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New C_{21} Δ^{20} pregnanes, inhibitors of mitochondrial respiratory chain, from Indopacific octocoral *Carijoa* sp.

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Abstract—Two new compounds, pregnanes **1** and **2**, the known pregnane **3** and a series of known chlorinated prostanoids (**4**–**9**) have been isolated from the Indian octocoral *Carijoa* sp. Their structures have been elucidated by spectroscopic methods, mainly by 1D and 2D NMR. The new compounds were potent inhibitors of the mitochondrial respiratory chain.

Steroidal compounds from marine organisms show a wide array of unusual structures.[1,2] Among these, C_{21} pregnanes and their glycosides, all of which are characterized by the uncommon vinyl side chain, represent a minor group of metabolites. Octocorals are the main source of this kind of compounds[3–6] even though pregnanes have been isolated also from sponges and echinoderms.[1]

As part of our investigation on bioactive compounds from marine benthic invertebrates, we have examined an Indopacific octocoral *Carijoa* sp., collected off the coast of Rameshwaram (Krusadi Islands, India), during January 2001. Previous chemical studies on octocoral genus *Carijoa* (Cnidaria: Anthozoa: Octocorallia: Alcyonacea: Clavulariidae) have proven the presence of chlorinated prostanoids, punaglandins, in different populations of *Carijoa* (=*Telesto*) *riisei*[7,8] as well as of chlorinated pregnanes in *Carijoa multiflora*.[6]

We describe here the structure elucidation of two new pregnanes, compounds **1** and **2**, isolated together with seven known metabolites, pregna-1,4,20-triene-3-one (**3**), previously found in soft corals,[9–12] punaglandins 1–3 (**4**–**6**) and acetyl punaglandins **7**–**9**, all of which already reported from *C. riisei*.[7,8]

Specimens of *Carijoa* sp. (dry weight 113 g) were extracted exhaustively with acetone, and the extract was partitioned between water and Et_2O. The Et_2O-soluble fraction (2.5 g) was analyzed by TLC, revealing the presence of a series of compounds along with usual fatty acid and sterol components. An aliquot of Et_2O extract (860 mg) was fractionated on a Sephadex LH-20 column eluted by $CHCl_3$/MeOH (1:1) to yield fractions I–VII. Fraction III (93 mg), containing chlorinated prostanoids as it was indicated by preliminary ^{1}H NMR analysis, was submitted to Si-gel column chromatography (light petroleum ether/diethyl ether gradient) followed by reverse-phase HPLC (CH_3CN/H_2O gradient) giving pure punaglandins 1 (**4**, 6.1 mg), 2 (**5**, 6.5 mg), 3 (**6**, 1.1 mg) and punaglandin-3-acetate (**7**, 12.1 mg), punaglandin-4-acetate (**8**, 3.7 mg) and 7*Z*-punaglandin-4-acetate (**9**, 1.0 mg).

A preliminary NMR analysis of fractions IV (132 mg) and V (165 mg) revealed the presence of pregnane mixtures along with sterols and fatty acids. Pure pregnane **3** (3.7 mg) was obtained from fraction IV by subsequent Si-gel column and reverse-phase HPLC (CH_3CN/H_2O gradient). Analogously, purification of fraction V in the same conditions gave pure compounds **1** (9.1 mg) and **2** (7.0 mg). The known metabolites **3**–**9** were easily identified by comparison of their spectral values with literature data, whereas compounds **1** and **2** were submitted to spectral analysis.

^{1}H and ^{13}C NMR spectra of both compounds **1** and **2** (Table 1) showed strong similarities with those of

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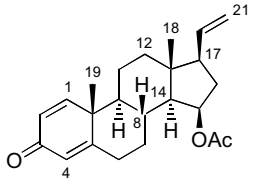

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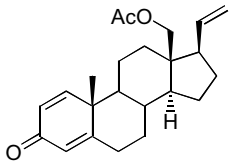

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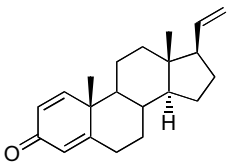

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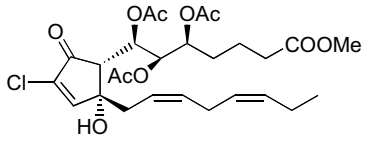

