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Chemistry

The pseudopterosins: Anti-inflammatory and analgesic natural products from the sea whip *Pseudopterogorgia elisabethae*

(marine natural products/diterpenoid glycosides/anti-inflammatory drugs)

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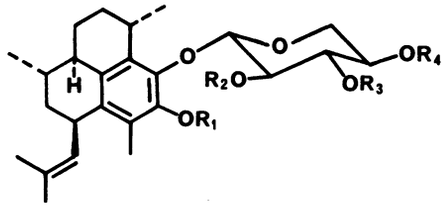
ABSTRACT **The Caribbean sea whip *Pseudopterogorgia elisabethae* (Octocorallia, Cnidaria) has been found to contain the pseudopterosins, a newly described class of natural products, which have been characterized as diterpene-pentose-glycosides. The pseudopterosins possess anti-inflammatory and analgesic properties that exceed, in our assays, the potencies of existing drugs such as indomethacin. As anti-inflammatory agents, the pseudopterosins appear to modify the arachidonic acid cascade by an as yet undefined mechanism of pharmacological action.**

As part of a continuing program to explore the chemical adaptations of marine organisms and to assess the biomedical applications of marine metabolites, we have focused considerable attention on the chemically rich sea fans and whips (gorgonian soft-corals) found in the Caribbean Sea (1–5). During an expedition on board the research vessel *Calanus*,[a] our attention was drawn to several sea whips of the genus *Pseudopterogorgia* found generally at depths in excess of 20 m. The highly branched *Pseudopterogorgia elisabethae* was of particular interest since ship-board chemical and biological assays revealed this animal to contain large quantities of unknown, polar-lipid metabolites that were apparently coupled with strong inhibitory activities in field-oriented antimicrobial and cytotoxicity assays.[bc] Subsequent mass collection, extraction, and fractionation of the extract of *P. elisabethae* has now resulted in the isolation of pseudopterosins A–D (**1–4**), highly bioactive diterpenoid glycosides, which are responsible for these potent activities.



(A) **1** $R_1R_2R_3R_4 = H$

(B) **2** $R_1R_3R_4 = H$; $R_2 = Ac$

(C) **3** $R_1R_2R_4 = H$; $R_3 = Ac$

(D) **4** $R_1R_2R_3 = H$; $R_4 = Ac$

5 $R_1R_2R_3R_4 = Ac$

Scheme I

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Chemistry of the Pseudopterosins

Purification of pseudopterosins A–D was achieved by sequential silica gel chromatography including HPLC. Pseudopterosin A (**1**), the most polar metabolite, was assigned the molecular formula $C_{25}H_{36}O_6$ by high-resolution mass spectrometry and by analysis of ^{13}C NMR data (Table 1). The eight degrees of unsaturation in this molecular formula were assigned to a fully substituted benzene ring, one trisubstituted olefinic bond (*gem*-dimethyl), a cyclic hemiacetal, and two carbocyclic rings on the basis of NMR and other spectral data.[d] Characteristic ^{13}C NMR features, infrared spectral data for hydroxyl (*ca.* 3400 cm^{-1}), and a base-induced bathochromic shift of the UV absorption of **1** showed this metabolite to be phenolic.

Acetylation of pseudopterosin A (Ac_2O/py/RT) yielded a tetraacetate, **5**, which proved that **1** contained at least four hydroxyl groups. In a consistent fashion, acetylation of each of the pseudopterosins, B(**2**), C(**3**), and D(**4**), produced the same tetraacetate, **5**, in nearly quantitative yield. Since the molecular formulas of these latter compounds were that of pseudopterosin A ($C_{25}H_{36}O_6$) + C_2H_2O, the less polar pseudopterosins were concluded to be monoacetate positional isomers of **1**.

Inspection of the high resolution mass spectrometry data for **1** showed that the base peak represented $M^+ - C_5H_8O_4$ fragmentation. This behavior, coupled with the presence of three nonphenolic hydroxyl groups and an acetal constellation, strongly suggested that **1** was a pentose-glycoside bonded with a C_{20} compound of probable terpenoid origin. Mild acid hydrolysis, which occurred with concomitant air oxidation, confirmed these suspicions and yielded D-xylose, $[\alpha]_D = +29°$, and the *o*-quinone **6** in good yield.[e]

The complete structure of pseudopterosin A (**1**) could not be unambiguously assigned on the basis of spectral and

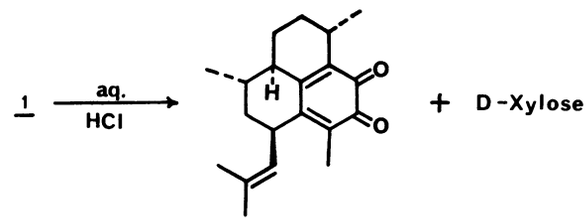
[a]The expedition of the research vessel *Calanus* (Univ. of Miami) took place in the central Bahama Islands in June 1982.

