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Carijenone, a Novel Class of Bicyclic Prostanoid from the Eastern Pacific Octocoral *Carijoa multiflora*

Enrique Dorta, Ana-R. Díaz-Marrero, Mercedes Cueto, Luis D'Croz,[†,‡] Juan L. Maté,[†,§] and José Darias*

Instituto de Productos Naturales y Agrobiología del CSIC, Avda. Astrofísico F. Sánchez, 3, 38206 La Laguna, Tenerife, Spain, Smithsonian Tropical Research Institute (STRI), P.O. Box 2072, Balboa, Panama, Departamento de Biología Marina y Limnología, Estafeta Universitaria, Universidad de Panamá, Panama, and Division of Marine Biology and Fisheries, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, Florida 33149

jdarias@ipna.csic.es

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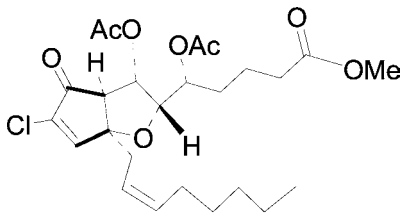
ABSTRACT



Carijenone

An unprecedented biogenetically interesting bicyclic prostanoid 1, carijenone, has been isolated from the eastern Pacific octocoral *Carijoa multiflora*. The C-12 oxygenated function, characteristic of the coral cyclopentanone fatty acid derivatives, is involved in the formation of a five-membered oxane ring fused to the cyclopentane network. Its structure and stereochemistry were determined on the basis of spectral studies and molecular mechanics calculations.

Prostanoids are a group of potent bioactive substances produced from either (a) the arachidonic acid via the cyclooxygenase (COX) pathway or (b) from one of the several related polyunsaturated fatty acids via the lipoxygenase pathway (LOX). These oxidized fatty acids play important roles as signaling molecules in cell proliferation, differentiation and apoptosis. They exert their biological actions through specific protein cognate receptors on the surface of responding cells.[1]

The marine prostanoids clavulones, isolated from the octocoral coral *Clavularia viridis*,[2,3] their halogenated derivatives from *C. viridis*[4,5] and *Dendronephthya* sp.,[6] and punaglandins from *Carijoa risei* (= *Telesto risei*)[7] and their related bromo analogues from *Tubipora musica*[6] have received much attention due to their considerable biological and structural interest.[8] These prostanoids, all of which are

[†] Smithsonian Tropical Research Institute.
[‡] Universidad de Panama.
[§] University of Miami.

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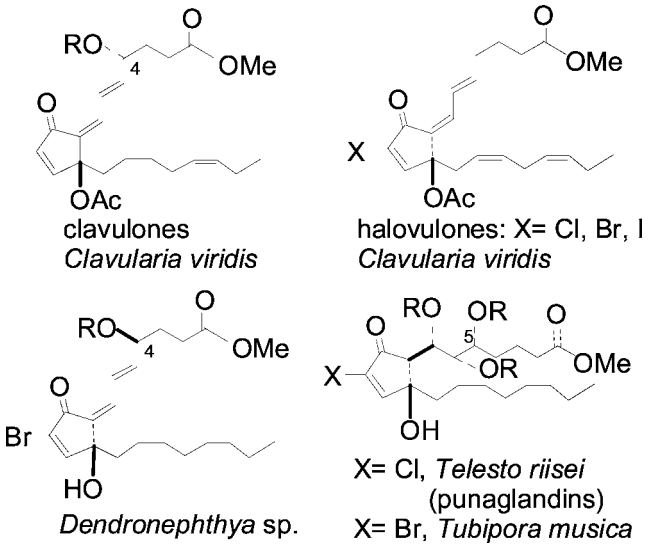
cyclopentane fatty acid derivatives, possess significant biosynthetic differences despite their relatively small structural differences. For instance: (a) the dienic α-side chain of the clavulone family is characterized by carrying an oxygenated functionalization at C-4. However, the related halovulones (chloro, bromo, and iodo derivatives), although isolated from the same source, are devoid of oxidation at C-4. Whereas, strikingly, the brominated analogue from *Dendroneohyta* sp. is C-4 oxygenated (b) in the punaglandins family, as well as in the bromo analogues from *T. musica*, a C-5 oxidized carbon appears to be characteristic of both species. Thus, punaglandins and halovulones possess the same α-halo-γ-hydroxy cyclopentenone ring and ω-side chain, the oxygenation regiochemistry of the respective α-side chain being specific LOX-dependent.