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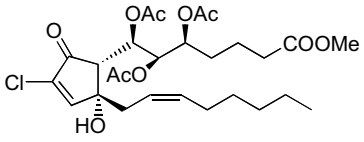

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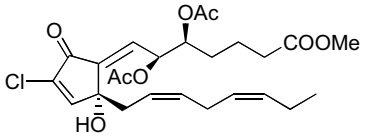

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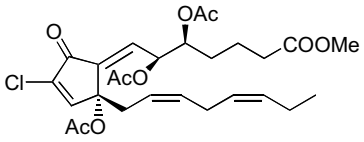

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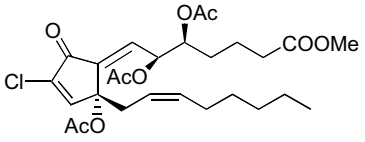

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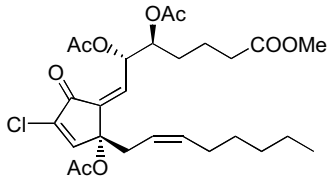

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pregna-1,4,20-triene-3-one, **3**[‡], immediately suggesting a close structural relationship among them, and, in particular, revealing the presence in the frameworks of **1** and **2** of the typical cross-conjugated dienone system of steroidal $\Delta^{1,4}$-3-ones as well as the vinyl side chain feature, the same as **3**.

Steroid **1**[§] exhibited the molecular formula $C_{23}H_{30}O_3$ as deduced by HRFABMS (*m/z* 355.2247 $[M+H]^+$), indicating the presence of an additional acetoxy function with respect to compound **3**.

Accordingly, in the carbon spectrum of **1** signals due to an acetoxy group at δ 170.6 (s, OCOMe), 21.4 (q, –OCOMe) and 73.7 (d, C-15) were observed. ^{1}H NMR spectrum was characterized in low-field region by the presence of a multiplet at δ 5.12 1H (ddd, J = 7.5, 5.7, 1.0, H-15) along with dienone [δ 7.06 (1H, d, J = 10.2, H-1), 6.23 (1H, dd, J = 10.2, 1.9, H-2) and 6.07 (1H, br t, H-4)] and vinyl [δ 4.98 (1H, dd, J = 17.5, 1.6, H-21a), 5.03 (1H, dd, J = 10.2, 1.6, H-21b) and 5.73 (1H, ddd, J = 7.6, 10.2 and 17.5 Hz, H-20)] signals. The proton spectrum also showed an acetyl singlet at δ 2.05 in addition to two angular methyl singlets at δ 0.89 (C-18) and 1.27 (C-19). These data were consistent with a pregna-1,4,20-triene-3-one skeleton bearing a secondary acetoxy function. The location of –OAc group at C-15 in ring D, as reported in structure **1**, was clearly indicated by ^{1}H–^{1}H COSY experiment. In fact, the proton at δ 5.12 showed cross-peak correlations with both the angular methine at δ 1.05 (1H, dd, J = 10.9, 5.7, H-14) and the methylene at δ 2.50 (1H, m, H-16a) and 1.59 (1H, m, H-16b) that was further coupled with the allylic proton at δ 1.95 (1H, m, H-17). Analysis of 2D NMR experiments and in particular of HMBC correlation spectrum confirmed the proposed structure and allowed to assign all carbon and proton resonances (Table 1). The β-stereochemistry of the acetoxy group at C-15

