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A New γ-Dihydropyrone-Propionate from the Caribbean Sacoglossan *Tridachia crispata.*

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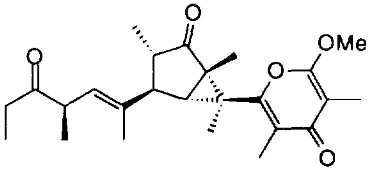
Abstract: A new polypropionate, tridachiahydropyrone (3), displaying an unprecedented bicyclic γ-dihydropyrone carbon skeleton, has been isolated from the Caribbean sacoglossan mollusc *Tridachia crispata*. The structure and the relative stereochemistry have been determined by spectroscopic methods.

Sacoglossan molluscs are very interesting models to investigate the ecology of benthic marine organisms.[1] They exhibit a complete evolutionary series of species from shelled molluscs (Oxynoidea and Cylindrobulloidea superfamilies) with a large or reduced shell to shell-less species (Elysioidea and Polybranchioidea superfamilies).[2] Many of them are able to sequester active chloroplasts from siphonaceous marine algae and to retain these organelles in their tissues where they carry out photosynthesis. Chemical studies of Pacific and Mediterranean Elysioidea sacoglossans have resulted in the isolation of diterpenoids, sequestered from green-algae and either accumulated or chemically modified,[3-5] as well as polypropionates *de novo* biosynthetized.[5-10]

In the course of our study, recently focused on Elysioidea sacoglossans, we have analyzed a *Tridachia crispata* population from Venezuela. Previous studies on the same mollusc collected at Belize and Panama[7] and at Jamaica[8] led to the finding of a group of propionate-derived γ-pyrones, crispatone (**1**) and crispatene (**2**) being the main metabolites. In this paper we report the structure elucidation of a polypropionate, tridachiahydropyrone (**3**), displaying a new bicyclic carbon skeleton.

Specimens of *T. crispata* were collected by SCUBA divers off Mochima (Venezuela) at a depth of 3-10 meters, during november 1993. They were immediately frozen and then transferred to Naples. The molluscs (25 individuals) were exhaustively extracted with acetone. The diethyl ether soluble fraction (g 1.16) of the acetone extract was analyzed by TLC, revealing the presence of a main UV positive spot (R_f 0.5, petroleum ether/diethyl ether, 9:1) together with other more polar minor metabolites. The liposoluble extract was fractionated by chromatographic column (SiO_2 gel, petroleum ether/diethyl ether gradient) and the fractions



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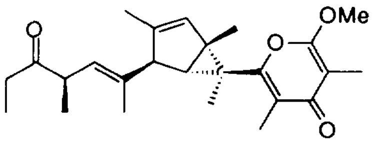

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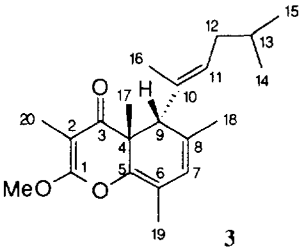
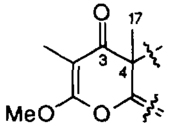
containing the main UV sensitive metabolite were combined and further purified by preparative TLC (benzene/petroleum ether, 8:2) to give pure tridachiahydropyrone (**3**, 15.8 mg).

Tridachiahydropyrone (**3**): $[\alpha]_D$ -476.1° ($CHCl_3$, c=0.49); m.p. 78-82 °C (*n*-hexane); IR (liquid film) ν_{max} 1592, 1659 cm^{-1}; UV (MeOH) λ_{max} 271 (ε=10,840) nm; EIMS, *m/z* (%): 330 (M^+, 30), 315 (93), 255 (21), 243 (91), 233 (76), 216 (33), 201 (31) 173 (100); HREIMS, *m/z* 330.2174 ($C_{21}H_{30}O_3$ requires 330.2195).

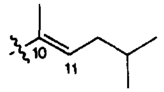


3

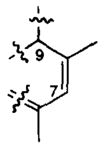
The molecular formula of **3**, $C_{21}H_{30}O_3$, was determined by HREIMS of the molecular ion observed at *m/z* 330.2174. The ^1H-NMR spectrum immediately suggested a polypropionate skeleton. In fact, it exhibited signals for eight methyls [a 3H singlet at δ 3.96, three 3H singlets at δ 1.20, 1.53 and 1.75, a 6H singlet at δ 1.63 and a 6H doublet (J=5.9 Hz) at δ 0.88], two olefinic protons (a singlet at δ 5.44 and a multiplet at δ 5.51), two methine protons (a singlet at δ 3.91 and a multiplet at δ 1.64) and, finally, two methylene protons [a double doublet at δ 1.90 (J=5.7 and 5.9 Hz)]. The presence of an α-methoxy-β-methyl-γ-pyrone system (partial structure **a**) was indicated by some typical ^{13}C-NMR resonances according to the presence of a conjugated carbonyl (C-3, δ 195.79), two quaternary carbons (C-2, δ 87.88 and C-1, δ 165.97), two methyl signals (MeO-, δ 55.08 and C-20, δ 7.40), attributable respectively to the methoxy group and to the β-vinyl methyl of the γ-pyrone system. A strong IR band at 1592 cm^{-1} and an UV absorption at 271 nm (ε=10,840) further supported this hypothesis. The ring was completed by two carbons (C-4, δ 46.56 and C-5, δ 145.33), the first bearing a methyl group (C-17, δ ^{13}C 21.21, δ ^1H 1.20).The long-range couplings of C-3, in the HMBC experiment, with both H_3-17 and H_3-20 was diagnostic for linking C-4 to C-3. ^1H-^1H COSY and ^1H-^1H decoupling experiments connected most of the remaining proton resonances (Table). In particular, according to the partial structure **b**, the olefinic proton at δ 5.51 (H-11), long-range coupled with a vinyl methyl at δ 1.53 (H_3-16), was linked to the methylene at δ 1.90 (H_2-12), which was in turn coupled with a methine (H-13, δ 1.64) of an isopropyl group (H_3-14 and H_3-15, δ 0.88). The *E* stereochemistry of the double bond was suggested by chemical shift of the vinyl methyl (C-16, δ 13.71). Finally, the partial structure **c** was supported by the following evidence: the signal at δ 5.44 (H-7) showed in the ^1H-^1H COSY experiment cross-peaks with signals at δ 1.63 (H_3-18) and δ 1.75 (H_3-19), attributed to two vinyl methyls, suggesting the presence of a conjugated substituted diene system. Furthermore, the broad methine singlet at δ 3.91 (H-9) displayed long-range couplings with both the methyl at δ 1.63 (H_3-18) and the olefinic proton at δ 5.44 (H-7).



