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Novel Pseudopteranoids of Pseudopterogorgia acerosa

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Abstract: The structures of seven pseudopteranoids isolated from the Caribbean octocoral Pseudopterogorgia acerosa have been investigated by 2-D NMR spectroscopy using our FLOCK pulse sequence to determine n-bond connectivities. Six of the compounds (2 - 7) are new.

The marine soft corals have excited a considerable amount of interest because of the diversity and complexity of their secondary metabolites, many of which are potentially valuable chemotherapeutic agents.[1] The common Caribbean octocoral *Pseudopterogorgia acerosa* (Pallas) was investigated in the laboratories of Fenical and Clardy[2] because extracts showed considerable cytotoxic activity. The major metabolite isolated from these extracts was the novel diterpenoid pseudopterolide, the first pseudopterane; a derivative of pseudopterolide, described as the pseudopterolide-methanol adduct 1, was also isolated from specimens stored in methanol. The same adduct was obtained by the action of acidic methanol on pseudopterolide. We subsequently investigated extracts of *P. acerosa* collected around the coast of Tobago.[3] We described several new diterpenoid metabolites, and we noted that there were considerable differences in the distribution of metabolites in specimens collected at different seasons. We now describe seven pseudopteranoids isolated from a collection made in August 1987.

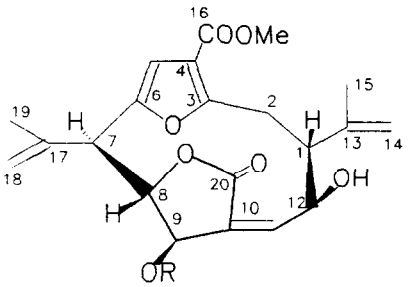
Isolated pseudopteranoids corresponded to about 0.2% of the dry weight of the specimen. The most abundant (43.2%) was identified as the pseudopterolide-methanol adduct 1 of Fenical and Clardy,[2] but pseudopterolide itself was not isolated. We note that our material was not stored in methanol (acetone was used), and methanol (85% aqueous) was used only in the final purification by reverse-phase HPLC. The methoxy signals of 1 (and 4 described below) were present in the NMR spectra of the mixture before chromatography, ruling out the possibility that these compounds were artifacts of the purification process. Two-dimensional NMR spectroscopy was used to make structural and spectroscopic assignments for all of the isolated compounds; one-bond ^{13}C-^{1}H connectivities were established by a standard HETCOR experiment, and longer-range *n*-bond connectivities were established from spectra

obtained using our FLOCK pulse sequence.[4] This pulse sequence, when appropriately optimized, provides a wealth of 2- and 3-bond connectivities that are usually more than sufficient for making complete structural and spectroscopic assignments since there is often a considerable amount of redundancy in the data, defining structural units in several different but self-consistent ways. The ^{13}C and ^{1}H chemical shifts that have been assigned for all of the compounds (**1** to **7**) by these procedures are gathered in Tables 1 and 2, respectively. Table 3 provides a list of ^{1}H-^{1}H coupling constants of stereochemical significance.

The next most abundant (21.9%) constituent is a new compound to which we have assigned structure **2** and the name gorgiacerone. The compound was isolated as a colorless gum, $C_{21}H_{22}O_6$ (determined by HREIMS), with infrared absorption at 1760, 1716, and 1713 cm^{-1}, suggesting the presence of an unstrained ketone moiety in addition to the unsaturated γ-lactone and ester (β-furoate) groups common to many of these metabolites. The NMR connectivity data establish structure **2** unequivocally; of particular importance was the observation that the ketonic carbon at δ_C 202.81 showed connectivities to protons at C-2 and C-11, leading to the assignment of the carbonyl to C-12.