[‡] 1,4,20-Pregnatrien-3-one, previously partially characterized[9–12] (**3**): $[\alpha]_D^{20}$ +36 ($CHCl_3$, *c* 0.23); ν_{max}: 2956, 2929, 2848, 1659, 1624 cm^{-1}; LRESIMS: 319 (M+Na); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.06 (H-1, d, 10.2), 6.23 (H-2, dd, 10.2, 1.6), 6.08 (H-3, t, 1.6), 5.75 (H-20, ddd, 17.0, 10.5, 7.6), 5.00 (H-21a, dd, 10.5, 1.5), 4.95 (H-21b, dd, 17.0, 1.5), 2.48 (H-6ax, ddd, 13.0, 10.8, 5.1), 2.37 (H-6eq, ddd, 13.0, 6.0, 2.3), 1.98 (H-7a, m), 1.95 (H-17, m), 1.80 (H-16a, m), 1.76 (H-12a, m), 1.70 (H_2-11 and H-15a, m), 1.65 (H-8, m), 1.58 (H-16b, m), 1.28 (H-15b, m), 1.24 (H_3-19, s), 1.10 (H-9, m), 1.08 (H-7b, m), 1.07 (H-12b, m), 1.02 (H-14, m), 0.67 (H_3-18, s); ^{13}C NMR (75.13 MHz, $CDCl_3$): δ 186.5 (C-3, s), 169.0 (C-5, s), 155.9 (C-1, d), 139.3 (C-20, d), 127.5 (C-2, d), 123.8 (C-4, d), 114.9 (C-21, t), 55.1 (C-17, d), 54.6 (C-14, d), 52.7 (C-9, d), 43.6 (C-10 and C-13, s × 2), 37.1 (C-12, t), 35.7 (C-8, d), 33.7 (C-7, t), 32.9 (C-6, t), 27.1 (C-16, t), 24.9 (C-15, t), 22.5 (C-11, t), 18.7 (C-19, q), 12.9 (C-18, q).

[§] Compound **1** (15-*O*-acetyl-1,4,20-pregnatrien-3-one) was obtained as a yellow oil, R_f 0.4 (benzene/diethyl ether 3:2); $[\alpha]_D$ −44.7 (*c* 0.9, $CHCl_3$); IR (KBr) ν_{max} 3076, 2975, 2940, 2856, 1732, 1659, 1624, 1250, 752 cm^{-1}; UV (MeOH) λ_{max} 243 (10420); HRFABMS 355.2245 $(M+H)^+$ (calcd for $C_{23}H_{31}O_3$ 355.2273); ^{1}H and ^{13}C NMR data in Table 1.

Table 1. NMR data for compounds **1** and **2**[a,b,c]

Position	Compound **1**			Compound **2**		
	δ_H, m	δ_C, m[c]	HMBC with H	δ_H, m[b]	δ_C, m[c]	HMBC with H
1	7.06 d (10.2)	155.6 d	H_3-19	7.03 d (10.1)	155.6 d	H_3-19
2	6.23 dd (10.2, 1.9)	127.6 d	H-4	6.23 dd (10.1, 1.9)	127.6 d	H-1, H-4
3	—	186.3 s	H-1	—	186.3 s	H-1
4	6.07 br t	124.0 d	H_2-6	6.07 br t	124.0 d	H_2-6
5	—	168.2 s	H_3-19, H_2-6, H_2-7	—	168.5 s	H_3-19, H_2-6, H-1, H_2-7
6	2.48ax m 2.36eq ddd (13.3, 4.1, 2.5)	32.6 t	H_2-7	2.46ax m 2.36eq ddd (13.3, 4.1, 2.5)	32.7 t	H_2-7
7	1.93 m 1.10 m	33.7 t	H-9	1.98 m 1.08 m	33.7 t	H-9
8	1.98 m	31.9 d	H-9	1.73 m	35.6 d	—
9	1.12 m	52.9 d	H_3-19, H-12, H-8	1.12 m	52.6 d	H_3-19, H-12, H-8
10	—	43.6 s	H_3-19, H_2-6, H_2-7	—	43.5 s	
11	1.77 m	22.6 t	—	1.78 m 1.58 m	22.5 t	—
12	1.78 m 1.08 m	38.2 t	H_3-18	2.17 m 0.98 m	32.5 t	H_2-18
13	—	43.6 s	H-17, H-14, H-15, H_3-18	—	46.3 s	H_2-18
14	1.05 dd (10.9, 5.7)	57.5 d	H_3-18, H_2-16, H-17, H-8, H_2-12	1.28 m	54.3 d	—
15	5.12 ddd (7.5, 5.7, 1.0)	73.7 d	H_2-16	1.89 m 1.26 m	24.8 t	—
16	2.50 m 1.59 m	38.3 t	—	1.90 m 1.73 m	27.1 t	H-20
17	1.95 m	54.8 d	H_3-18, H_2-16, H-20, H_2-21	2.08 m	54.6 d	H_2-18, H-20, H_2-21
18	0.89 s	14.8 q		4.07 Abq (11.8)	62.1 t	
19	1.27 s	18.8 q		1.24 s	18.8 q	
20	5.73 ddd (17.5, 10.2, 7.6)	137.6 d	H-17, H_2-16, H_2-21	5.80 ddd (17.2, 10.2, 7.7)	138.8 d	H_2-16, H-17
21	5.03 dd (10.2, 1.6) 4.98 dd (17.5, 1.6)	114.1 t	H-17, H_2-16	4.96 dd (17.2, 1.6) 4.93 dd (10.2, 1.6)	114.0 t	H-17
$COCH_3$	2.05 s	170.6 s 21.4 q	—	2.05 s	171.2 s 21.1 q	—

