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ISOLATION OF EUPALMERIN, A MINOR CEMBRANOID DITERPENE FROM THE CARIBBEAN GORGONIAN *EUNICEA MAMMOSA*

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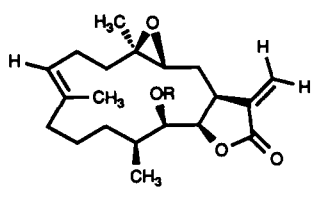
ABSTRACT.—Eupalmerin [**1**] has been found as a natural product in extracts of the gorgonian coral *Eunicea mammosa* collected in Desecheo Island, Puerto Rico. The structure of the new γ-lactonic cembranolide was assigned on the basis of spectral analysis.

A number of gorgonians (class Anthozoa, subclass Alcyonaria, order Gorgonacea) yield crystalline terpenoid lactones and sesquiterpene hydrocarbons when extracted with solvents such as hexane, Et_2O, or MeOH. In our search for biologically active secondary metabolites from marine organisms from Puerto Rico, we have isolated from a Caribbean gorgonian, *Eunicea mammosa* (Lamouroux) (phylum Cnidaria), a minor constituent identified as eupalmerin [**1**], a new crystalline natural product of the cembranoid series. Although the acetate isomer of **1** has been isolated earlier as a natural product from various species of *Eunicea* (1,2), this is the first report of the isolation of eupalmerin from a marine organism. The structure of this compound has been established by consideration of its combined spectral data and by direct comparison with the spectral data of eupalmerin acetate [**2**], also isolated from the same specimen of *E. mammosa* (3). In addition, careful comparison of the physical, chemical, and spectral properties of eupalmerin with those of the known cembranolide 12,13-bis-*epi*-eupalmerin [**3**] also helped to establish structure **1** for the new cembranolide (4,5).

The gorgonian, collected in Desecheo Island, a few miles off the northwest coast of Puerto Rico, was extracted with MeOH. The residue from the MeOH extract was subjected to solvent partitioning followed by repeated cc. Final purification was accomplished by preparative tlc.

Eupalmerin [**1**] was isolated as a crystalline solid, mp 148–149°, that analyzed for $C_{20}H_{30}O_4$ by combined hrfabms and ^{13}C-nmr methods. The compound showed ir spectral characteristics consistent with the presence of the α-methylene-γ-lactone (1767 br) and hydroxyl functionalities (3500 br). Consideration of ^{13}C- and ^{1}H-nmr data, and specifically the results of COSY analyses (Table 1), allowed the complete structure of **1** to be assigned.

The ^{1}H-nmr spectrum of eupalmerin showed two peaks at δ 6.10 and 5.38 due, respectively, to H-17α and H-17β of an α-methylene-γ-lactone, and signals at δ 5.03 (H-7), 4.62 (H-14), 3.55 (H-13), 3.06 (H-1), and 2.90 (H-3). The two singlets at δ 1.60 and 1.33 have



1 R=H
2 R=-Ac

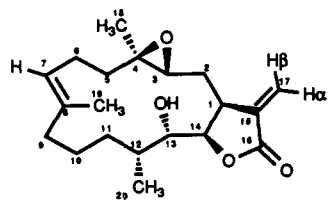


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TABLE 1. ^{13}C- (75 MHz) and ^{1}H- (300 MHz) nmr Spectral Data of Eupalmerin [**1**] in $CDCl_3$.[a]

Position	^{13}C (mult)[b]	^{1}H [mult, J (Hz), integration]
1	40.68 (d)	3.06 (m, 1H)
2	28.37 (t)	2.25 (m, 1H)
		1.75 (m, 1H)
3	59.06 (d)	2.90 (dd, 8.8, 5.0, 1H)
4	60.06 (s)	—
5	38.30 (t)	2.15 (m, 1H)
		1.30 (m, 1H)
6	23.18 (t)[c]	2.17 (m, 1H)
		2.05 (m, 1H)
7	125.96 (d)	5.03 (t, 6.3, 1H)
8	135.39 (s)	—
9	37.86 (t)	1.92 (m, 2H)
10	23.26 (t)[c]	1.52 (m, 2H)
11	31.70 (t)	1.31 (m, 2H)
12	34.82 (d)	1.78 (m, 1H)
13	74.21 (d)	3.55 (t, 6.6, 1H)
14	79.01 (d)	4.62 (dd, 8.0, 1.0, 1H)
15	138.51 (s)	—
16	170.29 (s)	—
17α	118.38 (t)	6.10 (d, 3.2, 1H)
17β		5.38 (d, 3.2, 1H)
18	16.78 (q)	1.33 (s, 3H)
19	16.44 (q)	1.60 (s, 3H)
20	16.08 (q)	0.98 (d, 6.9, 3H)