[b]Several of the pseudopterosins are active in agar-plate/disk-antimicrobial assays and in the urchin egg cytotoxicity bioassay (6, 7). These activities are quantitatively less important in relation to their anti-inflammatory and analgesic properties.

[c]Cytotoxicity was measured using the fertilized egg of the California sea urchin *Lytechinus pictus* (Verrill). Pharmacological aspects of this assay have been discussed (6, 7).

[d]Pseudopterosin A (**1**), an amorphous solid, showed $[\alpha]_D = -85°$ (*c* 0.69, $CHCl_3$) and had the following spectral characteristics: UV λ_{max} (MeOH) 230 nm (ε = 11,200), 278 nm (ε = 2060), 283 nm (ε = 2200), and after base addition: 247 nm, 285 nm, and 295 nm, respectively; IR ($CHCl_3$) 3500, 3030, 2920, 1200 cm^{-1}; HRMS: M^+ *m/z* (relative intensity) observed 432.2491 (1.1), $C_{25}H_{36}O_6$ requires 432.2512, 300.2088 ($C_{20}H_{28}O_2$, 100), 285.1852 ($C_{19}H_{25}O_2$, 72).

[e]D-Xylose was identified by comparison with an authentic sample treated in precisely the same manner, $[\alpha]_D = +23°$. The *o*-quinone was identified on the basis of its spectral properties. Details of the spectroscopy, chemistry, and x-ray crystallographic experiments will be presented in a forthcoming comprehensive publication.



6

Scheme II

chemical methods alone. Hence, the final structure assignments of **1** and of the remaining pseudopterosins were achieved on the basis of an x-ray structural assignment of the only crystalline metabolite, pseudopterosin C(**3**). This experiment is summarized in Fig. 1, a computer generated perspective drawing of the final x-ray model. The earlier identification of the sugar component as D-xylose allowed the absolute stereochemistry of **3**, as well as those of the other pseudopterosins, to be fully assigned.

Bioactivity of Pseudopterosin A

Pseudopterosin A possesses potent anti-inflammatory and analgesic activities that appear to be previously uncharacterized properties of this class of compounds. When applied to the skin of mice, pseudopterosin A is significantly more potent than indomethacin in blocking phorbol myristate acetate-induced topical inflammation (Fig. 2*A*, $K = 8.93 \times 10^{-4}$ M for pseudopterosin A versus 40 mM for indomethacin). *In vitro* studies suggest a unique, as yet unidentified, mechanism of pharmacological action for this compound. Concentrations of >100 μM do not inactivate hydrolysis of phosphatidylcholine by bee venom or by liver microsomal phospholipase A_2. Pseudopterosin A has been shown, however, to inhibit pancreatic phospholipase A_2 (IC_{50} = 3.0 μM) *in vitro*. In other experiments, inactivation of pancreatic phospholipase A_2 was achieved at much higher concentrations (IC_{50}, > 80 μM). Prostaglandin E_2 and leukotriene C_4 release was observed in response to zymosan stimulation in mouse peritoneal macrophages, indicating that both the cyclooxygenase and lipoxygenase pathways are effected by pseudopterosin A. As an analgesic, administered subcutaneously, pseudopterosin A is several times more potent than

Table 1. ^{1}H and ^{13}C NMR assignments for pseudopterosin A (**1**)

C no.	^{1}H	^{13}C	C no.	^{1}H	^{13}C
1	3.56 (1H, m)	26.8	16	1.73 (3H, s)‡	25.6
2	*	39.4	17	1.64 (3H, s)‡	10.9
3	1.47 (1H, m)	30.5	18	1.02 (3H, d,	
4	3.59 (1H, bs)	35.6‡		J = 5.7)	23.0
5	*	27.5‡	19	1.12 (3H, d,	17.6
6	*	29.3‡		J = 7.4)	
7	3.53 (1H, m)	41.7‡	20	1.99 (3H, s)	20.9
8	—	133.9‡	1′	4.49 (1H, d,	
9	—	140.8		J = 7.2)	
10	—	144.4	2′	3.73 (1H, m)†	75.9
11	—	121.2	3′	3.76 (1H, m)†	74.0
12	—	135.4‡	4′	3.75 (1H, m)†	69.5
13	—	128.8‡	5′	3.14 (1H, d, d,	
14	5.10 (1H, d,			J = 10.5, 10.2)	65.9
	J = 8.2)	129.8		3.95 (1H, d, d,	
15	—	129.6‡		J = 5.4, 10.4)	

