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M. Alam, G. E. Martin, A. S. Zektzer, A. J. Weinheimer, R. Sanduja, and M. A. Ghuman

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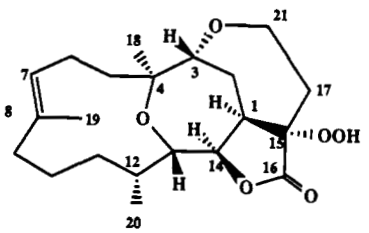
PLANAXOOL: A NOVEL CYTOTOXIC CEMBRANOID FROM THE MOLLUSK *PLANAXIS SULCATUS*

M. ALAM,* G.E. MARTIN,[1] A.S. ZEKTZER,[2] A.J. WEINHEIMER, R. SANDUJA,[3] and M.A. GHUMAN[4]

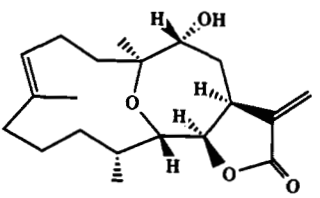
Department of Medicinal Chemistry and Pharmacognosy, University of Houston, Houston, Texas 77204-5515

ABSTRACT.—A novel cytotoxic cembranoid, planaxool [**1**], was isolated from the marine mollusk *Planaxis sulcatus*, and its structure determined by 2D nmr spectroscopy.

Cembranoid diterpenes constitute one of the major classes of lipoidal compounds found in marine coelenterates and are characterized by the presence of a 14-membered ring system. In addition to coelenterates (1,2), cembranoids have been isolated from plants (1), insects (1,3), and the mollusk *Ovula ovuum* (4). Recently, we reported the isolation and structure of a cytotoxic epoxysterol (5) and two novel cembranoid diterpenes from the mollusk *Planaxis sulcatus* Born.



1



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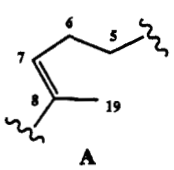
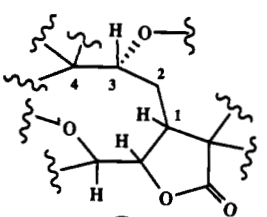


A



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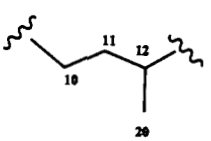

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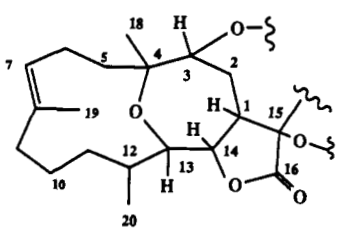


D

(Family Planaxidae) (6,7). We now report the isolation and structure of a cytotoxic homoditerpene with a modified cembranoid skeleton from this mollusk.

Repeated chromatography (LH-20,

[1–2]Present Addresses: [1]Burroughs Wellcome & Co., Research Triangle Park, North Carolina; [2]University of South Florida, Tampa, Florida; [3]Department of Hematology, University of Texas Medical School, Houston, Texas.

[4]On leave from the Institute of Chemistry, The University of Punjab, Lahore, Pakistan.

medium pressure and hplc) of the $CHCl_3$ extract of *P. sulcatus* resulted in the isolation of a low-melting solid, mp 58°, $[\alpha]D = 219°$, $C_{21}H_{32}O_6$. The ^1H-nmr spectrum of **1** showed the presence of a vinylic methyl (δ 1.71, 3H, s), three methine protons on oxygen-bearing carbons (δ 3.55, 3.85, 5.39), a methyl on a methine carbon (δ 0.94, d, $J = 6.9$ Hz), and a methyl on a quaternary carbon (δ 1.25). The ^{13}C-nmr spectrum showed the presence of 21 carbons, including an ester or lactone (δ 172.5), eight methylenes (δ 21.5, 22.8, 25.3, 27.9, 29.1, 31.6, 39.1, 78.3), two vinylic carbons (δ 123.9 and 135.1), and six oxygen-bearing carbons (δ 71.9, 74.6, 78.3, 80.2, 86.4, 102.1). The ^{13}C-nmr spectrum of **1** (Table 1) was similar to that reported for jeunicin [**2**] (8), but showed no resonance for an exocyclic methylene (δ 120.0, 137.6). The presence of additional resonances at δ 21.5, 78.3, and 102.1 suggested that the exocyclic methylene of jeunicin was replaced in **1** by a methylene and two oxygen-bearing carbons. An APT spectrum showed that of the six oxygen-bearing carbons in **1**, three were methine carbons (δ 71.9, 74.6, 86.2), one a methylene carbon (δ 78.3), and two quaternary carbons (δ 80.2, 102.1), along with eight methylenes and four quaternary carbons, suggesting that **1**

