

Horiolide, a Novel Norditerpenoid from Indian Ocean Soft Coral of the Genus *Sinularia*

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A novel norditerpenoid, horiolide (**1**), has been isolated from an Indian Ocean soft coral of the genus *Sinularia*. The structural elucidation was achieved by a study of its spectral characteristics. This compound is structurally characterized by a new carbon skeleton having one six-membered cyclohexane ring bearing an isopropylene moiety, a carbonyl group, and one seven-membered ring attached to a five-membered lactone moiety.

Soft corals are a rich source of structurally unique and biologically active diterpenoids.¹ Among soft corals, for example, the genus *Sinularia* is especially known as a source of unconventional 18-norcembranolide derivatives such as ineleanolide (**2**), from the Formosan soft coral *Sinularia ineleigans*,² which does not have a C-18 carbon at C-4. In our continuing interest³ in bioactive secondary metabolites from marine organisms of the Indian Ocean, we have undertaken the chemical examination of *Sinularia* species⁴ collected at Hori Island, Diglipur, off the coasts of Andaman and Nicobar Islands. This paper presents the structure of a new type of norcembranolide, named horiolide (**1**), having a strained tetracyclic ring system.

Specimens of the soft coral were hand-picked from the intertidal zone in rocky areas of Hori Island, cut into thin slices, and preserved in ethanol. The ethanol-soluble materials were concentrated in vacuo, and the dark residue was re-extracted with ethyl acetate. The ethyl acetate-soluble material of the soft coral was chromatographed over a silica gel column, which on elution with hexane/ethyl acetate furnished horiolide as a colorless powder from methanol. The molecular formula was determined as C₁₉H₂₂O₅ by high-resolution EI mass spectrometry. The ¹H and ¹³C NMR data were obtained in DMSO-*d*₆ as solvent. The 19 carbon signals in the ¹³C NMR APT spectrum were analyzed on the basis of HMQC and ¹H–¹H COSY data as two methyls, four methylenes, eight methines, and five quaternary carbons, suggesting it to be a norditerpenoid. Four carbonyl functionalities were responsible for signals at δ 207.9, 205.1, 203.9, and 175.8, three of them accounting for ketone carbonyls and the remaining for an ester. Both methyl groups appear as singlets. Only one carbinolic methine proton was noticed in the ¹H NMR spectrum at δ 5.45, thus excluding ethers. As also a free OH group was absent, an ester or lactone was further confirmed.

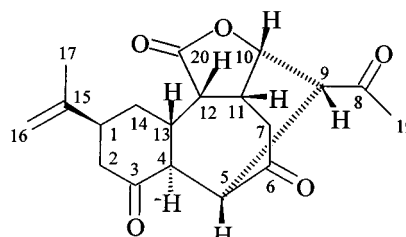
The double bond signals at δ 146.7 (s) and 111.9 (t) (δ _H 4.40, 4.75) and the presence of a singlet at δ 1.65 in the ¹H NMR spectrum of a quaternary methyl on a double bond accounted for an isopropenyl group, which was further confirmed by ¹H–¹H COSY and HMQC data (fragment A). Due to its shift (δ 2.18) and the HMBC couplings, the second methyl must belong to an acetyl group. Thus, five of the nine double-bond equivalents were assigned, suggesting the molecule to be tetracyclic.

The ¹H–¹H COSY spectrum (Table 1 and Figure 1) led to the fragments B and C, which together with A accounted

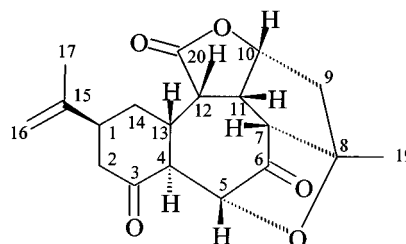
for 14 of the 19 carbons in the molecule. The four carbonyls and the acetyl methyl completed the C-count in the molecule.

The HMBC data (Table 1 and Figure 2) confirmed the correlations obtained by the ¹H–¹H COSY spectra and also consolidated the structure by linking up the three segments. The isopropenyl methyl (δ _H 1.65) showed correlations with C-1 (δ _C 39.7),⁵ whereas the 1-H (δ _H 2.70) coupled with C-2 (δ _C 43.1), C-14 (δ _C 30.6), and C-15 (δ _C 146.9), thereby proving the linkage between segment A and segment B across C-1–C-15.

