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MARINE NATURAL PRODUCTS: PYRROLOLACTAMS FROM SEVERAL SPONGES

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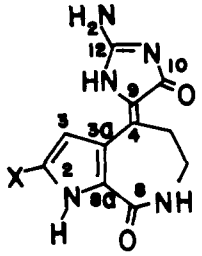
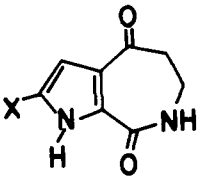
ABSTRACT.—Two pyrrololactams, likely degradation products of more complex known guanidines, were isolated from several marine sponges. 5-Bromo- and 4,5-dibromopyrrole-1-carboxylic acid methyl esters were also isolated. A complex guanidine metabolite, poorly soluble in DMSO, was found to give sharp, reproducible nmr spectra in acid media.

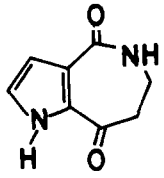
Pyrrole- and guanidine-based alkaloids have been isolated from marine sponges (1,2). Among the more unusual of these are the guanidines **1a** (3,5) and **2** (4,5). In our continuing search for cytotoxic metabolites, we have studied several sponges from the Pacific and report here the isolation of two pyrrololactams that appear to be cleavage products of **1a** and **2**, namely **3** and **4**. Guanidine **1a** was reisolated, and we observed that an acidic solvent is more suitable for obtaining nmr data of this poorly soluble compound.



1a X=H (HCl salt)
1b X=H
2 X=Br



3 X=H
4 X=Br



3'

RESULTS AND DISCUSSION

The first of the sponges examined was *Hymeniacidon aldis* de Laubenfels, which was collected at Guam Island and kept frozen until work up. $CHCl_3$-MeOH (1:1) extracts of freshly thawed specimens were concentrated and then partitioned between hexane and 10% aqueous MeOH. The aqueous MeOH phase was diluted to 20% H_2O and extracted with $CHCl_3$. Chromatography of the $CHCl_3$ soluble fraction over silica gel using a $CHCl_3$-MeOH step gradient yielded some fractions containing pure **3**, for which we propose the name aldisin. Several fractions eluting just before **3** were combined and rechromatographed to give 2-bromoaldisin (**4**).

Aldisin, mp 269° (d), was shown to have the molecular formula $C_8H_8N_2O_2$ by hrms (M^+ 164.05706, +0.51 mmu error). The ir spectrum of **3** indicated the presence of -OH or NH groups (3220 cm^{-1}, brd) and conjugated ketone or amide groups (1662, 1650 cm^{-1}). The uv spectrum, λ max (MeOH) 295 (6770), 245 (8370), 216 nm (19100), was changed upon addition of a few drops of a $NaBH_4$ in MeOH solution to λ max 272 (34500), thus giving additional evidence for the presence of a conjugated ketone in **3**. Signals in the ^{13}C-nmr spectrum at δ 194.5 and 162.2 also supported the presence of one ketone and one amide group, hence the 3200 cm^{-1} ir absorption could be ascribed to NH group(s).

In the ^1H-nmr spectrum (C_5H_5N) of **3**, two isolated spin systems were evident. One corresponded to a -CH=CH- group, δ 7.12, 7.29, doublets with J=2.5 Hz. The

chemical shift positions and small vicinal coupling were consistent with that of the α- and β-protons in a pyrrole ring. The second spin system was shown by decoupling and deuterium exchange (MeOD) to correspond to the aliphatic system -NH-CH_2-CH_2-[δ 9.41, 1H, m (exchangeable), 3.55, m (simplified by MeOD addition), 2.89, m].

The ^{13}C-nmr spectrum of **3** contained, in addition to the two carbonyl signals mentioned above, doublets at δ122.3, 109.0, singlets at δ 123.9, 128.3, and two triplets for aliphatic carbons at δ 36.6 and 43.9. The chemical shifts of the doublet signals at δ 122.3 and 109.0 were compatible with those expected for C-2, -3 in a pyrrole and, combined with the proton data, provided a basis for inferring the presence of this moiety in **3**. Since the ketone in **3** is conjugated, this must be attached to the pyrrole ring at an α- or β-position. Uv data suggested that the amide carbonyl was also attached to the pyrrole ring since the longest wave length absorption, 295 nm, corresponded closely to that of 2,3-dicarbethoxy 4,5-dimethylpyrrole (λ max 293) whereas pyrroles with a carbethoxy group at only position -2 or -3 exhibit shorter wave length maxima (e.g., 270 and 283, respectively) (6). Insertion of the -NH-CH_2-CH_2- unit between the carbonyl groups of a 2,3-dicarbonyl substituted pyrrole inferred from the above led to two possible structures for aldisin, **3** or **3'**.