The third isolated constituent (14.8%) was also a colorless gum, $C_{21}H_{24}O_7$ (by HREIMS), that was assigned structure **3** (a gorgiacerodiol) on the basis of NMR data. The observed connectivities establish the structure unambiguously, but the stereochemistry is more difficult to assign on the basis of NMR data alone. Vicinal ^{1}H-^{1}H coupling constants provide evidence for dihedral angles at stereochemically significant sites (see Table 3), but molecular models of the macrocyclic ring can be manipulated to provide the required dihedral angles in more than one way. The action of acetic anhydride and pyridine on **3** converts it to its diacetate, which is identical with the major diacetate obtained from tobagolide.[3] Tobagolide and its derivatives have been correlated with pseudopterolide in several ways,[3,5] and we have considered the relative configuration at C-12 to be established from these correlations. The coupling constants between the C-11/C-12 and C-12/C-1 pairs of vicinal protons are all small (<2.5 Hz) in **3** and its diacetate, and a model with suitable dihedral angles can be constructed if the oxygen functions have a *cis* relationship. The two OH signals in the nmr spectrum of **3** are very different; the C-11 OH signal (δ 4.18) is well resolved, showing coupling to the C-11 proton (δ 4.65), while the C-12 OH appears at higher field (δ 2.68) as a very broad signal. On dilution of the sample, the C-11 OH signal also broadens, indicating that it is involved with intermolecular hydrogen bonding at higher concentrations.

The fourth compound (10.5%), also non-crystalline, $C_{22}H_{26}O_7$ (by HREIMS), was assigned structure **4** (a methoxygorgiacerol) from the nmr data. This compound corresponds to an allylic-rearrangement isomer of the major constituent, **1**. Its stereochemistry is presumed to correspond to that of **3**. The C-11/C-12 protons exhibit a slightly larger vicinal coupling (3 Hz), but the major difference is seen at the C-12 OH signal, which is a well-resolved doublet (11.2 Hz) resulting from coupling with the C-12 proton. The next constituent (6.5%) was obtained as colorless crystals, mp 180-181 °C, and showed infrared absorption at 1754, 1742, and 1722 cm^{-1}. This was assigned structure **5** (a diepoxygorgiacerone) from the NMR data, and this structure was confirmed by single-crystal X-ray crystal structure analysis; a preliminary account of this compound has been published.[6] Since its relative configuration is secure, its NMR parameters provide a benchmark for stereochemical assignments made to the other compounds from NMR data. The presence of an unusually stable diepoxyfuran moiety is a noteworthy feature of this structure. Diepoxyfurans are normally very susceptible to solvolytic reactions



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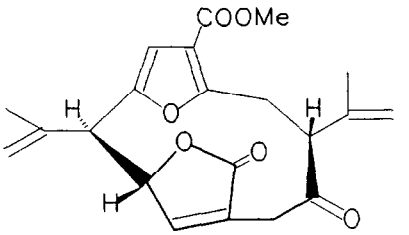

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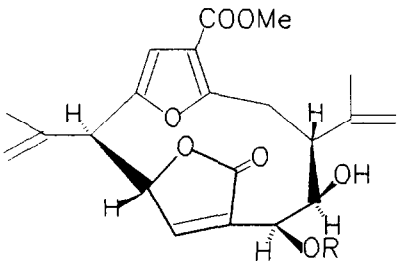

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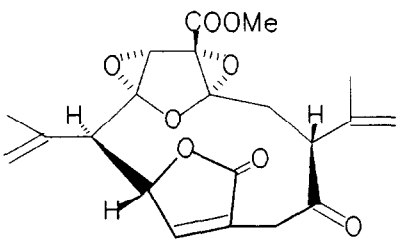

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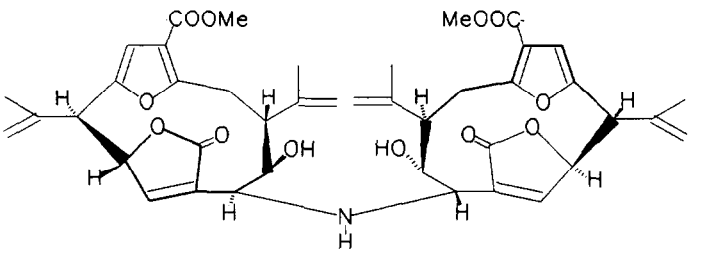

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Table 1: ^{13}C Chemical Shifts of Compounds 1 to 7

