MHz): δ 42.9 (C-1), 45.4 (C-2 or C-9), 212.9 (C-3), 46.5 (C-4), 78.4 (C-5 or C-10), 207.8 (C-6), 48.0 (C-7), 78.6 (C-8), 40.5 (C-9 or C-2), 74.7 (C-10 or C-5), 151.1 (C-11), 130.6 (C-12), 145.8 (C-15), 112.8 (C-16), 25.2 (C-17), 18.0 (C-18), 174.0 (C-19), 20.7, 27.5 (C-13, C-14).

Norcembrenolide 3

Norcembrenolide 3, as isolated from extracts of *S. inelegans* (PSC-217), showed mp. 176-7°, $[\alpha]_D$ -36° (c 0.009, $CHCl_3$) and had the following additional spectral features: HRMS: (m/z) 348.15696 obs M^+, calc. 348.15687 for $C_{19}H_{24}O_6$; IR ($CHCl_3$): 1770, 1760, 1710 cm^{-1}.

Norcembrenolide 4

Norcembrenolide 4, as isolated from extracts of *S. inelegans* (PSC-217), showed mp. 196-7°, $[\alpha]_D$ +3.8° (c 0.004, $CHCl_3$) and had the following spectral features: IR ($CHCl_3$): 3600, 3020, 2925, 1760 br, 1720, 1375, 1270, 1220, 1180, 1090 cm^{-1}.

Norcembrene 5

Norcembrene 5, as isolated from extracts of *S. querciformis* (PSC-30), showed mp 183-5°, $[\alpha]_D$ -77° (c 1.36, $CHCl_3$) and the following spectral features: HRMS (m/z): 362.17291 (M^+ for $C_{20}H_{26}O_6$, calc. 362.17295); IR ($CHCl_3$): 3020, 2950, 1780 br, 1720 br, 1440, 1290, 1095, 900 cm^{-1}; ^{13}C NMR ($CDCl_3$, 50 MHz): δ 39.6 (C-1), 214.8 (C-3), 75.8 (C-5), 206.6 (C-6 or C-10), 49.4 (C-7), 80.3 (C-8), 206.1 (C-10 or C-6), 128.1 (C-12), 140.2 (C-13 or C-15), 29.6 (C-14), 144.9 (C-15 or C-13), 112.0 (C-16), 28.7 (C-17), 21.8 (C-18), 167.4 (C-19), 51.9 (OMe), 45.7, 45.7, 45.5, 44.7

(all triplets for C-2, C-4, C-9, C-11).

X-Ray Structure Elucidation of Norcembrenolide 2

Preliminary X-ray photographs on the clear, colorless crystals of norcembrenolide 2 showed orthorhombic symmetry. Accurate lattice constants of a = 6.049(1), b = 11.889(4) and c = 24.212(7)Å were determined from a least-squares fit of fifteen diffractometer measured 2θ-values. The structure was analyzed in space group $P2_12_12_1$ with one molecule of $C_{19}H_{24}O_5$ forming the asymmetric unit. All unique diffraction maxima with 2θ ≤ 114° were collected on a computer controlled four circle diffractometer with graphite monochromated CuKα x-rays (1.54178Å) and a variable speed, 1° ω-scan technique. After correction for Lorentz, polarization and background effects, 1150 (88%) of the 1420 reflections were judged observed ($I \geq 3\sigma(I)$) and used in subsequent refinements.[5] A phasing model was achieved using a multisolution weighted tangent formula approach and the resultant E-synthesis showed twenty-one of the twenty-four nonhydrogen atoms. The remaining nonhydrogen atoms were located on a subsequent F-synthesis and hydrogens were located on a difference F-synthesis following partial refinement. Block diagonal least squares refinement with anisotropic nonhydrogen atoms and isotropic hydrogens have converged to a standard crystallographic residual of 0.063 for the observed reflections. Additional crystallographic data, bond distances and angles are available for this compound at the Cambridge Crystallographic Data Centre.

X-Ray Structure Elucidation of Norcembrenolide 3

Preliminary X-ray photographs of norcembrenolide 3 showed monoclinic symmetry and accurate lattice constants of a = 6.496(1), b = 13.873(2), c = 10.085(1)Å and β = 98.48(1)° were determined from a least-squares fit of fifteen diffractometer measured 2θ-values. The structure was successfully analyzed in space group $P2_1$ with one molecule of composition $C_{19}H_{24}O_6$ forming the asymmetric unit. All unique diffraction maxima with 2θ ≤ 114° were collected on a computer controlled four-circle diffractometer using a variable speed 1° ω-scan and graphite monochromated CuKα x-rays (1.54178Å). After correction for Lorentz, polarization and background effects, 1205 (95%) were judged observed.[5] Phasing the data was done uneventfully with a multisolution weighted tangent formula approach. Twenty-four of the twenty-five nonydrogen atoms were located on the first E-synthesis. Hydrogens were located on a difference F-synthesis following partial refinement. Block diagonal least-squares refinement with anisotropic nonhdrogens and isotropic hydrogens have converged to a standard crystallographic residual of 0.068 for the observed reflections. Additional crystallographic data, bond distances and angles, are available for this compound from the Cambridge Crystallographic Data Centre.

