

New Furanocembranoid Diterpenes from the Soft Coral *Sinularia maxima*[†]

Y. Venkateswarlu,* K. V. Sridevi, and M. Rama Rao

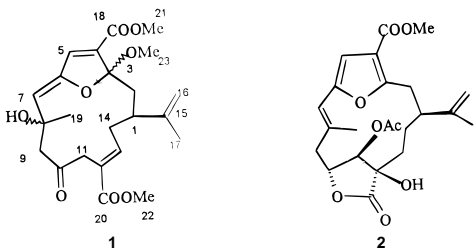
Natural Products Laboratory, Organic Chemistry Division-I, Indian Institute of Chemical Technology, Hyderabad, India, 500 007

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The soft coral *Sinularia maxima* yielded five terpenoids and one sterol, including two new furanocembranoid diterpenoids (**1** and **2**). The structures of these new compounds were determined by the interpretation of their spectral data.

Gorgonians and soft corals are two groups of coelenterates that belong to the orders Gorgonacea and Alcyonacea and are found in the coral-reef environment.¹ The ease of collection of these invertebrates has facilitated the extensive investigation of their secondary metabolites.² Sesquiterpenes and diterpenes having a wide variety of both well-known and rare carbon skeletons are the most common gorgonian and soft coral metabolites. Cembranoid diterpenes account for the majority of compounds reported thus far from both the orders of the subclass Alcyonaria. Among the soft corals, members of the genus *Sinularia* are prolific in nature, with 90 known species, of which about 35 species have been chemically examined.³ It was from *Sinularia abrupta* that the furanocembranoid diterpene pukalide was first reported in 1975,⁴ and since that time several related compounds such as 11 β ,12 β -epoxypukalide,⁵ 13 α -acetoxy-pukalide and 13 α -acetoxy-11 β ,12 β -epoxypukalide,⁶ lophotoxin⁷ and related compounds,⁸ and the bipinnatins⁹ and corralloides¹⁰ have been described. In our continuing interest in the bioactive secondary metabolites of soft coral species,^{11,12} we have examined *Sinularia maxima* Verseveldt, 1971 (Alcyoniidae) collected from the Mandapam coast during October 1997.

The 1:1 dichloromethane and methanol extract of *S. maxima* was subjected to gel filtration chromatography (Sephadex LH-20, 1:1 dichloromethane and methanol) followed by Si gel chromatography afforded the known sesquiterpene, $\Delta^{9(15)}$ africanene,^{13,14} the tetracyclic diterpene, isomandapamate,¹⁵ a norcembrene derivative,¹⁶ 24-methylenecholesterol,¹⁸ and two new furanocembranoid diterpenes, named sethukarailin (**1**) and sethukarailide (**2**).¹⁹



Compound **1** was obtained as pale yellow viscous liquid with $[\alpha]_D^{25} -23.4^\circ$ (*c* 0.25, CHCl₃) and analyzed for C₂₃H₃₀O₈ by HRFABMS. The UV maximum at 295 nm and IR bands at 3450, 1720, and 1680 cm⁻¹ indicated the presence of hydroxyl, unconjugated carbonyl, and dienoid

ester groups. The ¹³C NMR spectrum of sethukarailin (**1**) (Table 1) was typical of a furanocembranoid^{3–9} and indicated the presence of four double-bond carbons between δ 115 and 150, in addition to the presence of two ester carbonyls at δ 162.0 (s) and 167.3 (s), a ketone carbonyl at δ 210.6 (s), and a hemiacetal carbon at δ 115.9 (s). The ¹H NMR spectrum (Table 1) of compound **1** indicated the presence of two trisubstituted α,β -unsaturated double-bond protons at δ 6.99 (1H, t, *J* = 8 Hz) and 6.99 (1H, s), a trisubstituted-double bond proton at δ 5.16 (1H, s), an isopropenyl group [δ 4.58 (1H, br s), 4.73 (1H, br s), and 1.60 (3H, s)], and two isolated methylene protons [δ 3.87 (1H, d, *J* = 20 Hz) and 2.70 (1H, d, *J* = 20 Hz) and δ 3.38 (1H, d, *J* = 20 Hz) and 3.58 (1H, d, *J* = 20 Hz)], respectively. Further, its ¹H NMR spectrum also revealed the presence of two ester methyls at δ 3.80 (3H, s) and 3.75 (3H, s), a methoxyl group at δ 3.20 (3H, s), a tertiary methyl connected to an oxygen-bearing carbon at δ 1.38 (3H, s), and a D₂O-exchangeable proton at δ 4.26 (1H, br s).

In a series of decoupling experiments, irradiation of signals at δ 3.87 (1H, d, *J* = 20 Hz) and 3.38 (1H, d, *J* = 20 Hz) caused the collapse of the doublets at δ 2.70 and 3.58, respectively, to singlets, and further irradiation of the signals at δ 6.99 (t) and 1.80 (m) caused a change in the multiplicity pattern at δ 2.20 and 2.56, respectively, and established a connectivity between the C-13–C-14–C-1–C-2 protons.

