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Unusual chlorinated pregnanes from the eastern Pacific octocoral *Carijoa multiflora*

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Abstract—Two unique chloro-pregnane steroids have been isolated from the eastern Pacific octocoral *Carijoa multiflora* and their structures and stereochemistries were determined on the basis of spectral studies and molecular mechanics calculations.

Pregnane steroids and their glycosides are rare in the marine environment and, although the first pregnane derivatives were isolated from starfish,[1–3] the richest sources of these types of compounds appear to be corals,[4] sponges,[4] and less frequently, echinoderms.[5–7] In spite of the relatively few pregnane steroids isolated so far from marine invertebrates, several differing structural features deserve to be pointed out. For instance: (a) all pregnane derivatives from starfish possess a $\Delta^{9(11)}$-nucleus simultaneously oxygenated at C-3, C-6, and C-20, (b) all pregnane steroids from sponges have a $\Delta^{5(6)}$-nucleus, (c) a 5α-pregnane nucleus with a vinyl side chain appears to be characteristic of corals.

In pregnanes from sponges and terrestrial plants[8] the C-3 hydroxyl group is always β- oriented. However, in octocoral pregnanes the 3-OH may have indistinctly an α- or β-stereochemistry.

In this work we report on the structure elucidation of two chloro-pregnane steroids **1** and **2** isolated from the eastern Pacific octocoral *C. multiflora* (= *Telesto multiflora*) collected at Isla Iguana, Panama. The compounds are epimeric at C-2, having both a fully functionalized ring A with a unique 1,2-chlorohydrin and a 3-ketoenone.

The octocoral genus *Carijoa* (Cnidaria: Anthozoa: Octocorallia: Alcyonacea: Clavulariidae)[9] has proven to be a source of chlorinated metabolites, for example, punaglandins, isolated from *Carijoa riisei* (= *Telesto riisei*).[10] *C. multiflora* is a common non-zooxanthellate octocoral from the Pacific coast of Panama usually found with polyps expanded during the day time in sheltered and shaded crevices, or in shallow caverns covering basaltic and coralline substrata.

From the crude methanolic extract of *C. multiflora* compounds **1** and **2** were obtained after flash chromatography followed by gel filtration and successive HPLCs.

Compound **1** was isolated as a colorless oil.[11] The EIMS spectrum of **1** showed the molecular ion at m/z 348/350, with relative intensities suggestive of a chlorine atom. NMR data coupled with the $[M]^+$ peak in the HREIMS of **1** suggested a molecular formula of $C_{21}H_{29}O_2Cl$, indicating seven degrees of unsaturation. The ^{13}C NMR spectrum of **1** (Table 1), together with the information

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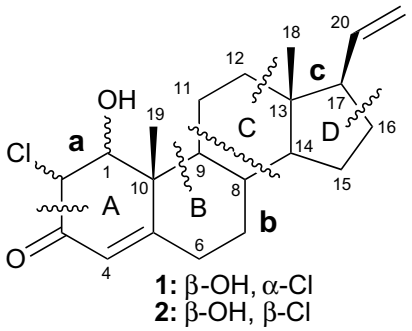
Table 1. ^{1}H, ^{13}C NMR and HMBC data of compounds **1** and **2** [500 MHz, δ ppm, (J) Hz, $CDCl_3$]

#	**1**			**2**		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
1	3.79 dd (2.1, 11.5)	78.8	C-2, C-9, C-19	4.23 dd (2.4, 4.2)	76.3	C-5, C-3
2	4.59 d (11.5)	68.7	C-1, C-3, C-10	4.79 d (2.4)	65.5	C-3, C-10
3		189.7			189.8	
4	5.89 d (1.4)	121.9	C-2, C-6, C-10	5.87 s	119.9	C-2, C-6, C-10
5		170.7			170.5	
6	α: 2.35 dddd (2.4. 2.4. 2.4, 13.4)	33.8	C-8, C-10	α: 2.26 ddd (2.5, 4.4, 12.4)	33.4	C-4, C-5, C-7
	β: 2.50 dddd (1.4, 5.4, 13.4, 13.4)		C-4, C-5, C-7	β: 2.50 ddd (4.0, 12.4, 12.4)		C-4, C-5, C-10
7	α: 1.08 m	32.7	C-8, C-14	α: 1.06 m	35.4	C-8, C-14
	β: 1.88 m			β: 2.02 m		
8	1.62 m	36.3	C-9, C-14, C-15	1.07 m	36.3	
9	1.16 ddd (4.3, 12.2, 12.2)	56.2	C-8, C-10	1.32 m	52.9	C-1, C-10
10		47.2			46.7	
11	2.10 dddd (3.8, 3.8, 3.8, 14.3)	24.0	C-9, C-12	1.59 m; 1.64 m	22.9	
	1.58 m					
12	α: 1.07 m	37.8	C-14, C-18	α: 1.10 m	37.5	
	β: 1.72 m		C-11, C-13	β: 1.77 m		C-18, C-11
13		43.5			44.0	
14	1.03 m	55.4	C-8, C-9, C-13, C-15, C-17, C-18	1.03 m	55.3	
15	1.65 m; 1.22 m	23.9	C-13, C-16; C-8, C-14, C-17	1.69 m; 1.59 m	25.1	
16	1.80 m; 1.56 m	27.4	C-13; C-14, C-15, C-17	1.83 m; 1.60 m	27.4	
17	1.94 dd (8.6, 8.6)	55.6	C-12, C-13, C-18, C-20, C-21	1.94 m	55.5	C-12, C-13, C-18, C-21, C-20
18	0.62 s	12.9	C-12, C-13, C-14, C-17	0.65 s	13.3	C-12, C-13, C-17
19	1.30 s	13.1	C-1, C-5, C-9, C-10	1.35 s	17.2	C-1, C-5, C-9, C-10
20	5.75 ddd (2.8, 10.5, 17.3)	139.8		5.75 ddd (2.8, 10.5, 17.3)	139.5	C-17
21	a: 4.95 m	115.2	C-17, C-20	a: 4.96 m	115.4	C-17, C-20
	b: 4.99 m			b: 4.99 m		
O*H*	2.58 d (2.1)		C-1, C-10	2.30 d (4.2)		

