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ISOLATION AND STRUCTURE OF ACEROPTERINE, A RARE PSEUDOPTERANE ALKALOID FROM THE CARIBBEAN SEA PLUME *PSEUDOPTEROGORGIA ACEROSA* (PALLAS).

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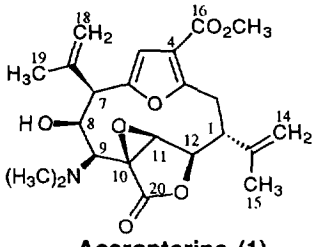
Abstract: Aceropterine (**1**) is a new tetracyclic pseudopterane alkaloid isolated from the purple sea plume *Pseudopterogorgia acerosa* collected in Puerto Rico. Its structure has been determined by spectroscopic methods. The unique transposed nature of the lactone function in **1** was established by two-dimensional ^{1}H-^{1}H and ^{1}H-^{13}C NMR correlation experiments and confirmed by mass spectral analysis.

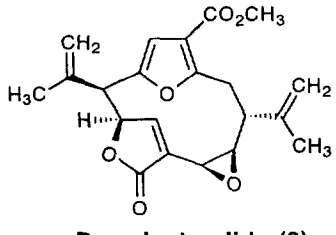
A large number of biologically active and structurally complex compounds have been found in marine organisms, especially in invertebrates. Caribbean gorgonian octocorals of the genus *Pseudopterogorgia* have been a well-recognized source of structurally unique and biologically active natural products.[2] Our anticancer drug discovery efforts have focused recently on a large collection of Puerto Rican specimens of *Pseudopterogorgia acerosa*, the crude extract of which was found to be cytotoxic against HeLa and CHO-K1 cell lines. Herein, we report the isolation and structure elucidation of a new marine natural product alkaloid, which we have named aceropterine (**1**).

Processing of a 6.7 kg batch of the *P. acerosa* crude $CHCl_3$/MeOH (1:1) extract by partitioning with hexane (6 X 1L; 368 g) and $CHCl_3$ (6 X 1L; 46.8 g), followed by two successive tiers of silica gel column chromatography of the $CHCl_3$ extract, gave **1** contaminated by known pseudopterolide (**2**).[3] This fraction was further purified by HPLC to yield 20.2 mg of **1** showing $[\alpha]_D$ = +0.44° (c= 2.0, $CHCl_3$).

High-resolution FAB MS (positive ion, 3-NBA+Na) gave a major $[M + Na]^+$ ion at *m/z* 454.1834 analyzing for $C_{23}H_{29}NO_7Na$ (0.8 mDa deviation). Hence, aceropterine, of $C_{23}H_{29}NO_7$ composition, possessed ten degrees of unsaturation, six of which were due to double bonds (Table 1) and four due to rings. An Attached Proton Test (APT) experiment established that the ^{13}C spectrum was comprised of 5 methyl, 3 methylene, 7 methine, and 8 non-protonated carbon signals, indicating that **1** contained 23 carbons and 28 carbon-bonded hydrogens. One exchangeable hydrogen had to be present and at least one nitrogen, since **1** had an odd molecular weight.[4] The presence of an exchangeable hydrogen was supported by a strong absorption at 3390 cm^{-1} in the IR spectrum[4] and the fact that the ^{1}H NMR spectrum in $CDCl_3$ revealed a signal (δ 4.97, d, 1H, J = 9.6 Hz) which lacked a ^{1}J-correlation with a ^{13}C signal in a HETCOR experiment. Further analysis of the NMR data (Table 1) indicated the



Aceropterine (1)



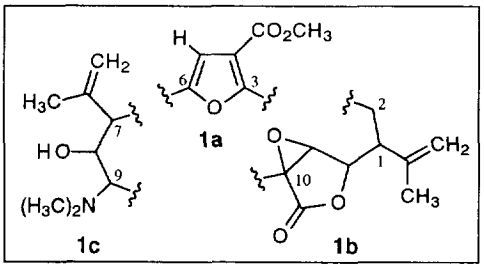
Pseudopterolide (2)

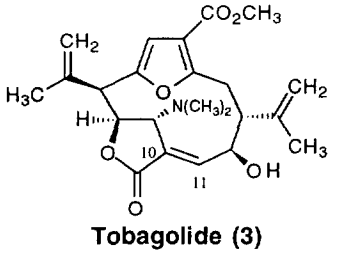
presence of two ester carbonyls (one a lactone carbonyl carbon), two terminal methylenes, and two inner double bonds (one trisubstituted and one tetrasubstituted).