Position	1	2	3	4	5	6	7
1	50.34	58.47	43.24	42.88	55.35	50.32	43.63
2	29.34	28.19	31.49	31.86	30.09	29.19	32.23
3	160.31	159.19	160.47	160.71	101.09	160.17	160.74
4	116.12	114.16	116.14	116.20	59.77	116.25	116.16
5	111.97	110.47	110.43	110.09	54.17	112.06	110.07
6	150.75	149.25	150.17	150.29	96.03	150.70	150.16
7	47.91	48.22	48.11	48.25	47.95	47.82	48.24
8	86.43	82.27	81.47	79.91	81.62	87.96	80.25
9	81.32	152.49	147.64	151.12	154.74	73.22	151.29
10	130.12	126.23	131.84	133.53	125.13	133.15	133.40
11	143.19	39.47	74.50	83.06	38.04	141.08	62.00
12	69.84	202.81	77.36	76.36	200.61	69.51	76.67
13	145.15	139.69	144.31	144.52	140.04	144.92	145.40
14	114.05	115.91	116.62	116.20	115.91	114.21	115.99
15	18.72	19.48	22.95	23.91	18.70	18.70	22.92
16	163.72	163.77	163.75	163.85	164.47	163.75	163.81
17	139.09	140.94	140.72	141.23	140.98	139.11	140.97
18	115.95	116.10	115.45	115.32	115.60	115.88	115.36
19	21.91	22.35	21.51	21.55	24.82	21.92	21.51
20	170.99	173.13	174.38	170.67	172.90	171.38	172.23
(CO)O$\underline{C}H_3$	51.69	51.42	51.59	51.53	53.30	51.72	51.56
O$\underline{C}H_3$	55.03[a]	--	--	58.10[b]	--	--	--

[a] C-9 methoxyl [b] C-11 methoxyl

Table 2: ^{1}H Chemical Shifts of Compounds 1 to 7

Position	1	2	3	4	5	6	7
1	3.54	3.52	3.25	3.29	3.24	3.51	3.26
2	3.59 2.62	3.40 3.24	3.68 2.77	3.57 2.81	3.05 1.64	3.58 2.61	3.62 2.68
5	6.41	6.34	6.38	6.36	4.20	6.40	6.33
7	3.70	3.68	3.88	3.84	3.29	3.67	3.77
8	5.05	5.42	5.51	5.43	5.41	5.02	5.34
9	3.66	7.32	6.81	6.98	7.37	4.14	6.79
11	5.95	3.84 3.16	4.65	4.32	3.84 3.12	5.93	3.66
12	4.00	--	3.04	2.92	--	3.98	3.11
14	5.00 4.96	5.08 4.99	5.18 5.11	5.21 5.08	4.96 4.92	4.99 4.98	5.12 5.06
15	1.82	1.78	1.96	2.01	1.62	1.81	1.98
18	5.07 4.88	5.18 5.17	5.05 4.82	5.04 4.82	5.33 5.04	5.08 4.88	5.03 4.78
19	1.92	1.96	1.96	1.97	1.84	1.94	1.94
(CO)OC<u>H</u>$_3$	3.82	3.79	3.80	3.80	3.89	3.84	3.79
OC<u>H</u>$_3$	3.15[a]	--	--	3.34[d]	--	--	--
O<u>H</u>	4.42	--	4.18[b] 2.68[c]	2.55[c]	--	4.59[c] 2.08[c]	3.32[c]
N<u>H</u>	--	--	--	--	--	--	3.04[f]

[a] C-9 methoxyl [b] C-11 hydroxyl [c] C-12 hydroxyl [d] C-11 methoxyl [e] C-9 hydroxyl [f] C-11 NH

Chemical shifts listed in Tables 1 and 2 are for solutions in $CDCl_3$. The assignments are based on ^{13}C-^1H one-bond and *n*-bond (*n* = 2 or 3) shift correlation experiments. The procedures used have been described in detail previously[3].

Tables of connectivity data are available from the authors at the Toronto address.