from a DEPT spectrum, showed the presence of 21 carbon signals assigned to two methyls, seven methylenes (one olefinic), eight methines (two olefinic and two bearing a heteroatom), and four non-protonated carbons (one carbonyl and one olefinic). The IR absorptions at 3422 and 1677 cm^{-1} were consistent with both oxygenated functionalities: a hydroxyl and a carbonyl. Since the IR spectrum revealed no absorption for additional unsaturations, the molecule must be tetracyclic.



Although the NMR data featured a pregnane network with a vinyl side chain, the presence of an unprecedented halogen atom in this skeleton, given by the molecular formula, raised some doubts about whether the molecule was indeed a pregnane steroid. Thus, a careful spectral analysis was undertaken.

All C–H correlations for **1** were detected in the HSQC spectrum. The ^{1}H NMR J-coupling and COSY measurement located vicinal protons geminal to the heteroatoms. As the HMBC NMR experiment correlated H_3-19 with a carbon (C-1) bearing a heteroatom a fragment **a** was thus established. The regiochemistry of the functionalities in fragment **a**, as depicted in **1**, was deduced by the ^{13}C chemical shift of C-1 and C-2 and by the coupling between protons H-1 and HO–C-1, and also by the HMBC correlations of HO–C-1 with C-1 and C-10. The whole functionalization of ring A and of the decalin moiety **b** was assessed by: (a) HMBC correlation of H-1/C-2, C-9, C-19; H-2/C-1, C-3, C-10; H-4/C-2, C-6, C-10; H-6/C-8, C-10; H_3-19/C-1, C-5, C-9, C-10 and (b) ^{1}H COSY NMR correlation of the portion H-6 $\rightarrow$ H-9 of a spin system from H-6 through H-12.

The HMBC correlation of downfield H-17 with both the C-18 methyl group and the vinylic residue established fragment **c**. The similarity of the resonances of the remaining C/D ring carbon atoms with those of a pregnane congener,[12] whose structure was secured by single-crystal X-ray diffraction, as well as the COSY connectivities from H-17 through H-8, and the HMBC correlations allowed us to establish a 3-keto-$\Delta^{4(5)}$ pregnane framework, with a fully functionalized ring A and a vinyl side chain at C-17.

Chemical shift arguments and 2D NOESY experiments established the relative configurations of the chiral

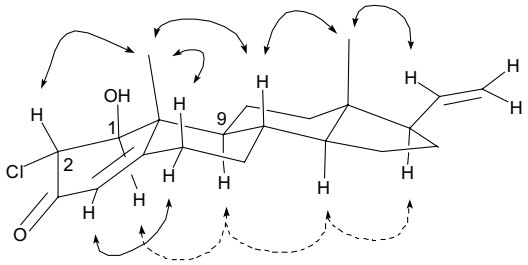


Figure 1. Selected NOEs of compound **1**.