Our interest in the chemical study of the eastern Pacific octocoral *Carijoa multiflora* (= *Telesto multiflora*) led us recently to the isolation of an unusual chlorinated pregnane from specimens collected at Isla Iguana, Panama.[9] This finding prompted us to study *C. multiflora* from other localities in Panama. In this paper we report on the isolation of a new oxylipin **1** from this species.

Vacuum flash chromatography of the acetonic extract of *C. multiflora* gave a fraction (1:2 hexane−ethyl acetate) from which carijenone **1** and the known pregnane steroid **2**[10] were obtained by standard chromatographic procedures involving gel filtration, Si gel chromatography, and HPLC. Compound **1** shared with the punaglandins an identical eicosanoid-derived skeleton but had an additional oxane ring, fused to the carbocyclic network, featuring a chlorinated bicyclic prostanoid of a novel structural class. The oxygenated functionality at C-12, characteristic of these compounds, is involved in the formation of the new ring.

Carijenone, **1**, was a colorless oil.[11] The EIMS spectrum showed a peak at *m/z* 480/482 $[M - H_2O]^+$, with relative intensities suggestive of a chlorine atom. NMR data coupled with the $[M - H_2O]^+$ peak in the HREIMS of **1** suggested a molecular formula of $C_{25}H_{35}O_8Cl$, indicating eight degrees of unsaturation. The ^{13}C NMR spectrum of **1** (Table 1) showed signals for 25 carbons. Multiplicities of the carbon signals were determined from the DEPT spectrum: 4 methyl groups, 8 methylenes, 7 methines (three olefinic, three bearing a heteroatom), and 6 nonprotonated carbons (four carbonyl groups). The IR spectrum suggested that no oxygen of the molecular formula was a hydroxyl group, and revealed absorptions for an α,β-unsaturated carbonyl group (1705 cm^{-1}) and for a methyl ester (1736, 1235 cm^{-1}).

Table 1. ^1H and ^{13}C NMR, HMBC, and NOESY Data of Compound **1** [500 MHz, δ (ppm), J (Hz), $CDCl_3$]

	δ_H	δ_C	HMBC	NOESY
1		173.4		
2	2.29 m	33.5	C-1, C-3, C-4	
3	1.58 m	20.5		
4	1.58 m	29.8		
5	4.97 ddd (3.4, 6.4, 6.4)	72.5	C-7	H-6, H-7
6	4.33 dd (2.5, 2.5)	88.0	C-7, C-12	H-5, H-7
7	5.08 bs ($W_{1/2}$ = 2.9)	77.5	C-5, C-6, C-9, C-12, C=O	H-5, H-6, H-8
8	2.94 s	58.0	C-6, C-7, C-9, C-11, C-12, C-13	H-7, H-14
9		196.1		
10		135.8		
11	7.34 s	156.0	C-8, C-9, C-12	H-13
12		89.6		
13	2.71 dd (8.5. 14.3)	35.7	C-8, C-11, C-12, C-14, C-15	H-8, H-11, H-14, H_2-16
	2.61 dd (7.0. 14.4)			H-8, H-11, H-14
14	5.32 m	121.7	C-16	H-8, H_2-13, H-15
15	5.61 ddd (7.3, 7.3, 10.9)	134.8	C-13, C-16	H-14, H_2-16, H_2-17
16	2.06 m	27.3	C-14, C-15, C-17	H_2-13, H-15
17	1.36 m	29.0	C-15, C-16, C-18, C-19	H-15
18	1.29 m	31.4		
19	1.29 m	22.4		
20	0.88 t (6.8)	13.9	C-18, C-19	
OMe	3.65 s	51.4	C-1	
5-OCO*Me*	1.97 s	20.8		
5-O*CO*Me		170.1		
7-OCO*Me*	2.09 s	20.7		
7-O*CO*Me		169.8		