In the NOESY spectrum (Table 1) of compound **1**, the C-1 methine proton showed correlations with H_a-2, H_a-14, and H_a-16, respectively. In turn, the H-7 vinylic proton at δ 5.16 correlated with H-13 at δ 6.99, the hydroxyl proton at δ 4.29, and CH₃-19 at δ 1.38. Further, the CH₃-19 protons showed a correlation with one of the C-9 methylene protons at δ 3.87. From the foregoing spectral data and a literature survey,^{3–9} the structure of this isolate was established as **1**. While studying the chemistry of lophotoxin,⁶ Bandurraga et al. observed the addition of methanol at C-3 with concomitant opening of the epoxide to obtain ketal analogues in the C-3 to C-8 region.¹⁷ Accordingly, compound **1** might be an artifact, resulting from methanol addition during extraction.

Compound **2** was obtained as colorless crystalline solid, mp 177–180 °C, $[\alpha]_D^{25} +2.6^\circ$ (*c* 0.5, CHCl₃) and analyzed for C₂₃H₂₈O₈ by HRFABMS, requiring 10 degrees of unsaturation. The UV maximum at 235 nm and IR bands at 3495, 1680, and 1755 cm⁻¹ indicated the presence of hydroxyl, α,β -unsaturated ester and γ -lactone carbonyl groups. The ¹H and ¹³C NMR spectral data (Table 1) of compound **2** showed close resemblances to those of known furanocembranoids.^{3–9} The ¹³C NMR spectrum (Table 1) of compound **2** contained signals for 23 carbons, of which

* To whom correspondence should be addressed. Tel.: (040) 7170512. Fax: +91-40-7173757/7173387. E-mail: root@csiict.ren.nic.in.

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Table 1. Spectral Data for Compounds **1** and **2**^a

position	compound 1			compound 2		
	δ_{H}	δ_{C}^b	NOESY	δ_{H}	δ_{C}^b	NOESY
1	1.80 br m	41.4 d	H _b -2, H _b -14, H _a -16	2.20 ^c	43.8 d	
2	Ha 2.56 dd (16,4)	33.2 t	H _a -14	3.45 dd (16, 8)		H _b -2
	H _b 2.20 m			2.72 dd (16, 4)	28.2 t	H _a -2
3		115.9 s			160.9 s	
4		131.2 s			116.0 s	
5	6.99 s	138.9 d	H-7	6.68 s	109.5 d	H-7
6		150.0 s			149.4 s	
7	5.16 s	116.9 d	H-5, H-19, OH	5.80 s	148.5 d	H-5, H-10 H-19
8		70.7 s			133.3 s	
9	H _a 3.87 d (20.0)	51.4 t	H-19, H _b -9	1.97 dd (16, 8)		H _b -9, H-11
	H _b 2.70 d (20.0)		H _a -9	2.60 dd (16, 4)	43.2 t	
10		210.6 s		4.95 br d	78.4 d	H _a -9, H-10
11	H _a 3.38 d (20.0)	40.9 t	H _b -11	5.60 s	75.7 d	H _b -9
	H _b 3.58 d (20.0)		H _a -11			H _a -9, H-19
12		128.1 s			72.4 s	
13	6.99 t (8.0)	143.3 d	H _a -14, H-19, OH	1.50 ^c		
				1.83 ^c	31.8 t	
14	H _a 2.08 dd (20,5)	39.8 t	H-1, H _a -2	2.10 ^c		
	H _b 2.20 m		H _a -2, H _b -11, H-13	2.20 ^c	21.5 t	
15		145.8 s			146.0 s	
16	H _a 4.58 br s	112.8 t		4.82 s	112.4 t	
	H _b 4.73 br s		H-17	4.86 br s		H-17
17	1.60 s	19.5 q	H _b -16	1.88 s	20.8 q	H _b -16
18		162.0 s			163.6 s	
19	1.38 s	27.3 q	H _b -9	1.50 s	18.9 q	H-7, H-11
20		167.3 s			173.3 s	
21	3.80 s	51.8 q		3.85 s	51.5 q	
22	3.75 s	52.2 q			169.4 s	
23	3.20 s	49.9 q		2.15 s	20.8 q	H _a -13

^a ¹H NMR (400 MHz and 200 MHz) and ¹³C NMR (50 MHz); δ in ppm, J in Hz. Spectra were recorded in CDCl₃ with TMS as internal standard. ^b Assignment of carbon types (s = C, d = CH, t = CH₂, q = CH₃) was made by DEPT. ^c Signal pattern unclear due to overlapping.

eight double-bond carbons resonated between δ 110 and 162, in addition to the presence of a methyl ester at δ 163.6 (s) and 51.5 (q), an acetoxy at δ 169.4 (s) and 20.8 (q), and a γ -lactone at δ 173.3 (s) and 78.4 (d). The ¹H NMR spectrum of compound **2** displayed signals for the presence of two trisubstituted double-bond protons at δ 6.68 (1H, s) and 5.80 (1H, s); an isopropenyl group at δ 4.82 (1H, s), 4.86 (1H, br s), and 1.88 (3H, s); and two methine proton signals at δ 5.60 (1H, s) and 4.95 (1H, br d), bearing an acetoxy group and a lactone oxygen, respectively. Further, its ¹H NMR spectrum showed a methylene proton multiplet at δ 3.45 (1H, dd, J = 16 Hz, 8 Hz) and 2.72 (1H, dd, J = 16 Hz, 4 Hz) and a vinylic methyl at δ 1.50 (3H, s).