As there was only one oxygen-bound methine group present, fragments B and C must overlap. This is further confirmed by cross signals between H-5 and 10-C. In addition to the expected couplings within the fragments themselves, the 10-H (δ _H 5.45) showed correlations with C-20 (δ _C 175.8); 5-H (δ _H 3.75) coupled with C-3 (δ _C 205.1), C-4 (δ _C 49.3), C-6 (δ _C 207.9), C-7 (δ _C 35.6), C-8 (δ _C 203.9), C-9 (δ _C 50.2), and C-13 (δ _C 34.6); 9-H (δ _H 3.42) showed correlations with C-4 (δ _C 49.3), C-6 (δ _C 207.9), C-8 (δ _C 203.9), and the methyl group C-19. From the foregoing and other HMBC correlations (Table 1 and Figure 2, structure D) as well as the COSY correlations, the structure of horiolide was fully established as **1**.



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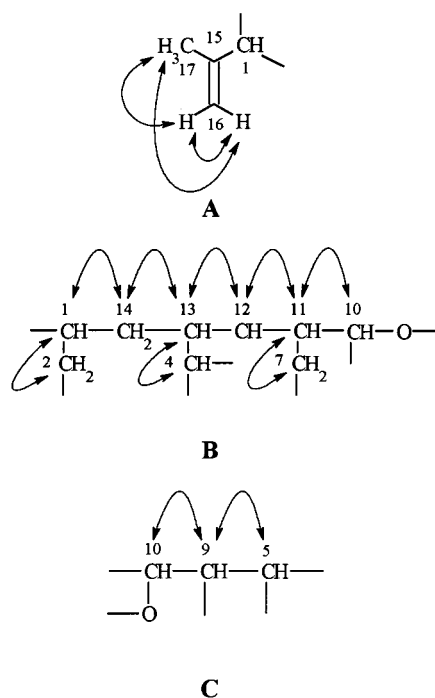
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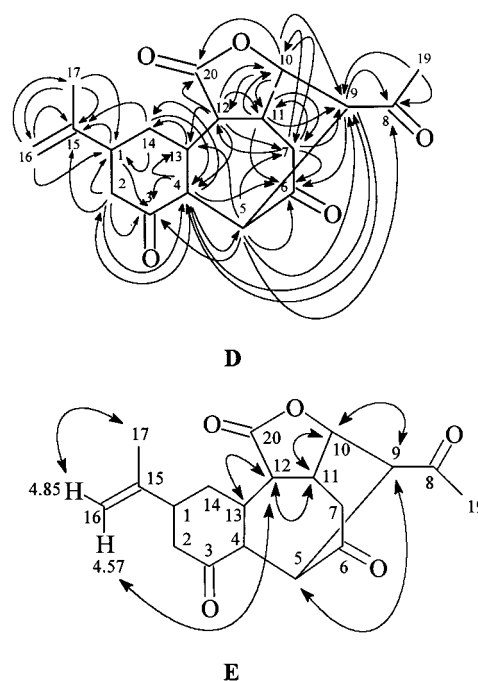
Table 1. ^1H , ^{13}C , ^1H - ^1H COSY, HMBC, and ^1H - ^1H NOESY Data of Horiolide (**1**)

carbon no.	$\delta^{13}\text{C}^a$	$\delta^{13}\text{C}^c$	$\delta^1\text{H}^b$	$\delta^1\text{H}^d$	^1H - ^1H COSY ^b	HMBC ^b	^1H - ^1H NOESY ^d
C-1	39.7	40.2	2.70	2.78	13-H, 14-H ₂ , 16-H ₂ , 17-H ₃ ,	C-3, 15	
C-2	43.1	43.6	2.67 2.43 (d, $J = 14.2$ Hz)	2.71, 2.46	2-H _a	C-1, 3, 4, 15	
C-3	205.1	204.3					
C-4	49.3	49.8	2.43 (d, $J = 14$ Hz)	2.61	13-H	C-2, 3, 5, 6, 9, 13, 14	
C-5	42.8	43.5	3.75	4.08	9-H	C-3, 4, 6, 7, 8, 9, 13	9-H
C-6	207.9	206.7	-				
C-7	35.6	35.93	2.52, 2.33 (ABX, $J =$ 19.5, 4.5, 2.3 Hz)	2.44, 2.20	7-H _a	C-6, 9, 10, 11, 12	13-H
C-8	203.9	201.8	-				
C-9	50.2	51.5	3.42 (dd, $J = 2.8,$ 5.9 Hz)	2.99 (dd, $J = 6, 3$ Hz)		C-4, 6, 8	
C-10	76.5	76.3	5.45	5.40 (dd, 2×8 Hz)	9, 11-H	C-7, 9, 11, 12, 20	9, 11-H
C-11	34.9	35.2	3.34	3.34	7, 12-H	C-6, 7, 9, 10	7, 12-H
C-12	46.0	46.9	2.80 (dd, $J = 3.8,$ 8.6 Hz)	2.69	13-H	C-4, 7, 10, 11, 13, 20	13-H
C-13	34.6	35.9	1.80	1.96		C-14, 20	
C-14	30.6	31.5	2.05, 1.92	2.16, 1.99	13-H	C-1, 2, 4, 13, 15	
C-15	146.7	145.6					
C-16	111.9	113.8	4.70, 4.40	4.85, 4.57	1, 14, 17 _a , 17 _b -H	C-1, 15, 17	17-H ₃ , 12-H
C-17	21.9	22.7	1.65	1.65		C-1, 15, 16	
C-19	28.4	28.8	2.18	2.30		C-8, 9	
C-20	175.8	175.2					