Among the punaglandins, the reported data of punaglandin-2 acetate[7] are those that best fit in with carijenone. The ^1H NMR and ^{13}C NMR spectra of **1**, Table 1, indicate the presence of two acetate groups and one ester, and this

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(11) Data: $[\alpha]^{25}_D = +22.2$ (*c* 0.1, $CHCl_3$); IR (film) ν_{max} 1736, 1705, 1235 cm^{-1}; EIMS *m/z* 480/482 $[M - H_2O]^+$ (1.4, 0.5), 387/389 $[M - C_8H_{15}]^+$ (100, 34), 327/329 $[M - C_8H_{15} - AcOH]^+$ (69, 25), 267/269 $[M - C_8H_{15} - 2\ AcOH]^+$ (43, 15); HREIMS 480.1892 (calcd for $C_{25}H_{33}O_7Cl$, 480.1914), 387.0806 (calcd for $C_{17}H_{20}O_8Cl$, 387.0846); ^1H and ^{13}C NMR, see Table 1.

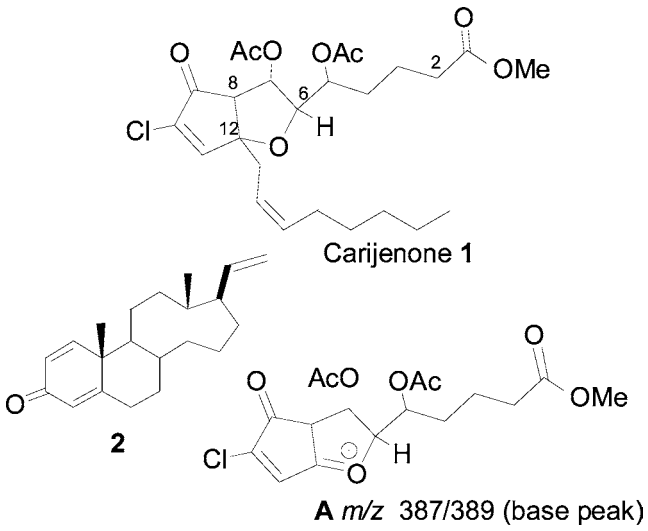

Figure 1. Compounds **1** and **2** and MS fragment **A** of **1**.

together with the ketone account for seven of the eight oxygens given by the molecular formula. Considering the unsaturation degrees and the absence of a hydroxyl group, the remaining oxygen must be a part of a ring. The downfield chemical shift of C-12 (δ_C 89.6) as well as the high-field chemical shift of H-6 (δ_H 4.33) of **1**, relative to the respective carbons ($\delta_C \sim 83.5$) and protons ($\delta_H \sim 5.6$) of congeners,[7] enabled us to locate an oxygen linking C-12 and C-6.

All C−H correlations for **1** were detected in the HSQC spectrum. The C-2−C-7 spin system, measured by COSY experiments, allowed the regiochemistry of the vicinal oxygenated functionalities of the α-side chain and oxane ring to be established. The fused bicyclic network was confirmed by HMBC experiments (Table 1), particularly by the long-range correlation H-6/C-12, C-8, and corroborated by the MS of **1** which showed a peak at *m*/*z* 387/389 (base peak) corresponding to fragment **A**, Figure 1. This completes the planar structure of carijenone as depicted in **1**.