The ^{1}H NMR spectrum was recorded at 360 MHz in C^2HCl_3. Assignments were aided by spin-decoupling experiments. J values are reported in Hz, and chemical shifts are given in δ units (ppm downfield from tetramethylsilane). The ^{13}C NMR spectrum was recorded in C^2HCl_3 at 50 MHz. Multiplicities were obtained by single frequency off resonance decoupling, and assignments were made based on J_R values when applicable and/or comparison to models.
*Nonassignable proton resonances.
†Peak broadening in the spectrum run at room temperature (20°C) did not allow for an assignment of J values.
‡Signals within a column may be reversed.

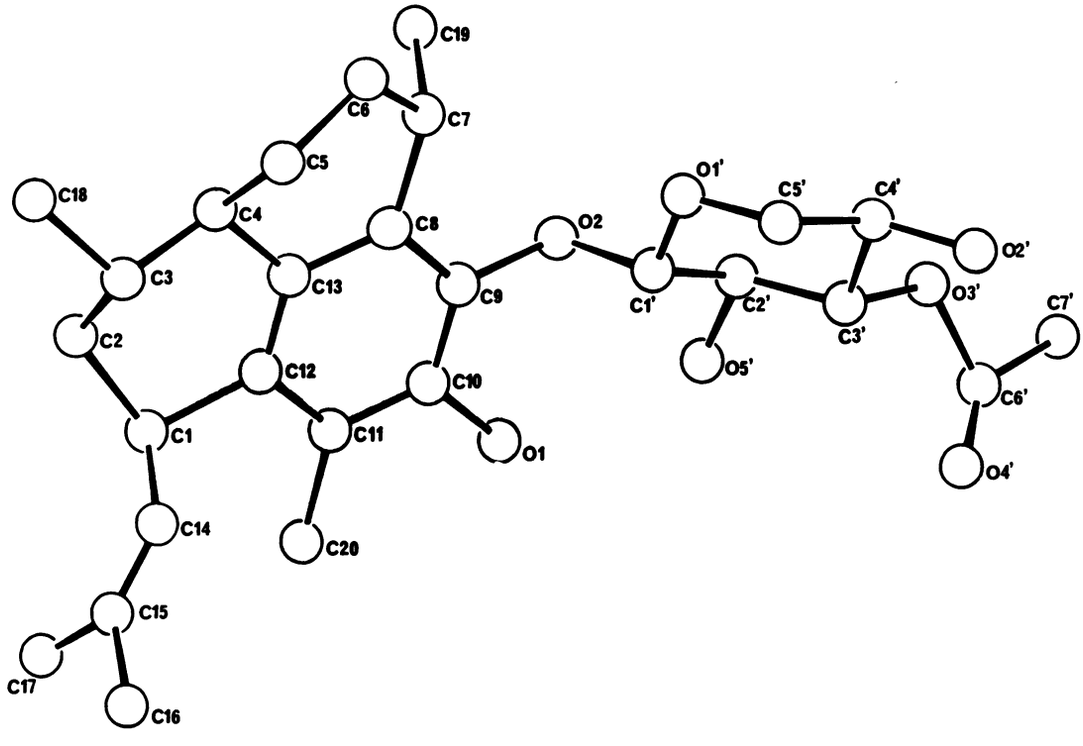


FIG. 1. Computer-generated perspective drawing of pseudopterosin C. Hydrogen atoms have been excluded, and the absolute configuration shown was chosen on the basis of chemical results presented in the body of the paper.