A search of the literature revealed that a compound having structure **3** had been obtained as a degradation product of **1a** by permanganate oxidation (3). The reported spectral properties of this degradation product matched those of **3** and hence this structure was confirmed for aldisin, although the reported (3) mp (275-277°) differs significantly from that observed in this work (269°).

2-Bromoaldisin (**4**) mp 243°, $C_8H_7N_2O_2Br$ by hrms measurements, exhibited ir, uv, ^{1}H-nmr and ^{13}C-nmr spectra very similar to **3** with a few notable exceptions. Thus **4** lacked the H-2 doublet signal present in **3**, the H-3 signal in **4** was only a singlet, and the ^{13}C-nmr signal assigned to C-2 in **3** was shifted to δ 106 in **4** and was now a singlet. These data, as well as biosynthetic considerations, indicated structure **4** for this natural product. This conclusion was confirmed when catalytic hydrogenolysis (Ra/Ni) of **4** yielded **3** (ms and ^{1}H-nmr comparison).

Aldisin (**3**) and 2-bromoaldisin (**4**) displayed an interesting difference in reactivity towards acetylation conditions which was discovered as a result of preliminary charac-

TABLE 1. ^{1}H-nmr Data for **3**, **4**, **5** and **6**[a].

Proton	Compound					
	3[b]	**3**[c]	**4**[b]	**4**[c]	**5**[d]	**6**[d]
H-2	7.29 d(2.5)	7.0 d(2.5)	— —	— —	7.49 d(3.2)	7.08 br t (W½ 4.4)
H-3	7.12 d(2.5)	6.89 d(2.5)	7.08 s	6.72 s	6.26 d(3.2)	6.27 br t (W½ 4.4)
H-5	2.89 m	2.85 m	2.89 m	2.84 m	6.15 t(7.0)	5.89 t(6.5)
H-6	3.55 m	3.55 m	3.55 m	3.55 m	4.21 m	4.39 m
N(7)H	9.41	—	—	—	—	—
Ac	—	—	—	—	2.64s 2.50s 2.29s	2.46s 2.23s

[a]Figures in parentheses are coupling constants reported in Hz.
[b]300 MHz, C_5D_5N.
[c]300 MHz, $CDCl_3$-CD_3OD.
[d]300 MHz, $CDCl_3$.

terization studies. When **3** was treated with Ac_2O and pyridine at 70° overnight, the tri- and diacetates **5** and **6**, respectively, were obtained and separated by hplc. The ir spectrum of **5** showed strong absorption at 1750 cm^{-1} with a distinct shoulder at 1765 (vinyl acetate, pyrrole N-acetate, acetate of N-acyl amide) and broad absorption from 1710-1665 cm^{-1} (ring amide and C=C unsaturation). In the ^{1}H-nmr spectrum of **5**, the pyrrole proton signals were shifted relative to those in **3** (see Table 1). An olefinic proton signal (δ 6.15, t) coupled to a methylene multiplet (4.21) provided evidence for the presence of the enol acetate moiety. ^{13}C-nmr data of **5** (see Table 2) confirmed the presence of the amide carbonyl (δ 159.7), the absence of any ketone carbons, and the presence of the two sp^2 carbons expected for the enol acetate (δ 117.5, d; 146.8, s). The chemical shift assignments for carbons-3a and -8a (see Table 1) are based on the assumption that the carbon α to nitrogen in a pyrrole will be farther downfield than the β-carbon.

TABLE 2. ^{13}C-nmr Data for **3**, **4**, and **5**[a].