The ^{1}H NMR spectrum of **1** showed a singlet for H-8. The energy-minimized conformation of **1** deduced by mo lecular mechanics[12] is shown in Figure 2. In this conformer, the H-8/H-7 dihedral angle was predicted to be 88.9°, which is in concordance with the observed value $J_{H-8/H-7}$ of 0 Hz. Further, molecular mechanics energy minimization done on the C-7 epimer of **1** led to an H-8/H-7 dihedral angle of 33.6°, which is incompatible with the measured *J* value for these protons. The small coupling constant of H-6 (dd, J = 2.5, 2.5 Hz) suggested a *syn*-periplanar relationship with vicinal H-7 and H-5 protons, Figure 2, which is in agreement with the shape of the ^{1}H NMR signal of H-7 ($W_{1/2}$ = 2.9 Hz) and with the theoretical coupling constants ($J_{H-8/H-7}$ = 0.48; $J_{H-7/H-6}$ =2.08; $J_{H-6/H-5}$ = 1.4 Hz) given by the program. This enabled a relative stereochemistry to be delineated for C-5, C-6, C-7, and C-8. The *J*-coupling (10.9 Hz) of the olefinic protons of the ω-side-chain suggested a *Z* stereochemistry.

(12) *PCModel* (version 7.0); Serena Software: Bloomington, IN.

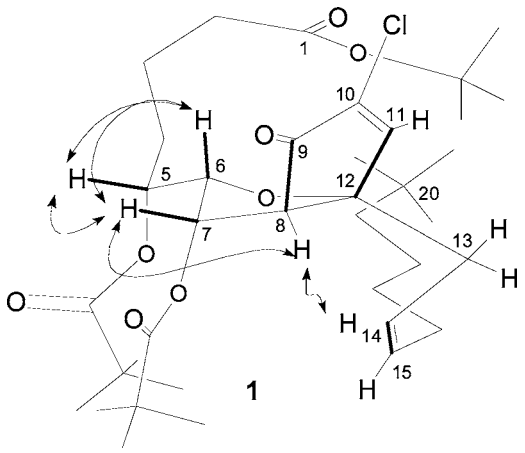

Figure 2. Selected NOEs of carijenone **1**.

The NOESY experiments, Figure 2, aided in establishing the stereochemistry of the ring fusion and corroborated the stereochemistry of the substituents on the oxane ring as well as the stereochemistry at C-5 on the carboxylate side chain. The NOEs observed between H-6 and both H-5 and H-7 protons and between H-5 and H-7 are compatible with an *all-threo* configuration. This is in agreement with the magnitude of their respective coupling constants.[6] On the other hand, the NOEs between H-8 and one of the protons of H_2-13 and with the olefinic H-14 secured a cis relationship between H-8 and the ω-side-chain. These data indicated that **1** is the only possible structure consonant with all the NMR data; thus, the overall stereochemistry of carijenone is 5**R*, 6**S*, 7**S*, 8**S*,12**R*, as depicted in **1**, Figure 2.

Corals use two parallel routes for the initial oxidation of the polyenoic acid substrates to be transformed into cyclopentane derivatives: (a) COX-pathway that catalyzes transformations of archidonic acid into prostaglandins[13] and (b) lipoxygenase/allene oxide pathway that biosynthesizes five-membered carbon rings akin to the prostaglandins such as preclavulone-A, a possible precursor of preclavulones and punaglandins.[14,15] Coral allene oxide synthase (AOS) converts 8*R*-hydroperoxyeicosatetraenoic acid (8*R*-HPETE) to the corresponding allene epoxide, the enzyme being identified as a catalase-like heme peroxidase,[16] unlike plant AOS which is a cytochrome P450 hemoprotein belonging to a new CYP74A subfamily.[17] Plant and coral AOS enzymes, although different, can catalyze reactions with essentially identical chemistry. The main difference is the stereoconfiguration of the substrate they catalyze: most plant lipoxygenases specifically catalyze the biosynthesis of products in the *S* form, whereas invertebrate lipoxygenases, such as the

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8-LOX of starfish oocytes[18] and the 5-LOX of the surf clam *Spisula solidisima*,[19] generally catalyze the biosynthesis of *R* enantiomers. Indeed, the biological activity of lipoxygenase products in invertebrates appears to be restricted to the *R* forms.