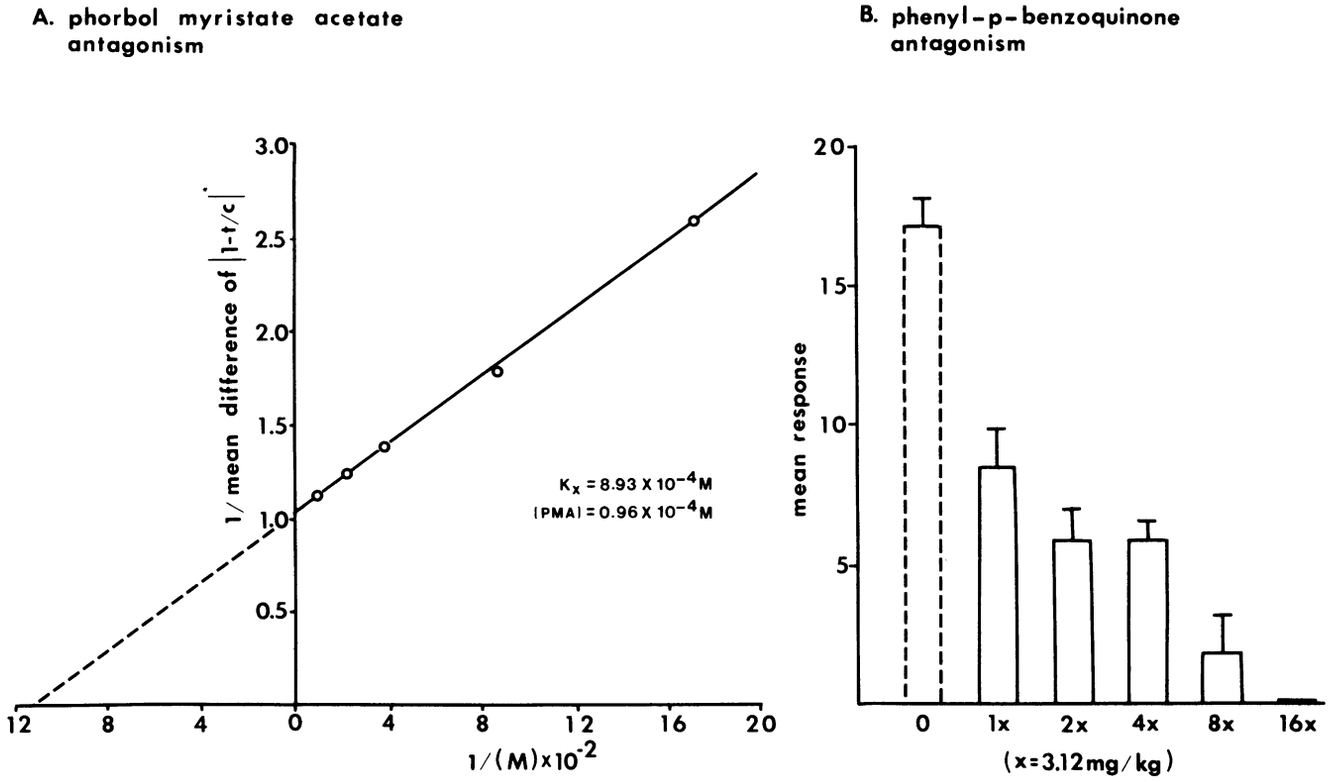


FIG. 2. (*A*) Double reciprocal plot of the inhibition of phorbol myristate acetate (PMA)-induced inflammation of the mouse ear by pseudopterosin A (1). The abcissa defines the molar concentration of pseudopterosin A, and the ordinate is the reduction of the degree of inflammation (t, ear weight in treated mice; c, ear weight of the untreated controls). (*B*) Antagonism of pseudopterosin A against the phenyl-quinone-induced stretch reflex in mice. The abcissa represents dose (mg/kg), and the ordinate is the mean number of responses.

indomethacin (i.e., the ED_{50} for pseudopterosin A is *ca.* 3.12 mg/kg versus *ca.* 10 mg/kg for indomethacin) in blocking the stretch–reflex response in mice induced by intraperitoneal injection of phenyl-quinone (8)[f] (Fig. 2*B*). *In vitro* data further indicate that pseudopterosin A neither mimics morphine in electrically driven preparations nor antagonizes histamine or bradykinin at concentrations up to 30 μM. Furthermore, pseudopterosin A did not prevent phorbol dibutyrate inhibition of bradykinin-induced contractions of the guinea pig ileum, an assay that is believed to be specific for pathways activated through phospholipase C (9). Hence, pseudopterosin A appears pharmacologically and qualitatively distinct from typical cyclooxygenase inhibiting nonsteroidal anti-inflammatory drugs.

On the bases of the experiments performed to date, the pseudopterosins appear to represent a newly described class of anti-inflammatory and analgesic agents. Although the putative mechanism of pharmacological action of these compounds is not yet fully defined, the pseudopterosins will no doubt prove to be important probes useful in defining the complex cellular responses associated with chemically produced pain and inflammation. There is a great need for additional drugs that control or modify the molecular signals that regulate the arachidonic acid biosynthetic cascade. Such drugs should prove useful in a variety of degenerative diseases that involve pain, inflammation, and the deterioration of membranes in which irregularities in phospholipid metabolism may be implicated.

[f]The ED_{50} for pseudopterosin in these assays was obtained at a dose of 3.12 mg/kg. For a comprehensive discussion of the methods involved see ref. 8.

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