The gross structure of **1** was determined by a detailed analysis of one and two dimensional NMR spectra (Table 1). The tracking of cross peaks in the ^{1}H, ^{1}H-COSY, and HETCOR NMR spectra led to three partial structures (**1a-c**) which are bounded by 3 quaternary oxygen-bearing carbons, one sp^3 and two sp^2 (Figure 1). The two ester units **1a-b** and the nitrogen-containing unit **1c** were connected together in the proper sequence by selective INEPT data. Confirmation of the structures of the three units as well as the sequencing was provided by HREIMS data (see Scheme 1).[4]

One of the ester units was a α,α'-disubstituted β-carbomethoxyfuran residue (**1a**). The signals at δ 163.6 (s), 156.9 (s), 150.4 (s), 115.6 (s), 110.3 (d), and 51.4 (q) in the ^{13}C NMR spectrum, coupled with the IR absorption at 1717 cm^{-1}, and two singlets in the ^{1}H NMR spectrum at δ 6.47 (1H) and 3.81 (3H), indicated that **1** possessed the same β-carbomethoxyfuran constellation found in **2**. The second ester unit was deduced to be a α,γ-disubstituted α,β-epoxy-γ-lactone unit (**1b**). COSY and CSCM analysis indicated that this unit possessed a hydrogen on the γ-carbon of the lactone [δ 4.70 (H12), 84.1 (C12)] that was connected to a methine group [δ 3.39 (H1), 46.5 (C1)] as well as to a trisubstituted epoxide-bearing methine group [δ 3.72 (H11), 59.6 (C11), 62.0 (C10)]. The COSY experiment also revealed an isopropylene group [δ 5.00 and 5.07 (H14αβ), 114.0 (C14); 1.91 (Me_{15}), 21.9 (C15)] and a methylene group [δ 2.91 and 3.48 (H2αβ), 27.7 (C2)] attached to this methine. The methylene group was in turn attached to **1a** through a quaternary oxygen-bearing sp^2 carbon, since selective INEPT correlations could be seen from the δ 2.91 and 3.48 signals to the carbon signal at 156.9 ppm (C3). The H11-H12 coupling response, however, led to a termination point that could not be linked to other spin systems through the normal COSY experiment alone.

The third unit in aceropterine was shown to possess a dimethylamino methine group (**1c**). COSY correlations revealed that this group was connected successively to two contiguous methines [δ 4.77 (H8), 69.5 (C8) and 3.59 (H7), 48.6 (C7)]. That an isopropylene unit was attached to C7 was shown by COSY correlations between the ^{1}H signals at δ 3.59 (H7), 5.45 (H18α), and 1.91 (Me_{19}). The hydroxyl proton showed coupling to the C8 methine as shown by the cross peak OH/CH δ: 4.97/4.77 in the ^{1}H-^{1}H COSY spectrum. Selective INEPT correlations from the methine proton signals at 3.44 (H9) and 3.72 ppm (H11) to the carbon signals at δ 62.0 (C10) and 174.4 (C20) showed that the dimethylamino-bearing methine was attached to the α,β-epoxy-γ-lactone ring. This suggested that **1** might possess a transposed γ-lactone residue. The C7 carbon of **1c** was connected to C6 of **1a**, as





Tobagolide (3)

Figure 1. The partial structures of aceropterine (**1**).

Scheme 1. Main Fragmentation Processes of Aceropterine with Suggested Ion Structures