The foregoing spectral data and a literature survey revealed that the structure of compound **2** is closely related to a furanocembranoid isolated from *Sinularia polydactyla*,⁵ except for the presence of a double bond between C-7, C-8 and a hydroxyl group at C-12 in compound **2** instead of C-7, C-8 epoxy and C-12, C-13 double-bond functionalities. The presence of the C-7, C-8 double bond in compound **2** caused a downfield chemical shift for H-5 by 0.48 ppm in its ¹H NMR spectrum as compared to that of the known furanocembranoid from *S. polydactyla*.⁵

The relative stereochemistry of the γ -lactone, acetoxy, and hydroxyl groups in **2** was established by NOESY experiments and by analogy with model compounds. Further, the magnitude of the coupling constant ($J_{10,11}$ = 0 Hz) suggested that H-10 and H-11 were disposed to each other with a dihedral angle of about 90°. This is only possible if

the protons are trans to one another. The structure proposed for sethukarailide (**2**) is in agreement with the observed spectral data.

Experimental Section

General Experimental Procedures. The melting point was determined on a Fisher–Johns instrument and is uncorrected. Optical rotations were measured on a JASCO DIP-370 polarimeter. UV and IR spectra were recorded on Shimadzu-240 and Perkin–Elmer 240-C instruments, respectively. The ¹H NMR (200 and 400 MHz) and ¹³C NMR (50 MHz) spectra were recorded on a Varian Gemini 400 MHz and a Varian Gemini 200 MHz spectrometer, respectively, using CDCl₃ as solvent and TMS as internal standard. Chemical shifts are reported in parts per million, and coupling constants (J) are expressed in Hertz. The MS were recorded on VG Autospec-M instrument.

Animal Material. The soft coral *Sinularia maxima* was collected on the Mandapam coast in the Gulf of Mannar, Tamilnadu, India, during October 1997, and a voucher specimen (IIC-260) is on deposit at the National Institute of Oceanography, Goa, India.

Extraction and Isolation. The freshly collected specimens were soaked in MeOH at the site of collection until workup. The soft coral *S. maxima* was freeze-dried (3 kg) and extracted with 1:1 CH₂Cl₂–MeOH (3 × 1.5 L) at room temperature. The combined extract was filtered, and the solvent was removed under reduced pressure to give a brownish green gummy mass (30 g). This crude extract (30 g) was subjected to gel filtration chromatography (Sephadex LH-20, 1:1 CH₂Cl₂–MeOH) fol-

lowed by silica gel chromatography using hexane, through hexane–EtOAc mixtures, to EtOAc, to yield $\Delta^9(15)$ africanene (1 g), 24-methylenecholesterol (100 mg), a norcembre derivative (60 mg), isomandapamate (180 mg), and compounds **1** (100 mg) and **2** (80 mg).

Compound 1: pale yellow, viscous liquid; $[\alpha]_D^{25} -23.4^\circ$ (c 0.25, CHCl_3); UV (MeOH) λ_{max} (ϵ) 295 (6945), 240 (7380) nm; IR (CHCl_3) ν_{max} 3450, 1720, 1680, 1195, 910, 710 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz), see Table 1; ^{13}C NMR (CDCl_3 , 50 MHz), see Table 1; EIMS (70 eV) m/z 434 [M^+]; HRFABMS m/z found 435.2029 [$\text{M}^+ + 1$], calcd for $\text{C}_{23}\text{H}_{30}\text{O}_8$ 435.2019 [$\text{M}^+ + 1$], R_f 0.50 (15% EtOAc in hexane).

Compound 2: colorless crystalline solid; mp 177–180 $^\circ\text{C}$; $[\alpha]_D^{25} +2.6^\circ$ (c 0.5, CHCl_3); UV (MeOH) λ_{max} (ϵ) 235 (5130), 250 (4680); IR (KBr) ν_{max} 3495, 2890, 1755, 1680, 1230, 1050, 885 cm^{-1} ; ^1H NMR (CDCl_3 , 200 MHz), see Table 1; ^{13}C NMR (CDCl_3 , 50 MHz), see Table 1; EIMS (70 eV) m/z 432 [M^+]; HRFABMS m/z found 433.1884 [$\text{M}^+ + 1$] calcd for $\text{C}_{23}\text{H}_{28}\text{O}_8$ 433.1874 [$\text{M}^+ + 1$], R_f 0.45 (20% EtOAc in hexane).

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