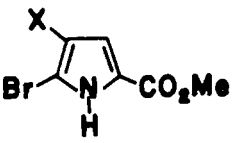
Carbon	Compound		
	3[b]	**4**[c]	**5**[d]
2	122.3	105.7 (5)	127.8 dd (192.6; 8.5)
3	109.0	111.7 d (180.9)	107.2 dd (177.0; 7.3)
3a	123.9[e]	124.9[e] brd s	125.5[e] s
4	194.5	194.3 brd s	146.8 brd s
5	36.6	36.5 t (135.0)	117.5 d (165.4)
6	43.9	42.6 t (127.5)	39.1 t (145.0)
8	162.2	162.1 brd s	159.7 s
8a	128.3[e]	128.3[e] (7)	129.2[e] brd s
Ac			171.5 s 168.8 s 168.4 s 26.6 q 24.4 q 20.4 q (all 130)

[a]Figures in parentheses are coupling constants reported in Hz.
[b]20 MHz, DMSO-d_6.
[c]75 MHz, $CDCl_3$-CD_3OD 1:1.
[d]22.5 MHz, $CDCl_3$.
[e]These values may be exchanged in any column.

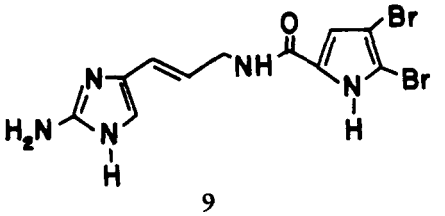
Diacetate **6** showed ir absorption at 1764, 1697, and 1660 cm^{-1}. The enol acetate moiety was clearly retained in **6** as shown by the ir absorption at 1764 and comparison of the ^{1}H-nmr signals of H-5 and -6 with those of triacetate **5** (see Table 1). The second acetate in **6** was placed on the lactam nitrogen because there is very little difference between **5** and **6** in the chemical shift for H-6, while there is significant difference in the shift of the H-2 signals. Furthermore, in the ^{1}H-nmr spectrum of **6** the H-2 and -3 signals are broad, but discernible triplets, $W_{1/2}$=4.4 Hz, attributable to coupling with the pyrrole NH, while in **5** these signals are sharp doublets.

When **4** was treated with Ac_2O/pyridine under the same conditions used for **3**, no acetylated products were obtained and starting material was recovered.

5 R=Ac
6 R=H



7 X=H
8 X=Br



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iPrOH extracts of an unidentified sponge from Fiji yielded **1a**, **3**, and **4** using a separation scheme similar to that described above. Guanidine **1a** ws obtained as nicely crystalline, lemon-yellow cubes, which decomposed at approximately 360°. ^{1}H- and ^{13}C-nmr spectra obtained in $CDCl_3$ and DMSO were useable, but not sharp. A switch to $CDCl_3$-TFA-d (9:1), in which **1a** was readily soluble, gave very sharp ^{1}H- and ^{13}C-

TABLE 3. Comparison of nmr Data of **1** in Neutral and Acidic Solvents.

Carbon	^{13}C nmr			^{1}H nmr			
	Compound			Compound			
	1a	**1a**	**1b**	**1a**	**1a**	**1a**	**1b**
	DMSO[a]	DMSO[b]	$CDCl_3$[c]-HOAc (2:1)	DMSO[d]	DMSO[e]	$CDCl_3$[e]-TFA-d (9:1)	$CDCl_3$[e]-TFA-d (0:1)
5	31.2	29.0	30.3	H-2, 7.20 d[f]	7.10 br s	7.20 d[g]	7.18 d[g]
6	39.2	40.0	40.8	H-3, 6.60 d[f]	6.40 br s	6.62 d[g]	6.60 d[g]
3	109.6	111.0	110.2	H-5, 3.3 br s	3.10 m	3.63 m	3.61 m
				-6	3.30 m	3.62 m	3.59 m
3a	119.9	121.8	120.5				
9	120.4	122.3	123.1				8.29, br s (NH)
2	123.2	123.7	123.8				
8a	126.8	125.1	124.4				
4	130.4	[h]	130.4				
12	155.2	157.7	154.7				
8	163.3	163.3	163.4				
10	164.0	175.0	165.3				

[a]25 MHz, Sharma *et al.* (3). Assignments as per this reference. Note that numbering scheme used in present paper differs from that of Sharma *et al.* (3).

[b]20 MHz, this work; mult. not determined, assignments by analogy to Sharma *et al.* (3).

