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Tetrahedron Letters
Tetrahedron Letters 45 (2004) 1995–1997

A novel metabolite from the hybrid soft coral *Sinularia maxima* × *Sinularia polydactyla*: a biosynthetically mixed skeleton linking cembrane and africanane terpenoids ☆

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Received 6 November 2003; revised 16 December 2003; accepted 17 December 2003

Abstract—A novel terpenoid, polymaxenolide, has been isolated from the hybrid soft coral *Sinularia maxima* × *Sinularia polydactyla*. The structure and stereochemistry were determined using spectroscopic methods and X-ray diffraction analysis. This terpenoid with a biosynthetically mixed skeleton is characterized by a C,C-linkage between a cembrane-type diterpene and an africanane-type sesquiterpene. A possible biogenesis is proposed.

The effects of hybridization on secondary chemistry have received considerable attention.[1] It was shown that hybridization results in progeny that differ quantitatively and qualitatively from the parents in the expression of secondary metabolites.[1,2] Data in the terrestrial environment indicated that 68% of the hybrid chemistry originated from one or both parent species, 27% of the parental chemistry is lost and 18% of hybrid chemistry is novel.[1] Although hybridization is known to occur in marine systems,[3] few studies have examined the phenomenon of hybrid resistance and no studies have been reported on the chemistry of hybrids.

Soft corals are a group of cnidarians that constitute a dominant part of the biomass in the tropical reef environments. One of the most abundant soft corals is of the genus *Sinularia*, which tends to form large monospecific 'carpets' of up to 10 m^2. Soft corals are known to produce several classes of structurally unique and complex secondary metabolites such as sesquiterpenes and diterpenes with wide variety of carbon skeletons and a range of biological activities.[4,5] Africanane-type sesquiterpenes and cembrane-type diterpenes are frequently encountered in soft corals of the genus *Sinularia*.[4] We have discovered hybridization between *Sinularia maxima* and *Sinularia polydactyla* (hybrids were characterized using molecular techniques[6]) and we report herein the isolation and the structure determination of a novel metabolite of a mixed biosynthetic pathway, which we named polymaxenolide. It is composed of a cembrane–africanane joined skeleton via a distinct C,C bond. This is the first report of novel hybrid chemistry from the marine environment.

The hybrid soft coral *S. maxima* × *S. polydactyla* was collected from Piti bomb holes, Guam. The methanol–dichloromethane extract of the coral was partitioned between hexane, ethyl acetate and methanol. The fraction eluted with 2:8 ethyl acetate–hexane was subjected to repeated column chromatography on silica gel to afford compound **1**, which was purified by HPLC on RP C_{18} silica using a gradient elution starting with 2.5:7.5 methanol–water to methanol. The structure of the novel compound was determined by extensive 1D and 2D NMR experiments and X-ray crystallography.

Compound **1** (polymaxenolide) was obtained as a white solid, mp 189–191 °C, $[\alpha]_D^{25}$ +83.9°. Its molecular formula $C_{38}H_{50}O_8$ was determined by HREIMS [$(M+H)^+$ m/z 635.3578, $(M+Na)^+$ m/z 657.3397] indicating 14 degrees of unsaturation. Its IR spectrum showed strong

Keywords: Marine natural product; Hybridization; Sesquiterpene; Diterpene; Guam; Biosynthesis.
☆ Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2003.12.108
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doi:10.1016/j.tetlet.2003.12.108