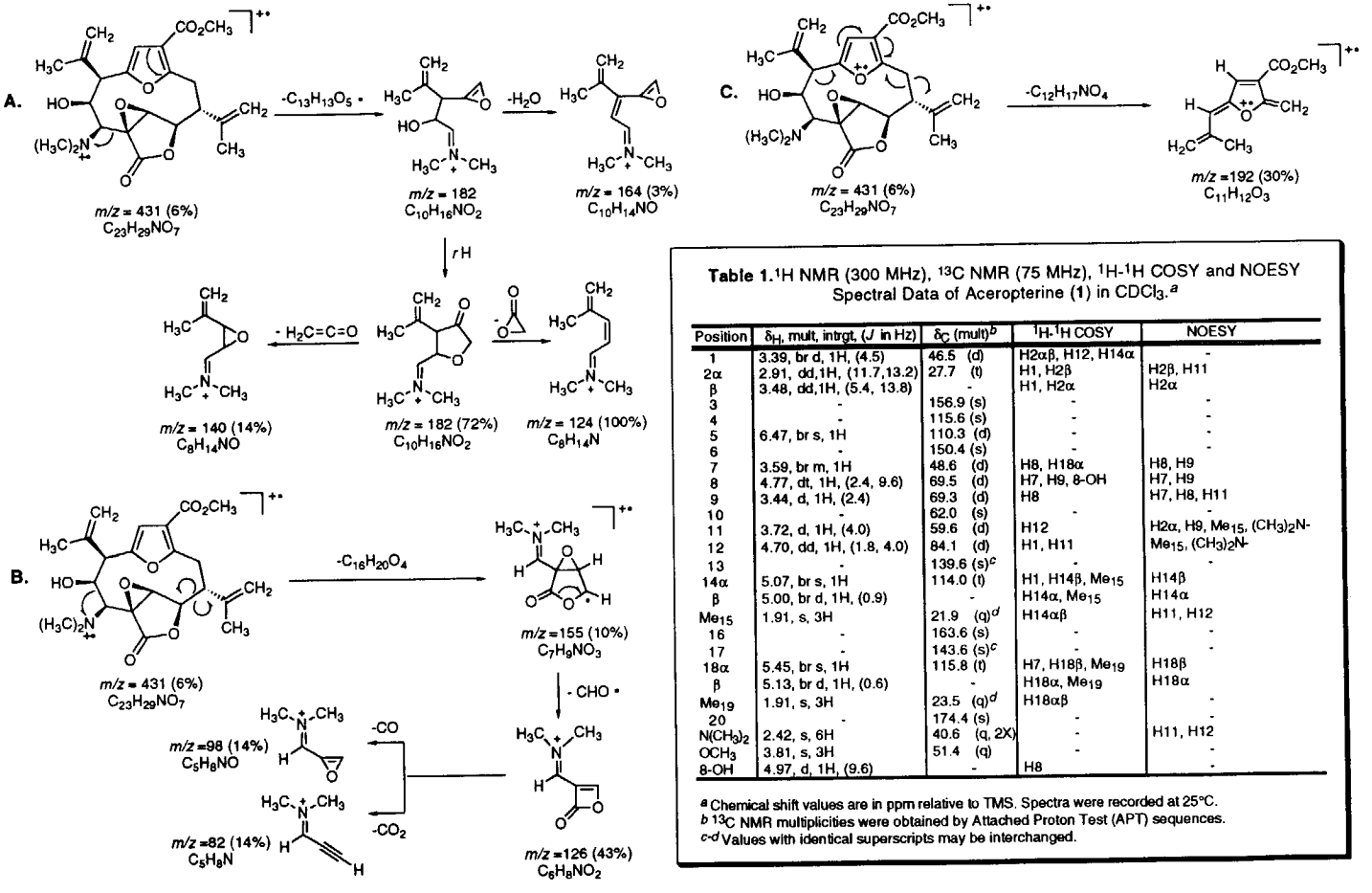


Table 1. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), ^{1}H-^{1}H COSY and NOESY Spectral Data of Aceropterine (**1**) in $CDCl_3$.[a]