^a DMSO- d_6 (75 MHz). ^b DMSO- d_6 (300 MHz). ^c CDCl_3 (125 MHz). ^d CDCl_3 (500 MHz); all spectra with TMS as internal standard.

**Figure 1.** Sequences A-C deduced from ^1H - ^1H COSY correlations.

All attempts to obtain crystals from various solvents and to perform an X-ray analysis failed. The relative stereochemistry at the respective chiral centers, however, could be confirmed by analysis of the coupling constants and NOESY correlations (Table 1 and Figure 2, structure E). According to the NMR data, the six- and seven-membered rings are *trans*-fused. In the case of *cis*-fused rings, a strong anisotropic effect between the 14-methylene protons due to the lactone CO can be expected and should result in a striking shift difference. This was not observed. Correspondingly, H-4 shows a 14 Hz coupling with H-13 (~ 0 with H-5, which is perpendicularly oriented according to the molecule model). Proton 10-H appeared as a dd signal

**Figure 2.** HMBC couplings (D) and structure-relevant NOESY correlations (E) of **1** in CDCl_3 (500 MHz).

with coupling constants of 8 Hz each, consistent with a *cis* position of 9-, 10-, and 11-H. The proton at C-12 gave a double doublet with $J = 3.7$ and 8.6 Hz in DMSO, indicating an axial/equatorial position of 11- and 13-H. The 11-H (δ_{H} 3.34) showed NOEs with 7-H_A (δ_{H} 2.44), 10-H (δ_{H} 5.40), and 12-H (δ_{H} 2.69), whereas 9-H (δ_{H} 2.99) showed NOEs with 5-H (δ_{H} 4.08) and 10-H (δ_{H} 5.40). The proton at C-12 (δ_{H} 2.69) showed an NOE with 11-H and 13-H (δ_{H} 1.96). The above data indicate that the molecule must have a bowl-shaped structure, with the hydrogens H-5,9,10,11,12,13 arranged on the convex outer side (Figure 2, structure E). There was no direct evidence for the configuration at C-1 from the NOE measurements. The

broad signal for 1-H, however, indicated small coupling constants and therefore its equatorial position in the chairlike six-membered ring. There was also a clear NOE cross signal between the 16-*cis* proton ($\delta_{\text{H}} = 4.57$) and 12-H.

The bridged molecule **1** has only a low degree of conformational freedom. Force field calculations delivered a stereostructure, which, therefore, seems to reflect the actual structure very precisely, since the calculated 3J coupling constants met the measured values within ± 0.5 Hz.

The configuration of **1** has close similarity to that of the norditerpene ineganolide (**2**): The structure of horiolide (**1**) differs from that of **2** only in that it has a carbon bond between C-5 and C-9; cleavage of the C-7–C-8 bond of the norcembranolide **2**; and absence of an ether linkage between C-5 and C-8. Horiolide (**1**) exhibited a positive Cotton effect (+5400) at higher wavelength, 295 nm, and a negative Cotton effect (–207) at lower wavelength, 227 nm. Due to the presence of two carbonyl functions in the molecule, it was not possible to predict the Cotton effect using the octant rule. Further, a sample of ineganolide² (**2**) used for direct comparison did not give any CD signal in methanol, leaving the absolute configuration of horiolide (**1**) uncertain.