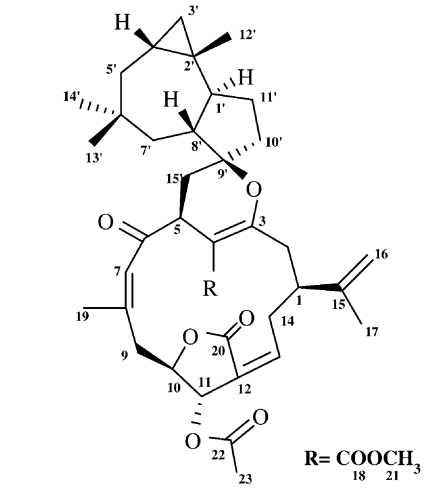
absorptions at 1759, 1738, 1715 and 1683 cm^{-1}, indicative of α,β-unsaturated, ester and keto-carbonyl functionalities. The UV spectrum showed absorptions at λ_{max} (ε 13,145) 230 nm indicating the presence of an enone. The ^{13}C NMR spectrum of **1** measured in C_6D_6, showed 38 carbon signals, and DEPT experiments indicated 7 methyls, 10 methylenes, 9 methines (including 3 oxymethines and 2 vinylic methines) and 12 quaternary carbons (Table 1). The signal appearing at δ 199.2 suggested a conjugated ketone and absorptions at 169.6, 167.2 and 167.0 were attributed to three ester carbonyls. The ^1H NMR spectrum of **1** indicated the presence of an isopropenyl group [δ 4.75 (s, 1H), 4.80 (s, 1H) and 1.80 (br s, 3H)], two trisubstituted double bonds [δ 7.17 (dd, $J = 11.4$, 6.4), 6.19 (br s)] and a lactone methine [δ 4.35 (dd, $J = 8.6$, 2.8 Hz)] characteristic of a cembranoid diterpene skeleton. In addition, its ^1H NMR spectrum contained signals for three cyclopropyl protons [δ 0.17 (t, $J = 4.2$), 0.56 (dd, $J = 8.2$, 4.2), 0.48 (m)] and three tertiary methyls [1.03 (s, 3H), 1.03 (s, 3H), 1.19 (s, 3H)], which are reminiscent of africanane sesquiterpene skeleton. Analysis of 1D and 2D NMR spectra including COSY, HMQC, HMBC and NOESY led to the conclusion that **1** is structurally characterized by a novel cembrane–africanane skeleton of mixed biosynthesis. ^1H–^1H COSY connectivity between H15′ and H5 and HMBC correlations of H15′ to C4, C5 and C6 established the connection through C5 of the cembrane moiety and C15′of the africanane skeleton.

Table 1. ^1H and ^{13}C NMR data of compound **1**[a]

Position	^{13}C δ	^1H δ (Mult.)	J (Hz)
1	49.6 (CH)	2.76 (br t)	11.2
2	41.3 (CH_2)	2.02 (br d)	13.0
		3.62 (d)	13.0
3	171.8 (C)		
4	104.6 (C)		
5	43.9 (CH)	3.68 (t)	8.2
6	199.2 (C)		
7	127.5 (CH)	6.19 (br s)	
8	150.3 (C)		
9	34.9 (CH_2)	2.55 (dd)	13.4, 8.6
		3.45 (br d)	13.4
10	80.9 (CH)	4.35 (dd)	8.6, 2.8
11	75.4 (CH)	5.70 (br s)	
12	125.5 (C)		
13	151.6 (CH)	7.17 (dd)	11.4, 6.4
14	34.4 (CH_2)	3.99 (br t)	11.4
		1.87 (dd)	12.0, 6.4
15	150.0 (C)		
16	109.7 (CH_2)	4.75 (s)	
		4.80 (s)	
17	21.0 (CH_3)	1.80 (br s)	
18	167.2 (C)		
19	27.4 (CH_3)	1.70 (s)	
20	167.0 (C)		
21	50.9 (CH_3)	3.49 (s)	
22	169.6 (C)		
23	20.5 (CH_3)	1.75 (s)	
1′	50.2 (CH)	1.0 (m)	
2′	20.0 (C)		
3′	23.5 (CH_2)	0.17 (t)	4.2
		0.56 (dd)	8.2, 4.2
4′	22.0 (CH)	0.48 (m)	
5′	43.4 (CH_2)	0.89 (m)	
		1.75 (m)	
6′	33.7 (C)		
7′	45.4 (CH_2)	2.06 (br d)	10.4
		0.96 (m)	
8′	47.1 (CH)	2.34 (m)	
9′	90.7 (C)		
10′	36.6 (CH_2)	1.31 (m)	
		1.59 (m)	
11′	24.2 (CH_2)	1.37 (m)	
		1.62 (m)	
12′	19.7 (CH)	1.03 (s)	
13′-α	24.0 (CH_3)	1.19 (s)	
14′-β	34.1 (CH_3)	1.03 (s)	
15′	30.7 (CH_2)	1.72 (m)	
		2.38 (dd)	14.0, 8.2

[a] Spectra recorded at 400 MHz in C_6D_6, chemical shift values are in part per million relative to TMS.