[a]The chemical shifts are given in δ units (ppm downfield from TMS). Assignments were aided by ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY, homonuclear spin-decoupling experiments, and comparison to known models.

[b]Multiplicities of the carbon atoms were revealed by an Attached Proton Test (APT) experiment.

[c]Values may be interchanged.

been attributed to the methyl groups placed, respectively, at C-8 and C-4, while the doublet at δ 0.98 is ascribable to the methyl group bonded at C-12. The ^{13}C-nmr spectrum of eupalmerin exhibited twenty signals divided by APT (6) into four quaternary carbons (C-4, C-8, C-15, and C-16), six CH groups (C-1, C-3, C-7, C-12, C-13, and C-14), seven methylenes (C-2, C-5, C-6, C-9, C-10, C-11, and C-17), and three Me groups (C-18, C-19, and C-20); one ester carbonyl carbon (δ 170.29); four oxygenated carbons (δ 79.01, 74.21, 60.06, 59.06); and four olefinic (δ 138.51, 135.39, 125.96, 118.38) carbons. The APT experiment indicated that three of the four oxygenated carbons (δ 79.01, 74.21, 59.06) are tertiary, while the fourth (δ 60.06) belongs to a quaternary carbon. Two of the olefinic carbons (δ 138.51, 135.39) are nonprotonated vinyls, one is singly protonated (δ 125.96), and a fourth olefinic signal is of an exocyclic methylene carbon (δ 118.38).

A heteronuclear chemical shift correlation with broad-band decoupling (CSCMBB) (7,8) experiment correlated all proton resonances with their corresponding carbon resonances, including those in the aliphatic (high field) region. By use of a ^{1}H-^{1}H correlation COSY (9,10) experiment coupled with homonuclear spin-decoupling experiments, the complete structure of **1** was assigned unequivocally, starting with the exocyclic methylene (δ 6.10 and 5.38, H-17α, -17β), which exhibits a COSY response corresponding (allylic coupling)

to the complex multiplet at δ 3.06 (H-1). The single proton of this multiplet has responses to the non-equivalent methylene protons (δ 2.25, 1.75, H-2) and a methine proton (δ 4.62, H-14). This latter proton and the chemical shift of its corresponding carbon (δ 79.01, C-14) indicate the location of the attachment of the oxygen of the γ-lactone. Irradiation of the proton at δ 3.06 (H-1) collapsed the doublets at δ 6.10 (H-17α) and 5.38 (H-17β) ppm to sharp singlets and the lactonic methine proton at δ 4.62 ppm (H-14) to a broad singlet. These findings revealed that the lactonic methine proton (H-14) at δ 4.62 ppm is also coupled, albeit weakly ($J = 1.0$ Hz), to the methine proton at δ 3.55 (H-13). The C-13–C-14 connectivity could not, however, be confirmed by autocorrelated proton 2D nmr, since cross-peaks connecting the H-13 and H-14 protons could not be observed in the contour plot of the ^{1}H-^{1}H COSY spectrum of **1**. This very closely resembles behavior reported previously for eupalmerin acetate [**2**]; no cross peaks connecting the H-13 and H-14 protons are observed in the contour plot of the ^{1}H-^{1}H COSY spectrum of **2** (3). During irradiation of the proton at δ 3.06 (H-1), modified absorption patterns were also observed distinctively around δ 2.25 and 1.75 ppm, thereby confirming the location of the methylene protons in position C-2. A nearby epoxide ring was indicated [δ 60.06 (s), 59.06 (d); δ 2.90, dd, $J = 8.8$ and 5.0 Hz, epoxymethine] also by double resonance nmr experiments. Irradiation of the protons at δ 2.25 and 1.75 (H-2) ppm collapsed the doublet of doublets at δ 2.90 ppm (H-3) to a doublet ($J = 8.8$ Hz) and a broad triplet, respectively. The presence of a 3,4-epoxide was thus established as shown in structure **1**. These double resonance experiments together with the ^{1}H-^{1}H COSY confirmed the chain of coupling between H-3 and the protons around the α-methylene-γ-lactone ring including H-12, H-13, and H-14 and the more remote exomethylene protons H-17α and H-17β.