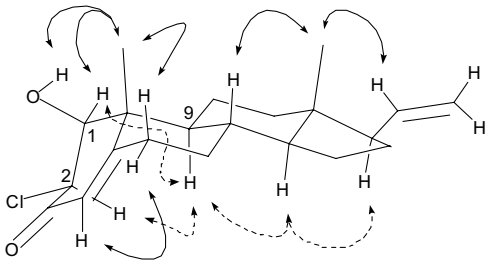


Figure 2. Selected NOEs of compound **2**.

centers of the steroidal nuclei as shown in Figure 1. The large J values for H-1 and H-2, Table 1, and the NOEs observed between H-2/H_3-19 and between H-1/H-9 indicated a *trans* stereochemistry for both the chlorohydrin group and H_3-19/H-9. The NMR data for H_3-18 (δ_H 0.62, δ_C 12.9) suggested that the methyl group was β and that rings C/D were *trans* fused. [13] This was corroborated by NOE effects, a selection of which is represented in **1**. Thus, the molecule is 4,20-pregnadien-1β-hydroxy-2α-chloro-3-one.

Compound **2** was isolated as a colorless oil.[14] The EIMS spectrum of **2** showed the molecular ion at m/z 348/350, with relative intensities indicative of a chlorine atom. The NMR data were very similar to those of **1**, the most significant differences being the proton and carbon resonances of the chlorohydrin and the C-19 methyl group suggesting, according to the identities of the molecular formulas, that both compounds must be epimeric.

The HBMC correlation of H_3-19/C-1 and of HO/C-1 suggested that compound **2** has the same chlorohydrin regiochemistry as **1**. Although the small J value ($J = 2.4$ Hz) of H-1 and H-2 pointed to vicinal axial–equatorial coupling, it did not enable us to discriminate which of the carbon-bearing heteroatoms was epimeric. However, the strong NOE observed between H-2 and H-9 and between H-1/H_3-19, H-9, suggested that ring A adopted an arrangement different from that of **1**, enabling protons H-2 and H-9 to be brought closer.

Molecular mechanics calculations were performed[15] in order to evaluate a conformation compatible with: (a) the small J coupling observed for the adjacent protons bearing a heteroatom, and (b) the observed NOE effects. The comparison of the well-resolved J-coupling of the protons of the energetically favorable conformation **2** (Fig. 2) with the theoretical coupling constants ($J = 1.8$ Hz) given by the program proved to be in good agreement. The conformation of ring A placed H-2 at a suitable distance to allow the observed NOE with H-9. Thus, **2** is 4,20-pregnadien-1β-hydroxy-2β-chloro-3-one.

Octocorals are the most prolific source of pregnane steroids among marine invertebrates, the biosynthetic origin of the frequently complex mixture of sterols being enigmatic. The conversion of cholesterol to pregnane steroids is accomplished by cleavage of the cholesterol side-chain catalyzed by the cytochrome P-450 (CYP) family of enzymes.[16] At least six cytochrome P-450 enzymes appear to participate in steroidogenesis, and the CYP11A1 single enzyme is involved in cholesterol side-chain cleavage, catalyzing sequential hydroxylation, and scission of the C-20–C-22 di-hydroxylated bond giving pregnenolone.[17]

Cytochrome P-450, and related oxidase enzymes, as well as C_{19} and C_{21} steroids concentration has been measured in scleractinian[18] and alcyonacean soft corals,[19] respectively, suggesting the metabolization of the steroids. Seasonal changes in estrogen concentration[20] and metabolization of radiolabeled progesterone into pregnenolone and testosterone have been detected in scleractinian corals. These studies indicate that corals may synthesize estrogen to regulate gametogenesis and spawning, supporting the hypothesis that steroids act as bioregulators of reproduction in invertebrates.

Chlorinated steroids are rare in nature. So far, six chloro-withanolides and two chloro-steroid glycosides, called blattellastanoside A and B, have been isolated, respectively, from terrestrial plants of the Solanaceae family,[21] and from a terrestrial invertebrate.[22] A few C-28 and C-29 chlorinated sterols from marine sources have also been isolated: two side-chain chlorinated C-29 sterols from an unidentified *Xetospongia* sp.;[23,24] three C-28 sterols from an unidentified *Strongylacidon* sp. sponge,[25] and finally yonasterols G and H (C-28) and yonasterol I (C-29) have recently been isolated from the octocoral *Clavularia viridis*,[26] all being chlorinated at the steroidal nucleus.

Metabolites **1** and **2** of *C. multiflora* are unusual chlorinated pregnanes steroids, and raise the issue of whether the chlorine atom was incorporated before or after side-chain cleavage during steroidogenesis.