Position	δ_H, mult, intrgt, (J in Hz)	δ_C (mult)[b]	^{1}H-^{1}H COSY	NOESY
1	3.39, br d, 1H, (4.5)	46.5 (d)	H2αβ, H12, H14α	-
2α	2.91, dd,1H, (11.7,13.2)	27.7 (t)	H1, H2β	H2β, H11
β	3.48, dd,1H, (5.4, 13.8)	-	H1, H2α	H2α
3	-	156.9 (s)	-	-
4	-	115.6 (s)	-	-
5	6.47, br s, 1H	110.3 (d)	-	-
6	-	150.4 (s)	-	-
7	3.59, br m, 1H	48.6 (d)	H8, H18α	H8, H9
8	4.77, dt, 1H, (2.4, 9.6)	69.5 (d)	H7, H9, 8-OH	H7, H9
9	3.44, d, 1H, (2.4)	69.3 (d)	H8	H7, H8, H11
10	-	62.0 (s)	-	-
11	3.72, d, 1H, (4.0)	59.6 (d)	H12	H2α, H9, Me_{15}, $(CH_3)_2N$-
12	4.70, dd, 1H, (1.8, 4.0)	84.1 (d)	H1, H11	Me_{15}, $(CH_3)_2N$-
13	-	139.6 (s)[c]	-	-
14α	5.07, br s, 1H	114.0 (t)	H1, H14β, Me_{15}	H14β
β	5.00, br d, 1H, (0.9)	-	H14α, Me_{15}	H14α
Me_{15}	1.91, s, 3H	21.9 (q)[d]	H14αβ	H11, H12
16	-	163.6 (s)	-	-
17	-	143.6 (s)[c]	-	-
18α	5.45, br s, 1H	115.8 (t)	H7, H18β, Me_{19}	H18β
β	5.13, br d, 1H, (0.6)	-	H18α, Me_{19}	H18α
Me_{19}	1.91, s, 3H	23.5 (q)[d]	H18αβ	-
20	-	174.4 (s)	-	-
$N(CH_3)_2$	2.42, s, 6H	40.6 (q, 2X)	-	H11, H12
OCH_3	3.81, s, 3H	51.4 (q)	-	-
8-OH	4.97, d, 1H, (9.6)	-	H8	-

[a] Chemical shift values are in ppm relative to TMS. Spectra were recorded at 25°C.
[b] ^{13}C NMR multiplicities were obtained by Attached Proton Test (APT) sequences.
[c-d] Values with identical superscripts may be interchanged.

the proton signal at δ 6.47 (H5) displayed a $^3J_{CH}$ correlation to the carbon signal at 48.6 ppm (C7).

Conformational analysis was required to solve the relative stereochemistry with a high degree of confidence. The vicinal ^{1}H-^{1}H coupling constants and NOE's observed for aceropterine (Table 1) correlated with a Dreiding model representing the relative stereochemistry shown in the structure of **1**. The coupling between H12 and H1 was found to be 1.8 Hz and this suggested that the dihedral angle was near 90°. By comparison, $J_{11,12}$ was considerably larger (4.0 Hz). Inspection of Dreiding models allowed us to conclude that H1 and the oxygen on the γ-carbon of the lactone must have a *cis* relationship. The $J_{11,12}$ value found was consistent with an epoxide function oriented in the β-configuration (coupling near 4 Hz is observed for a dihedral angle of 1.9°). If the epoxide function had been in the α-configuration (expected dihedral angle of 107.0°), a much smaller coupling would have been anticipated. The coupling constants between the H7/H8 and H8/H9 pairs of vicinal protons are both small (< 2.5 Hz) in **1**, and a model with suitable dihedral angles can be constructed if all the substituents on the C7-C9 segment have a *cis* relationship. Inspection of Dreiding models allowed us to conclude that the OH and C20 carbonyl were *cis* and thus capable of hydrogen bonding to each other. NOESY correlations observed for the H7/H8, H7/H9, H8/H9, and H9/H11 pairs confirmed the relative stereochemistry depicted in structure **1** (1*R**, 7*R**, 8*S**, 9*S**, 10*S**, 11*R**, 12*R**) (Table 1).

The structure of aceropterine (**1**), the first pseudopterane with a transposed lactone moiety, is closely related to that of tobagolide (**3**) which was isolated from a Trinidadian specimen of *P. acerosa*.[5] It might be possible that aceropterine is generated from **3** via epoxidation at C10,11 (on the β-face) followed by a translactonization rearrangement (or vice versa). In order to gain further insight into the conformational properties of **1** and its plausible biogenetic precursor "tobagolide epoxide", molecular mechanics calculations (MM3) were performed. After these were energy-minimized, the relative steric energies of the resultant conformations differed only by 1.03 kcal/mol. On the other hand, an acid or base-catalyzed translactonization experiment, which would have further supported this contention, was not carried out due to the paucity of material. More detailed studies of the bioactivity of aceropterine will be reported in due course.

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