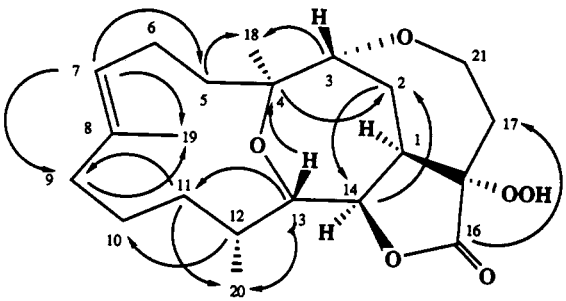
TABLE 1. ^1H and ^{13}C Chemical Shift Assignments of **1** and **2**.

Position	Compound			
	1		**2**[a]	
	δ ^1H (J in Hz)	δ ^{13}C	δ ^1H	δ ^{13}C
1	2.96 (dd, 8.0, 5.3)	41.2 (d)	3.36	40.0
2	1.68 (m)	27.9 (t)	1.89	30.3
	2.26 (dd, 8.4)		2.36	
3	3.55 (m)	74.6 (d)	3.67	71.9
4	—	80.2 (s)	—	80.0
5	1.32 (m)	31.6	1.50	32.3
	2.09 (m)		1.71	
6	2.13 (m)	22.8	2.08	22.8
7	5.47 (t, 7.1)	123.9 (d)	5.53	125.4
8	—	135.1 (s)	—	133.5
9	1.78 (m)	39.1 (t)	1.93	40.4
	2.02 (m)		2.04	
10	1.11 (m)	25.3 (t)	1.27	22.8
	1.85 (m)		1.63	
11	1.29 (m)	29.1 (t)	1.14	29.6
	1.45 (m)		1.72	
12	1.63	41.7 (d)	1.86	36.5
13	3.85 (d, 5.6)	71.9 (d)	3.18	72.1
14	5.39	86.4 (d)	4.42	80.0
15	—	102.1 (s)	—	137.6
16	—	172.5 (s)	—	169.7
17	1.55 (m), 2.05 (m)	21.5 (t)	5.61 (m), 6.31 (m)	120.0
18	1.28 (s)	21.5 (q)	1.25	19.1
19	1.71 (s)	16.3 (q)	1.58	15.5
20	0.94 (d, 6.9)	15.1 (q)	0.92	15.8
21	4.58 (ddd, 8.5, 8.5, 17.9)	78.3 (t)	—	—
	4.83 (m, 3.5, 9.5, 17.9)			

[a]Values for this compound are from Sanduja *et al.* (8).

has a modified jeunicin structure. A ^{1}H-^{13}C COSY spectrum was used to assign individual protonated carbons.

A ^{1}H-^{1}H COSY spectrum showed correlations between the vinylic proton resonating at δ 5.47 (H-7) and two protons resonating at δ 2.28 (H_a-6) and 2.13 (H_b-6) and also to the methyl at δ 1.71 (Me-19). The protons at δ 2.28 and 2.13 were coupled to two protons resonating at δ 2.09 (H_a-5) and 1.32 (H_b-5), which were also mutually coupled. These correlations suggested the presence of fragment **A** in **1**. The proton resonating at δ 5.39 (H-14) was coupled to two protons resonating at δ 3.85 (H-13) and 2.96 (H-1). The latter was coupled to a proton at δ 2.68 (H_a-2), which in turn was correlated to a proton resonating at δ 3.55 (H-3), and connectivity stopped there, indicating the presence of a quaternary carbon (C-4) next to the carbon bearing the δ 3.55 proton. These connectivities are compatible with fragment **B**. The presence of a four-carbon spin system (fragment **C**) was discerned by the utilization of a proton double quantum coherence [HDQC] spectrum, which showed connectivities within the highly congested upfield region. Thus the methyl at δ 0.94 (Me-20) was found to be coupled to a proton at δ 1.63 (H-12), which in turn was coupled to protons resonating at δ 1.29 (H_a-11) and 1.45 (H_b-11). The proton at δ 1.29 was further connected to a proton resonating at δ 1.85 (H_a-10). Careful interpretation of the long-range ^{1}H-^{13}C COSY spectrum (Figure 1) linked fragments **A** and **B** together through the quaternary carbon C-4 (δ 80.4). Both C-5 and C-3 were linked to H-18 (three