The reported 5*R*-LOX in an invertebrate, the surf clam *Spisula solidisima*, indicates that 5-LOX activity is not restricted to vertebrates. It is well-known that a single lipoxygenase can give rise to multiple products through frameshifts[20] along its substrate and it has been suggested that frameshifts in 8-LOX activity could explain the presence of 5-*R*-HETE in the surf clam.[19] On the basis of these considerations, the biogenetic origin of carijenone in *C. multiflora* is proposed to involve a 5*R*- or an 8*R*-lipoxygenase acting on preclavulone-A to oxygenate C-5 stereospecifically, Figure 3. If C-12 is also oxygenated by a lipoxygenase, a spontaneous or enzymatically catalyzed rearrangement of a trisubstituted olefin **4** to a cyclopentadienone **5** may be postulated to enable a free H_2-7 interrupted enoic system,[18] which is the ordinary LOX substrate for stereospecific hydrogen removal from methylene, antarafacial allylic rearrangement of the resulting free radical and binding of the molecular oxygen to the enyl radical at C-12. Since compounds derived from C-12-hydroxyl-promoted intermolecular displacement of C-6 substituents of the punaglandin/clavulone congeners have not been detected, it seemed appropriate to propose an intermediate epoxide **4** rather than its corresponding hydrolyzed C5/C6 vicinal diol derivative. Thus, assuming that 5*R*-hydroperoxide **3** is the natural substrate in *C. multiflora* evolving toward carijenone, a 5*R* stereochemistry and thereby the whole absolute stereochemistry of **1** could be supposed since its relative stereochemistry has been established. We are pursuing the collection of raw material to corroborate this hypothesis.

Carijenone, together with tricycloclavulone and clavubicyclone,[21] are unprecedented coral prostanoids whose structural diversity is generated from internal rearrangement of the parent network. Eicosanoid cyclopentane derivatives have a broad significance in animal physiology, and carijenone may represent an interesting structural model for biomedical research.

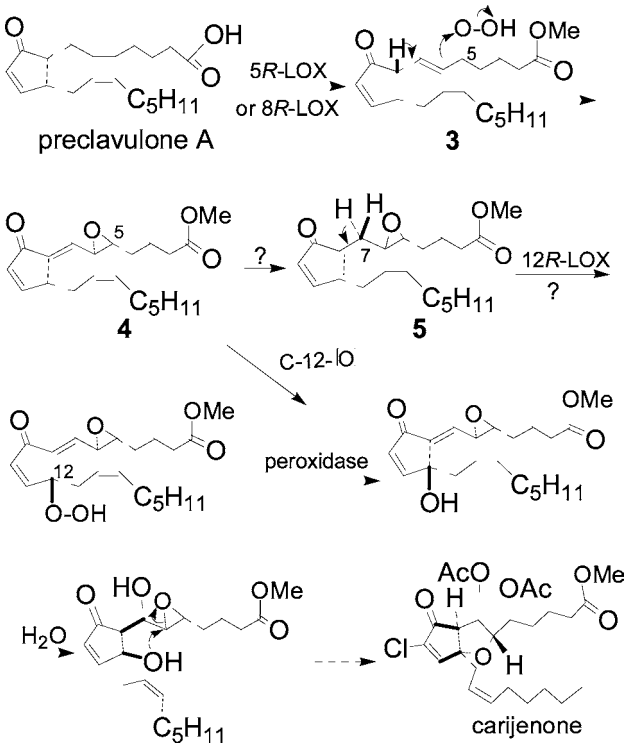

Figure 3. Possible biogenesis of carijenone **1**.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of carijenone **1** and experimental procedure. This material is available free of charge via the Internet at http://pubs.acs.org.

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