Table 3: Selected ^1H-^1H Coupling Constants

Protons	$J_{X,Y}$ (Hz)						
X,Y	1	2	3	4	5	6	7
1,2a	13	11	13	13	10	13	13
1,2b	2	2	4	4	<1.5	3	4
2a,2b	15	15	15.5	16	15	15	16
7,8	4	3	4.5	4	<1.5	4	<1.5
8,9	<1.5	<1.5	1.5	<1.5	<1.5	<1.5	<1.5
11,12	7	--	<1.5	3	--	7	4
12,1	10	--	<1.5	<1.5	--	10	<1.5

that open the epoxy rings,[7,8] but the steric constraints imposed by the macrocyclic ring of 5, preventing a nucleophile from approaching the C-O bond on a reactive trajectory, provide an explanation for the observed stability.

The remaining two compounds were isolated in smaller amounts (1.7% and 1.5%), but they were obtained crystalline. The first, mp 174-176 °C, $C_{21}H_{24}O_7$ (by HREIMS), was assigned structure 6 (isogorgiacerodiol), an allylic-rearrangement isomer of 3, on the basis of the NMR data. It may be noted that the UV spectrum included a peak at 274 nm, characteristic of the isomers with the double bond exocyclic to the γ-lactone ring. It also corresponds to 1 ("pseudopterolide-methanol adduct") except that H_2O has, formally at least, taken the place of MeOH. The close correspondence of the nmr spectra (especially ^{13}C) of these compounds may also be noted, with the small differences being those expected on replacement of OMe by OH. Examination of the data reveals that moving the double bond from the 9,10 to the 10,11 location leads to substantial spectroscopic changes, especially in the NMR and UV as cited above, that are of clear diagnostic value, and the optical rotation also undergoes a profound change.

The final compound, mp 248-249 °C, $[\alpha]_D$ +40°, at first presented a number of puzzling features. The ^{13}C and ^1H NMR spectra appeared to indicate a C_{21} compound generally similar to the other compounds isolated from the extract (see Tables 1 and 2); signals in the ^1H spectrum were, however, unusually broad. A careful examination of the mass spectrum then provided an explanation: the molecular formula is $C_{42}H_{47}O_{12}N$, corresponding to two diterpenoid units combined with one unit of ammonia. The NMR spectra and optical activity establish that the two C_{21} units are structurally and configurationally identical. The broadening of the ^1H signals can be attributed to the reduced rate of tumbling of this large molecule in solution. Despite the rather small amount of material available and the problems associated with the size of the molecule, the structure was assigned as 7 by the procedures used for the other compounds. All of the cross peaks necessary to determine connectivities within each diterpenoid unit were observed; a comparison of the ^{13}C and ^1H NMR spectra with those of 3 and 4 is also informative. If the latter compounds can be viewed as being formally the products of H_2O and

MeOH to a biosynthetic precursor, 7 can be seen as the product of addition of NH_3 to give the amino analog of 3, followed by the addition of this amine to a second C_{21} precursor unit.

We have previously noted that the distribution of metabolites isolated from this species have shown variations that appeared to depend on the season. We now can report that a collection of the octocoral made in March 1990 has afforded five of the metabolites described above from the August 1987 collection; the two not found were 2 and 7. It is apparent that other variables also affect the production of the metabolites, but we have no evidence at present concerning their nature. There seems little doubt that the compounds are links in a biosynthetic chain that is concerned with changing the oxidation level of the pseudopteranes, but the chronological order of the chain is not established. Questions of chemical and biosynthetic processes remain for the future, as do those of biological control and function.

EXPERIMENTAL

Pseudopterogorgia acerosa (dried weight 675 g) was collected in August 1987 at Lau's reef (-10m), Tobago. The collection and identification were made by Mr. R. S. Laydoo of the Institute of Marine Affairs, Chaguaramas, Trinidad. The sample was immediately stored in acetone for transportation. In the laboratory it was blended with fresh acetone (12 L), and the solvent was evaporated to provide an aqueous suspension, which was thoroughly extracted with chloroform. The extract yielded a dark viscous oil (91 g), which was chromatographed on silica gel with 3:1 hexane-acetone. Six major fractions were obtained, and the middle fractions were subjected to reverse-phase HPLC on a C-18 column with 85:15 MeOH-H_2O to provide the following compounds.