[a] Assignments determined by COSY, HSQC, HMBC.
[b] Multiplicity given in hertz.
[c] Multiplicity deduced by DEPT.

was suggested by both the coupling constants of H-15 (ddd, $J_{H\text{-}15\text{–}H\text{-}14} = 5.7$, $J_{H\text{-}15\text{–}16a} = 7.5$, $J_{H\text{-}15\text{–}16b} = 1.0$), which were in agreement with a pseudo-equatorial orientation of H-15, and the carbon value of C-8 (δ 31.9), upshifted in comparison with the corresponding carbon in **3** (δ 35.7) by the γ-gauche effects of the β-oriented substituent at C-15. The relative stereochemistry at all chiral centres was further supported by NOESY experiments. Expected NOE effects were observed among the angular methine H-8 and both H_3-18 and H_3-19, as well as between H-20 and H_3-18, according to the common steroid configuration. NOESY spectrum showed also diagnostic cross-peak correlations between H-15 and H_2-7 further confirming the β-orientation of acetoxy group at C-15.

Steroid **2**[¶] was isomeric with compound **1**, having the same molecular formula $C_{23}H_{30}O_3$ as inferred by HRFABMS (*m/z* 355.2262 $[M+H]^+$). Analogously with **1** and **3** the ^1H NMR spectrum showed the typical signals for dienone and vinyl protons (Table 1), and differed from steroid **3** in the presence of an AB quartet centred at δ 4.07 and an acetyl methyl signal at δ 2.05, in the place of the high-field angular methyl singlet H_3-18, according with the pregnane skeleton exhibiting an oxidized tertiary methyl. The placement of the acetoxymethyl group at C-13, suggested by the absence of the typical methyl signal at high field, was supported by diagnostic correlations in the HMBC spectra of **2** (Table 1). In particular, H_2-18 displayed long-range connectivities with C-12, C-13, C-14 and C-17. Analogously with **1**, all proton and carbon resonances were easily attributed by 2D-NMR experiments (^1H–^1H COSY, HMQC and HMBC) as reported in Table 1.

Compounds **1** and **2** showed a strong activity in the preliminary biological assay for the inhibition of the integrated electron transfer chain (NADH oxidase activity) in beef heart submitochondrial particles (SMP).[13] Inhibitory concentration 50% (IC_{50}) for compound **1** was $1.9 \pm 0.19\,\mu M$ and for compound **2** was $1.1 \pm 0.04\,\mu M$, whereas full inhibition of rotenone sensitive NADH oxidase activity was achieved at approximately $2\,\mu M$. Further experiments have been planned in order to evaluate other biological activities for these compounds.

[¶] Compound **2** (18-acetyl-18-hydroxymethyl-1,4,20-pregnatrien-3-one) was obtained as a pale yellow oil, R_f 0.35 (benzene/diethyl ether 3:2); $[\alpha]_D$ +32.9 (*c* 0.7, $CHCl_3$); IR (KBr) ν_{max} 3076, 2940, 2875, 1740, 1663, 1235, 1042, 884 cm^{-1}; UV (MeOH) λ_{max} 243 (ε 10,800); HRFABMS 355.2262 $(M+H)^+$ (calcd for $C_{23}H_{31}O_3$ 355.2273); ^1H and ^{13}C NMR data in Table 1.

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