A synthetic chloro-pregnane has been shown to increase the conversion of testosterone (T) to DHT producing androgen activity. The compound stimulated T conversion, suggesting a potential application in the treatment of androgen-dependent diseases.[27]

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References and notes

1. Ikegami, S.; Kamiya, Y.; Tamura, S. *Tetrahedron Lett.* **1972**, 1601–1604, and references cited therein.
2. Sheikh, Y. M.; Tursch, B. M.; Djerassi, C. *J. Am. Chem. Soc.* **1972**, *94*, 3278–3280.
3. Shimizu, Y. *J. Am. Chem. Soc.* **1972**, *94*, 4051–4052.
4. Ortega, M. J.; Zubia, E.; Rodríguez, S.; Carballo, J. L.; Salvá, J. *Eur. J. Org. Chem.* **2002**, 3250–3253, and references cited therein.
5. Findlay, J. A.; He, Z.-Q.; Jaseja, M. *Can. J. Chem.* **1989**, *65*, 2078–2080.
6. Findlay, J. A.; He, Z.-Q. *J. Nat. Prod.* **1990**, *53*, 710–712.
7. Chudil, H. D.; Seldes, A. M.; Maier, M. S. *J. Nat. Prod.* **2002**, *65*, 153–157.
8. Deepak, D.; Khare, A.; Khare, M. P. *Phytochemistry* **1989**, *12*, 3255–3263.
9. Fabricius, K; Alderslade, P. *Soft Corals and Sea Fans*; Australian Institute of Marine Science: Townsville, Qld., 2001; 264 pp.
10. Baker, B. J.; Okuda, R. K.; Yu, P. T. K.; Scheuer, P. J. *J. Am. Chem. Soc.* **1985**, *107*, 2976–2977.
11. $[\alpha]_D^{25}$ +92° (*c* 0.12, $CHCl_3$); IR ν_{max} (film) 3422, 2928, 1677, 1618, 1450 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 348/350 $[M]^+$ (76, 28), 313 $[M-Cl]^+$ (100), 270 (72), 201 (34), 175 (50), 133 (31), 121 (63), 119 (54), 107 (44), 105 (64), 95 (32), 93 (62), 91 (81), 81 (46), 79 (66), 77 (44), 67 (51), 55 (41); HREIMS 348.1915 (calcd for $C_{21}H_{29}O_2Cl$, 348.1856), 313.2169 (calcd for $C_{21}H_{29}O_2$, 313.2167).
12. Blackman, A. J.; Heaton, A.; Skelton, B. W.; White, A. H. *Aust. J. Chem.* **1985**, *38*, 565–573.
13. Smith, W. B. *Annu. Rep. NMR Spectrosc.* **1978**, *8*, 199.
14. $[\alpha]_D^{25}$ −164° (*c* 0.14, $CHCl_3$); IR ν_{max} (film) 3448, 2931, 1684, 1619, 1437 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* 348/350 $[M]^+$ (30, 10), 313 $[M-Cl]^+$ (67), 270 (74), 201 (47), 175 (43), 133 (30), 121 (59), 119 (50), 107 (44), 105 (63), 95 (37), 93 (65), 91 (100), 81 (55), 79 (76), 77 (49), 67 (59), 55 (58), 53 (34); HREIMS 348.1837 (calcd for $C_{21}H_{29}O_2Cl$, 348.1856), 313.2155 (calcd for $C_{21}H_{29}O_2$, 313.2167).
15. PCModel (version 7.0) Serena Software, Bloomington, IN, 47402-3076.
16. Miller, W. L. *Mol. Cell Endocrinol.* **2002**, *198*, 4–14.
17. Nebert, D. W.; Russell, D. W. *The Lancet* **2002**, *360*, 1155–1162.
18. Gassman, N. J.; Kennedy, C. J *Bull. Mar. Sci.* **1992**, *50*, 320–330.
19. Slattery, M.; Hines, G. A.; Watts, S. A. *Polar Biol.* **1997**, *18*, 76–82.
20. Tarrant, A. M.; Atkison, S.; Atkison, M. J *Comp. Biochem. Physiol.* **1999**, *122*, 85–92.
21. Nittala, S. S.; Velde, V. V.; Frolow, F.; Lavie, D. *Phytochemistry* **1981**, *20*, 2547–2552.
22. Sakuma, M.; Fukami, H. *Tetrahedron Lett.* **1993**, *34*, 6059–6062.
23. Shimura, H.; Iguchi, K.; Yamada, Y.; Yamagishi, T.; Matsumoto, K.; Yokoo, C. *Experientia* **1994**, *50*, 134–136.
24. Kobayashi, M.; Chen, Y.-J.; Higuchi, K.; Aoki, S.; Kitagawa, I. *Chem. Pharm. Bull.* **1996**, *44*, 1840–1842.
25. Carney, J. R.; Scheuer, P. J.; Kelly-Borges, M. *J. Org. Chem.* **1993**, *58*, 3460–3462.
26. Iwashima, M.; Nara, K.; Nakamichi, Y.; Iguchi, K. *Steroids* **2001**, *66*, 25–32.
27. Cabeza, M.; Gutiérrez, R.; Miranda, I.; Heuze, E.; Bratoeff, G.; Flores, E.; Ramírez, E. *Steroids* **1999**, *64*, 413–421.