Confirmation of the proposed structure as well as the determination of the relative stereochemistry was achieved independently via X-ray crystallography (Fig. 1). Crystals are monoclinic, space group $P2_1$, $R = 0.043$ for 4144 intensity data measured at 100 K.



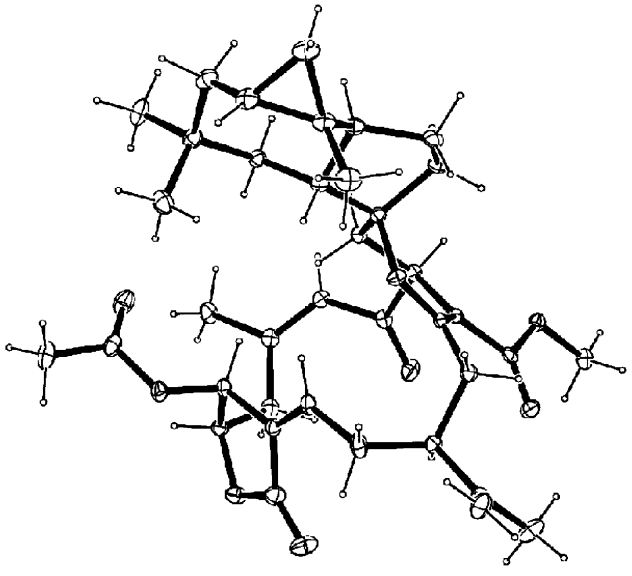
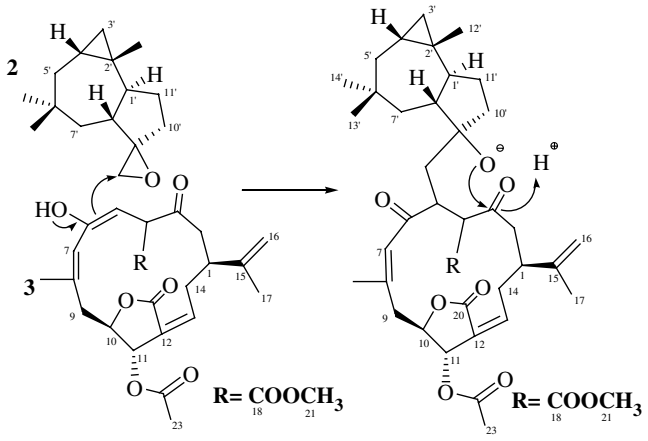


Figure 1.



Scheme 1.

We postulate a probable biogenetic route to compound **1** starting from the molecules **2** and **3** by the nucleophilic attack of an enolate on epoxide methylene carbon followed by the formation of a hemiacetal and an elimination reaction (Scheme 1).

It has been hypothesized that novelty in hybrids occurs via three different mechanisms: obstruction of biosynthetic pathway resulting in the buildup of transient substrates, elaboration of pathways leading to a combination of the basic skeleton of one parent chemical with a new side chain derived from the second parent and disruption of regulatory genes following hybridization causing a shift in where the chemical is produced.[2] Although africanane-type sesquiterpenes and cembrane-type diterpenes are common to the *Sinularia* genus, our data suggest a new mechanism of qualitative variation in hybrid secondary chemistry. Based on the assumption that this novel terpenoid is a natural metabolite and not a product of an autocatalytic reaction, it may represent a biosynthetic chimera formed by a combination of the basic skeleton of one parent with a basic skeleton from the other parental species. This terpenoid also shows the importance of hybridization as a source of molecular and structural diversity.

Supplementary material

Supplementary data including ^{1}H NMR, ^{13}C NMR and HREIMS are available online with the paper in Sciencedirect.

Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 221810. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: +44(0)-1223-336033 or email: deposit@ccdc.cam.ac.uk].

Acknowledgements

We greatly appreciate the contributions of Dr. Chuck Dunbar and Mr. Frank Wiggers for acquiring the HREIMS and the NMR data. We would also like to thank Dr. Deborah Gochfeld and Dr. Robert Thacker who were involved in collection and verification of the hybrids. This work was supported in part by a NOAANURO/NIUST grant (NA16RU1496) to M.S.

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