a



b



c

The partial structures **a**, **b** and **c** were supported by all ^1H- and ^{13}C-NMR resonances that were coupled by HMQC experiment (Table). Diagnostic long-range ^1H-^{13}C couplings in HMBC experiments (J=6 and 10 Hz) allowed to connect the partial structures **a**-**c**. In fact, the methine carbon of moiety **c** at δ 53.45 (C-9) displayed the expected long-range couplings with the protons at C-7 and C-18 but also cross peaks with the signals at δ 1.53 (H_3-16) and 5.51 (H-11), diagnostic to link C-10 of **b** to C-9 of **c**. Finally, an additional coupling between C-9 and H_3-17 linked C-4 of **a** to C-9 of **c**, whereas the long-range couplings between C-5 and H_3-19 linked C-5 of **a** to C-6 of **c**, leading to structure **3**.

Table - NMR Data[a] for tridachiahydropyrone (**3**)

Position	δ^1H	$\delta^{13}C$	m	Long-range connectivities[b]
1	----	165.97	s	H_3-20; OMe
2	----	87.88	s	H_3-20
3	----	195.79	s	H-9; H_3-17; H_3-20
4	----	46.56	s	H-9; H_3-17
5	----	145.33	s	H-7; H-9; H_3-17; H_3-19
6	----	115.74	s	H_3-19
7	5.44	121.28	d	H-9; H_3-18; H_3-19
8	----	134.42	s	H-9; H_3-18
9	3.91	53.45	d	H-7; H-11; H_3-16; H_3-17; H_3-18
10	----	133.42	s	H-9; H_2-12; H_3-16
11	5.51	130.37	d	H-9; H_2-12; H_3-16
12	1.90	37.24	t	H_3-14; H_3-15; H-11
13	1.64	28.72	d	H_3-14; H_3-15; H_2-12
14	0.88	22.59	q	H_2-12
15	0.88	22.47	q	H_2-12
16	1.53	13.71	q	H-9; H-11; H_2-12
17	1.20	21.21	q	H-9
18	1.63	21.72	q	H-7; H-9
19	1.75	14.62	q	H-7
20	1.63	7.40	q	----
-OMe	3.96	55.08	q	----

[a] Bruker AMX 500 MHz, $CDCl_3$, δ values (ppm) referred to $C\underline{H}Cl_3$ (δ 7.26) for proton and to $\underline{C}DCl_3$ (δ 77.00); [b] by HMBC experiments (J =6 and 10 Hz).

A series of n.O.e. experiments confirmed the proposed structure also revealing some stereochemical details. The relative configuration of C-4 and C-9 was suggested by a diagnostic n.O.e. effect between H-9 (δ 3.91) and H_3-17 (δ 1.20), that supported the same orientation for the methyl at C-5 and H-9. Strong n.O.e. effects were also observed between H_3-16 (δ 1.53) and H_2-12 (δ 1.90), according to the *E* stereochemistry of the alkyl chain double bond, and between H-9 and H-11, suggesting a favoured conformation with the vinyl proton sterically near to H-9. Irradiation of H-7 (δ 5.44) induced positive enhancement of the methyl signals at δ 1.63 (C-18) and δ 1.75 (C-19), confirming the assignments made. Finally, steric interactions were also observed between H_3-19 and the methyl of methoxy group (δ 3.96).

The carbon skeleton of **3** displays some interesting structural features, in particular the biogenetically anomalous alkyl chain and the γ-dihydropyrone ring. Apparently, the alkyl chain does not follow the biogenetic propionate rule, probably one of the terminal methyls is shifted from C-12 to C-13. Propionates containing a γ-dihydropyrone ring are very rare in marine organisms, they have been previously reported for the Pacific pulmonate mollusc *Siphonaria maura*[11-12] and, more recently, for the Mediterranean notaspidean opisthobranch *Pleurobranchus membranaceus*.[13] However, the carbon skeleton of **3** could derive from the cyclization of the lateral alkyl chain with a γ-pyrone system. Bearing in mind that the suggested biosynthesis of **1** and **2** from a regular polypropionate has been justified as a defensive mechanism in order to protect the mollusc from a too prolonged exposition to sun rays,[6] the synthesis of **3** in this population of *T. crispata* could play an analogous ecological role.

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