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[6] We recently became aware of the presence, in the Chinese literature, of a report which describes a closely related compound from the South China Sea soft-coral Sinularia foeta; see Long Kanghou and Lin Yongcheng, Zhongshan Daxue Xuebao Xiran Kexueban, 1981, 98-104 (in Chinese). The compound described was either 1 or 4, but the stereochemistry of this compound was not provided.

^{1}H NMR Assignments for Norcembrenolides 1-5[a]

C#	1[b]			2[c]			3[d]			4[b]			5[b]		
	δ	m	(J; Hz)	δ	m	(J; Hz)	δ	m	(J; Hz)	δ	m	(J; Hz)	δ	m	(J; Hz)
1.	3.03	dddd	(3.5,5,6,11)	2.3	m		2.53	dddd	(3,8.1,11,11)	2.81	dddd	(3.4,3.2,8,11)	2.85	dddd	(3,4.8,4.8,12)
2.	2.53	dd	(3.5,15)	1.96	dd	(2.7,11)	1.96	dd	(11,11)	2.54	dd	(3.4,14)	2.38	dd	(3,15.4)
	2.60	dd	(11,15)	1.83	t	(11)	2.20	dd	(3,11)	2.57	dd	(11,14)	2.58	dd	(12,15.4)
4.	2.56	dd	(8.3,16)	2.19	dd	(3.5,15)	2.37	dd	(6.2,16)	2.57	dd	(9.5,15)	2.60	dd	(9.3,17)
	2.85	dd	(3,16)	2.46	dd	(8.7,15)	2.20	dd	(4,16)	2.62	dd	(3,15)	2.69	dd	(3.5,17)
5.	4.28	dd	(3,8.3)	3.59	dd	(3.5,8.7)	3.92	dd	(4,6.2)	4.28	dd	(3,9.5)	4.42	dd	(3.5,9.3)
7.	2.48	s		1.61	d	(17.6)	2.05	d	(18)	2.41	d	(17)	2.28	d	(18)
				1.71	d	(17.6)	2.20	d	(18)	2.54	d	(17)	3.45	d	(18)
9.	2.15	dd	(2,16)	1.30	dd	(4.7,14.9)	1.59	dd	(6.2,15)	2.05	d	(15)	2.50	d	(13)
	2.50	dd	(7,16)	1.55	dd	(6.5,14.9)	2.05	dd	(6.2,15)	2.40	dd	(7,15)	3.02	d	(13)
10.	4.63	ddd	(2,2,7)	4.28	ddd	(1.3,4.7,6.5)	4.28	dd	(6.2,6.2)	4.65	d	(7)	—		
11.	4.61	d	(2)	6.68	d	(1.3)	3.56	s		4.61	s		3.52	d	(18.6)
													3.64	d	(18.6)
13.	6.47	dd	(5,11)	1.98	ddd	(2.5,5.4,14.1)	1.64	ddd	(4,7.7,16)	6.53	dd	(6,11)	6.76	dd	(7.8,7.8)
				2.4	m		2.14	ddd	(3.5,9,16)						
14.	2.21	ddd	(5,5,15)	1.48	dddd	(2.5,5.4,9.9,14.1)	1.23	dddd	(4,8.1,9,19)	2.16	ddd	(6,8,14)	2.32	dd	(4.8,7.8)
	3.76	ddd	(6,11,15)	1.87	tt	(2.5,14.1)	1.48	dddd	(3.5,7.7,11,19)	3.71	ddd	(3.4,11,14)			
16.	4.81	s		4.61	s		4.78	s		4.80	s		4.72	s	
	4.89	s		4.70	s		4.84	s		4.86	s		4.96	s	
17.	1.82	s		1.39	s		1.53	s		1.83	s		1.80	s	
18.	1.53	s		0.72	s		1.12	s		1.49	s		1.38	s	
OMe													3.72	s	

a. Recorded at 360 MHz in the indicated (most optimum resolution) solvents. Assignments by single frequency decoupling and difference decoupling techniques.

b. $CDCl_3$ solution.

c. Benzene-d_6 solution.

d. 1/1 $CDCl_3$/benzene-d_6 solution.