The relative stereochemical orientation of all six chiral centers in **1** was assigned based upon similarities of the ^{13}C-nmr shifts, coupling constants, and splitting patterns with those reported from eupalmerin acetate [**2**] and 12,13-bis-*epi*-eupalmerin [**3**] (3,5). The 8-Me signal in the ^{13}C-nmr spectrum (δ 16.44 ppm) was at high field, which indicated that the trisubstituted double bond is trans with respect to the continuous chain of carbons (11). Unfortunately, the non-reactivity of **1** towards acetylation under various reaction conditions (pyridine/Ac_2O and pyridine/AcCl in THF) did not allow us to correlate the structure of eupalmerin with that of acetate **2**. Similarly, attempts to convert 12,13-bis-*epi*-eupalmerin [**3**] into its acetate isomer led only to the recovery of unchanged starting material. Additional experiments aimed at the chemical correlation of structures **1** and **2** via careful hydrolysis of the acetoxy group of eupalmerin acetate [**2**] always gave complex mixtures of products. In no instance were we able to identify **1** (by tlc analysis) as one of the several products obtained. In vitro screening data for eupalmerin showed significant cytotoxicity against CHO-K1 cells ($ED_{50} = 5.46$ μg/ml).

EXPERIMENTAL

GENERAL PROCEDURES AND ISOLATION.—Instrumental parameters and methods employed in this study have been summarized elsewhere (3). *E. mammosa* was collected on 18 March 1989, at 50-ft depth at Desecheo Island, Puerto Rico. A voucher specimen is stored at the Chemistry Department of the University of Puerto Rico. The animal (0.38 kg) was immediately frozen and subsequently extracted with MeOH. The combined MeOH extract was concentrated, and the residue was triturated with hexane. The hexane extract, after evaporation (13.42 g), was chromatographed over Bio-Beads SX-2 (toluene), and a portion of the light-green residue (4.18 g) was further purified by successive chromatography over Sephadex LH-20 [500 g, MeOH-$CHCl_3$ (1:1)] and Si gel (200 g, 10% EtOAc/hexane) to give pure eupalmerin acetate [**2**] (1.41

g) as a white crystalline solid. The fraction eluting with 25% EtOAc/hexane contained impure eupalmerin [**1**]. Further purification by preparative tlc (10% EtOAc/hexane) afforded pure crystalline **1** (0.18 g).

(7*E*,1*S*,3*S*,4*R*,12*S*,13*R*,14*R*)-13-HYDROXY-3,4-EPOXYCEMBRA-7,15-DIEN-16,14-OLIDE (EUPALMERIN) [**1**].—After recrystallization from hexane/C_6H_6 mixtures, compound **1** was isolated as a white crystalline solid: mp 148–149°; ir ($CHCl_3$) 3500 (br), 2930 (s), 2857 (s), 1767 (br), 1452, 1264, 1106, 1032, 758 cm^{-1}; uv λ max $CHCl_3$ (log ε) 242 (2.92) nm; $[\alpha]^{27}D$ −79.27° (*c* = 1.36, $CHCl_3$); hrfabms *m/z* $[M+Na]^+$ 357.2044 for $C_{20}H_{30}O_4Na$; ^{13}C nmr (75 MHz, $CDCl_3$) and ^1H nmr (300 MHz, $CDCl_3$) see Table 1.

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