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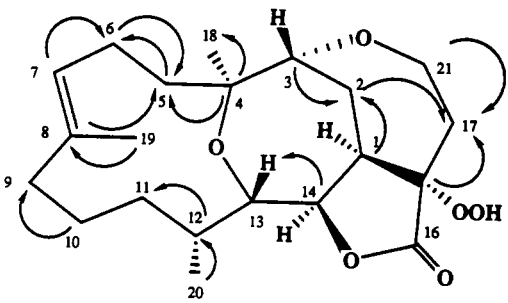

B

FIGURE 1. Three-bond (A) and two- and four-bond (B) long-range ^{1}H-^{13}C connectivities for **1**. In all cases, the arrows denote correlation pathways which originate from the carbon exhibiting the response and are directed to the location of the proton to which the carbon is coupled.

bonds), which placed the Me-18 on C-4. C-4 itself showed connectivities to H_a-2 (three bonds), and to H-18 and H-5 (two bonds). Fragment **C** was added by the observation in the long-range ^1H-^{13}C spectrum of two- and three-bond ^1H-^{13}C correlations linking C-11 to H-20, C-13 to H-20 and H_a-11, C-20 to H-12, and C-12 to H_a-11. Complementary utilization of the long-range ^1H-^{13}C COSY and HDQC spectra completed the closure of the fourteen-membered cembranoid ring as follows: Connectivities were observed in the long-range ^1H-^{13}C COSY spectrum, which linked C-9 (δ 39.1) to the Me-19 protons whose identity had been confirmed by an H-7: H-19 off-diagonal response in the COSY spectrum; similarly connectivities between H-7 and C-19, H-19 and C-7, H-19 and C-8, and C-7 and H_a-9 were also observed in the long-range ^1H-^{13}C COSY spectrum. The C-9–C-11 methylene bridge connectivities, as determined by the HDQC spectrum in which the proton at δ 1.85 (H_a-10) was coupled to H_a-9 (δ 2.02), were also confirmed by the long-range ^1H-^{13}C COSY spectrum. In the long-range ^1H-^{13}C COSY, both carbons C-10 and C-11 showed transfer of magnetization to H-11. This spectrum also showed a connectivity between C-4 (δ 80.2) and C-13 (δ 71.9), thereby establishing an ether linkage between these atoms. Allocation of the non-protonated oxycarbon (δ 102.1) to C-15 in the lactone ring completed the assignment of all carbon atoms of composite **D**. Only that atom and two methylenes (δ 4.58, 4.83 and 1.55, 2.05) remained unassigned, and the methylenes were excluded by the double doublet multiplicity of H-1, which has already been accounted for.

The two remaining methylenes formed an isolated ethylene bridge, consisting of the anisochronous oxymethylene protons [δ 4.58 (J = 17.9, 8.5, 8.5 Hz) and δ 4.83 (J = 17.9, 9.5, 3.5 Hz)] which were coupled to only two protons (δ 1.55 and 2.05) of a methylene carbon. The long-range ^1H-^{13}C COSY spectrum showed connectivities between H_a-21 and H_b-21 and C-17 (δ 21.5) and also showed correlations of the H_a-17 and H_b-17 resonances with C-16 and C-1. One of the H-17 resonances also showed correlations to C-15. This evidence permitted incorporation of the two methylenes between O-3 and C-15 as the balance of an ether ring, which accounted for the last unsaturation equivalent required by the molecular formula $C_{21}H_{32}O_6$ as determined by elemental analysis or $C_{21}H_{32}O_5$ suggested by the ^{13}C and APT spectra. However, the simple hydroxyl group at C-15 included in the second formula fails to account for the unusually high chemical shift (δ 102.1) of that carbon atom [carbons bearing hydroxyl groups are known to resonate between δ 50 and 80 (9)].

Since the largest ion observed in the mass spectra of **1** [hrms $C_{21}H_{32}O_4$ (calcd 348.2300, found 348.2281), cims, fabms] contained only four oxygen atoms, it was evident that the true molecular ion was not observed. Nevertheless, the mass spectral composition required the same number of unsaturation equivalents as the composition based on nmr spectra. Thus the loss of the missing oxygen could not have occurred via an elimination process, typical of alcohols, but must have occurred via a rearrangement process. A hydroperoxy group at C-15 would be compatible with the mass spectral behavior, as observed with cumene hydroperoxide [*m/z* (rel. int.) 152 (30%), 136 (34%), 120 (60%), 118 (88%), 105 (40%), and 91 (100%)], which undergoes a similar type of rearrangment to yield a major ion at *m/z* 120 $[M-32]^+$. This assignment is supported by the ir absorbance at 3560 cm^{-1} [hydroperoxide absorption ranges between 3560 and 3530 cm^{-1} (10)], the presence of an exchangeable proton resonance at δ 8.4 in the ^1H-nmr spectrum when recorded in DMSO, and a positive starch iodide test for peroxides. Repeated attempts, under

various conditions, to reduce the hydroperoxide functionality in **1** with $NaBH_4$ failed to give a product(s) which could be separated and identified. Natural hydroperoxides have been reported to be both non-amenable (11) and amenable (12) to $NaBH_4$ reduction. On the basis of 2D nmr spectroscopy, structure **1** was assigned to plaxanool.