Coll⁶ observed that almost all of the diterpenoids isolated from soft corals belong to the cembranoid or rearranged cembranoid skeleton. His observation has been further corroborated by isolation of several unusual and interesting cembranoids from soft corals. The first norcembranolide isolated from the Australian species of *Sinularia*⁶ and later reported from several other species⁷ was 5,8-epoxy-11 α -hydroxy-18-nor-3,6-dioxo-12,15-cembradien-20,10-olide. The C-5 diastereoisomers of this norcembranolide were isolated from a Southern Japanese species of *Sinularia*.⁸ A few more norditerpenoids, yonanolide,⁹ sinularianolide,¹⁰ dissectolide,¹¹ and ineganolide,² are recent additions to this group. In the agar diffusion test, horiolide (**1**) was biologically inactive against the bacteria *Escherichia coli*, *Streptococcus aureus*, and *Bacillus subtilis*, the fungus *Mucor miehei*, and the micro-algae *Chlorella vulgaris*, *Chlorella salina*, and *Chlorella sorokiniana*.

Experimental Section

General Experimental Procedures. Melting points were determined on a VEB-Analytic Dreader HMK hotplate and are uncorrected. IR spectra were measured on a Perkin-Elmer 1600 series FTIR spectrophotometer, UV spectra on an HP 8451A diode array spectrophotometer, optical rotations on a Perkin-Elmer Model 241 polarimeter, and EI mass spectra with Varian MAT 731 (70 eV) and AMD-402 (70 eV), high resolution with perfluoro kerosene for comparison. ^1H NMR spectra were recorded on a Varian VXR 200 (200 MHz) and Varian VXR 500 (499.8 MHz), and ^{13}C NMR spectra on a Varian VXR 300 spectrometer at 75 MHz with TMS as internal standard. 2D spectra were run on Varian VXR 300 and Inova 500 instruments using a 5 mm inverse probe. Column chromatography was performed on silica gel of 100–200 mesh and silica gel G (Acme) for TLC.

Animal Material. The soft coral (0.9 kg dry wt) was collected at Hori Island, Diglipur (93°02'E, 13°20'N) of the Andaman and Nicobar Islands by hand picking in the intertidal rocky region during March 1993. It was cut into small

pieces and stored in ethanol at room temperature. It was identified as *Sinularia* species by Dr. B. Grebnev, Biologist, PIBOC, Vladivostok-22, Russia. The voucher specimen was preserved at the above museum, and at the School of Chemistry, Andhra University, Visakhapatnam as MF-VA/31.

Extraction and Isolation. Ethanol used for storing the specimen was decanted, fresh ethanol was added, and the extraction of the specimen was carried out by percolation using fresh ethanol every 4 days. The procedure was repeated eight times. The combined ethanolic extracts (total 12 L) were concentrated under reduced pressure, and the residue was extracted into ethyl acetate, which was concentrated under reduced pressure to leave a gummy residue (40 g). The residue was chromatographed on a silica gel column (100–200 mesh, Acme) with an increasing gradient, starting from hexane and progressing through ethyl acetate to methanol. The fraction eluted with 4:1 hexane/ethyl acetate yielded horiolide, **1** (30 mg), as a colorless crystalline powder: R_f 0.75 ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$, 12:1); mp 210–212 °C; UV (CH_3OH) λ_{max} 270 nm (ϵ 5400); IR (KBr) ν 3402, 2924, 1774, 1735, 1707, 1454, 1365, 1290, 1227, 1162, 1097, 1065, 1011, 991, 890 cm^{-1} ; ^1H NMR and ^{13}C NMR (DMSO- d_6 and CDCl_3), see Table 1; CD $[\Theta]_{295} +5400$, $[\Theta]_{227} -207$ (CH_3OH , 2.2398 exp–4 M); $[\alpha]_{\text{D}} +81^\circ$ (c 0.037, CH_3OH); EIMS m/z (%) 330 $[\text{M}]^+$ (90), 287 (50), 245 (35), 217 (30), 199 (40), 171 (60), 157 (33), 135 (38), 91 (43), 77 (34), 43.1 (100); HREIMS m/z 330.1481 (calcd for $\text{C}_{19}\text{H}_{22}\text{O}_5$, 330.14810).

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Supporting Information Available: A table of comparison of ^1H and ^{13}C NMR data of horiolide (**1**) and ineganolide (**2**). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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