Melting points were determined on a micro hot stage. Selected IR absorptions (FTIR) are reported (in cm^{-1}). UV spectra were obtained for MeOH solutions; $\lambda_{max}(\epsilon)$ values are reported (in nm). NMR spectra were obtained for $CDCl_3$ solutions; ^1H measurements were made at 400 MHz and ^{13}C measurements were made at 100.6 MHz.

Pseudopterolide-methanol adduct (1): colorless gum (535 mg), $[\alpha]_D$ -116.6° (*c* 0.53, $CHCl_3$); IR ($CHCl_3$) 3440, 1745, 1717; UV 221 (8000), 235 (6500), 275 (3600); EIMS 402 (20), 370 (34), 338 (18), 259 (27), 246 (100), 215 (41), 195 (45), 133 (28); *Exact mass*: 402.1705, calcd. for $C_{22}H_{26}O_7$ 402.1679.

Gorgiacerone (2): colorless gum (271 mg), $[\alpha]_D$ -184° (*c* 0.36, $CHCl_3$); IR ($CHCl_3$) 1760, 1716, 1713; UV 220 (10,000), 250 (5400); EIMS 370 (24), 338 (25), 310 (10), 246 (21), 231 (7), 214 (22), 191 (100), 133 (35); *Exact mass*: 370.1423, calcd. for $C_{21}H_{22}O_6$ 370.1416.

Gorgiacerodiol (3): colorless gum (183 mg), $[\alpha]_D$ +98° (*c* 0.29, $CHCl_3$); IR ($CHCl_3$) 3501, 1735, 1716; UV 244 (4700); EIMS 388 (35), 370 (6), 356 (60), 339 (21), 259 (67), 246 (100), 215 (80), 193 (80); *Exact mass*: 388.1498, calcd. for $C_{21}H_{24}O_7$ 388.1522.

Methoxygorgiacerol (4): colorless gum (130 mg), $[\alpha]_D$ +124.4° (*c* 0.75, $CHCl_3$); IR ($CHCl_3$) 3450, 1764, 1715; UV 219 (10,000), 250 (7000); EIMS 402 (18), 384 (8), 370 (82), 319 (57), 276 (39), 247 (56), 192 (100), 133 (42); *Exact mass*: 402.1681, calcd. for $C_{22}H_{26}O_7$ 402.1679.

Diepoxygorgiacerone (5): colorless crystals (80 mg), mp 180-181 °C, $[\alpha]_D$ -319° (*c* 0.27, $CHCl_3$); IR (KBr) 1754, 1742, 1722; UV 291 (2900); EIMS 402 (23), 343 (12), 315 (16), 247 (24), 229 (56), 201 (59), 123 (79), 95 (100); *Exact mass*: 402.1310, calcd. for $C_{21}H_{22}O_8$ 402.1315.
Isogorgiacerodiol (6): colorless crystals (21 mg), mp 174-176 °C, $[\alpha]_D$ -60.5° (*c* 0.09, $CHCl_3$); IR ($CHCl_3$) 3402, 1740, 1718; UV 226 (5800), 234 (5500), 274 (2700); EIMS 388 (18), 370 (15), 356 (60), 338 (20), 305 (30), 276 (53), 192 (100), 133 (67); *Exact mass*: 388.1519, calcd. for $C_{21}H_{24}O_7$ 388.1522.
Bis(gorgiacerol)amine 7: colorless crystals (19 mg), mp 248-249 °C, $[\alpha]_D$ +40° (*c* 0.3, $CHCl_3$); IR ($CHCl_3$) 3400, 1750, 1714; UV 215 (14,400), 248 (8400); EIMS 757 (25), 674 (15), 632 (16), 549 (25), 388 (12), 370 (16), 275 (100); *Exact mass*: 757.3100, calcd. for $C_{42}H_{47}O_{12}N$ 757.3098.

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