The stereochemistry of **1** was assigned on the basis of an nOe difference spectrum [4K data points and 2.5 Hz line broadening]. Cis orientation of the H-3 and H-13 pair was based upon a large nOe of 15.2%. Similarly H-1 was cis-oriented to H-14 (8.4% nOe), and H-12 had a cis orientation in relation to H-13 (7.9% nOe). Irradiation of H-3, in addition to affording the large nOe observed at H-13, also gave a smaller (5.7% nOe) at one of the H-17 protons, suggesting a β orientation of the ethylene bridge. This smaller nOe is in agreement with a spatial distance of about 3.08 Å (between H-3α and one of the methylene protons) in a molecular model as compared to a distance of 2.15 Å between H-3α and H-13. A molecular model of **1** with ethylene bridge in α orientation places the closest H-17 proton at a 4.6 Å distance, thereby eliminating the possibility of observing any nOe enhancement (13). A trans orientation for H-14 and H-13 is based on a relatively small (1.7%) nOe. Similarly, absence of an nOe at H-18, when H-3 was irradiated, suggested an α orientation of Me-18. The possibility of **1** being an artifact was eliminated because extracts of six different samples, collected at various times and stored in different freezers, were found to contain **1** when analyzed by hplc. Planaxool showed cytotoxicity (IC_{100}) against L1210 (mouse murine leukemia) cell line at a level of 2.4 μg/ml.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp was recorded on a Fisher-Johns apparatus, and ir was recorded on a Perkin-Elmer Model 283 spectrometer. Rotation was recorded on a Perkin-Elmer Model 141 polarimeter. Both cims (CH_4 as carrier) and eims were recorded on a Finnigan model 1020 spectrometer equipped with an INCOS data system. All nmr spectra were recorded in $CDCl_3$ (unless otherwise specified) at observation frequencies of 300.068 and 75 MHz for ^1H and ^{13}C, respectively. The ^1H-^1H COSY spectrum (14) was recorded using phase cycling to give quadrature detection in both frequency domains (15); the HDQC spectrum was recorded using the pulse sequence and phase cycling previously described (15). ^1H-^{13}C COSY spectrum was recorded using the pulse sequence described by Bax and Morris (16), while the long-range ^1H-13C COSY was recorded according to the pulse sequence as described by Salazar *et al.* (17).

P. sulcatus was collected and identified by Dr. R.E. Schroeder from Heron Island off the coast of Queensland, Australia in 1974. A voucher specimen of *P. sulcatus* is deposited in the specimen collection of the Department of Medicinal Chemistry, University of Houston. The iPrOH extract of the mollusk was concentrated and stored in a freezer until processed. The residue from the iPrOH extract (9.7 g) was defatted with hexane and partitioned between $CHCl_3$ and 10% aqueous MeOH. The $CHCl_3$ extract was chromatographed on an LH 20 (1.5 × 60 cm) column using $CHCl_3$-MeOH (95:5) as the eluting solvent. Fractions 49–79 (8 ml each) were combined, on the basis of tlc [Si gel 60, $CHCl_3$-MeOH (95:5)], to give a residue (185.7 mg), which was rechromatographed on a Si gel column (1.5 × 60 cm). A linear gradient of MeOH in $CHCl_3$ (0.5 to 5%, 400 ml total volume) was used as eluting solvent. Fractions of 5 ml were collected and combined on the basis of tlc [Si gel 60, solvent $CHCl_3$-MeOH (95:5)]. Fractions 19–29 contained one major compound along with a small amount of impurity. Hplc [Si gel cartridge in a radial compression module (Waters Associates), 2% MeOH in $CHCl_3$ as the mobile phase] of the residue from the combined fractions 19–29 gave **1** as a low melting solid (29.15 mg): mp 58°, $[\alpha]_D = -219$ ($c = 0.621$, $CHCl_3$); ir (CCl_4) ν max 3560 (hydroperoxide), 3050 (C=C), 1765 (γ lactone), 1125 (C-O-C); ^1H and ^{13}C nmr see Table 1. Found C 65.95, H 8.52; calcd for $C_{21}H_{32}O_6$, C 66.31, H 8.42.

ACKNOWLEDGMENTS

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