[c]75 MHz, this work; mult. not determined, assignments by analogy to Sharma *et al.* (3).

[d]100 MHz, Sharma *et al.* (3).

[e]300 MHz, this work; mult. not determined, assignments by analogy to Sharma *et al.* (3).

[f]After exchange with D_2O.

[g]Sharp d, J=2.8 Hz.

[h]Not observed.

nmr spectra with only small changes in chemical shifts.[1] $CDCl_3$-CD_3OD solutions were tried for nmr analyses but did not give much better results than $CDCl_3$. Some comparisons are recorded in Table 3.

Since the decomposition point for **1a** did not closely match that reported (3), the free base of **1b** was prepared by treatment of **1a** in DMSO with 5% Na_2CO_3 solution. The initial heterogeneous mixture clarified, and then after standing overnight, pale yellow crystals of **1b** were deposited, mp 240-245° [Lit. (3) mp 220-225°, dec]. Solubility problems were again encountered in attempting to obtain good nmr spectra, and finally $CDCl_3$-TFA-d and $CDCl_3$-HOAc solutions were used. The nmr spectra were virtually the same as those obtained for **1a** (see Table 3), as was the mass spectrum.

MeOH extracts of fresh specimens of a *Lissodendoryx* sp. of sponge from Sri Lanka were concentrated, diluted with H_2O, and then partitioned sequentially versus CH_2Cl_2 and *n*-BuOH. From the CH_2Cl_2 soluble fraction, 2-bromoaldisin (**4**) and the pyrrole-2-carboxylic acid methyl esters **7** (7) and **8** (7) were obtained by a sequence of Sephadex LH-20, silica gel and reversed-phase C-18 chromatographies. 2-Bromoaldisin was identified by comparison of its spectral properties with those of samples isolated from the other sponges. Esters **7** and **8** were identified by their physical and spectral properties.

Aldisin and 2-bromoaldisin may be formed by oxidation of **1** and **2**, respectively, or could be formed by a conjugate addition of H_2O at C-4 followed by a reverse aldol reaction resulting in loss of the guanidine moiety. Likely progenitors for guanidines **1** and **2** would be the debromo- and monobromo forms of oroidin (**9**) (8,9).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected. Ir spectra were taken on Perkin-Elmer Model 298 and Digilab FXS-20 spectrophotometers; uv spectra were taken on a Perkin-Elmer Lamda-3 UV/VIS spectrophotometer. Nmr spectra were taken on Nicolet 300 MHz and Varian XL 300 MHz instruments in the solvents specified; signals are reported in parts per million (δ) downfield from internal TMS (^1H) or $CDCl_3$ taken as 77.0 (^{13}C). Low resolution mass spectra were taken on Hewlett-Packard 5985B and high resolution mass spectra on CEC 110 (Du Pont, Monrovia, CA) instruments. Chromatographic absorbent used was Brinkmann silica gel (230-400 mesh). Sponge identifications were made by Dr. K. Reutzler, Smithsonian Institution.

ISOLATION OF **3** AND **4** FROM *H. ALDIS*.—Specimens of *H. aldis* were collected around Guam and transported frozen to Oklahoma. Freshly thawed material (wet weight 7 kg) was extracted twice (3 days each) at room temperature with $CHCl_3$-MeOH (1:1, 4 liters). Evaporation of the extracts in vacuo afforded 416 g of residue. The total extract was dissolved in 10% aqueous MeOH and partitioned against $CHCl_3$. Evaporation of the $CHCl_3$ fraction in vacuo yielded 4.4 g of residue, which was chromatographed over 250 g of silica gel, using a $CHCl_3$-MeOH step gradient (0⟶50% MeOH) to give 21 fractions. Fractions 13 and 14 were combined and upon crystallization from MeOH-$CHCl_3$ (1:19) yielded 3 mg of pure **3**. The mother liquors were combined with fraction 10′ (see below under isolation of **4**) in MeOH and cooled to 5° to give 3 mg more of **3**. (Yield: 8.5×10^{-5}% of wet sponge wt.).

Fractions 6-12 (250 mg) were chromatographed over 30 g of silica gel using a $CHCl_3$-MeOH step gradient (0⟶2% MeOH) to give 10 fractions (1′-10′). Combined fractions 2′-4′ upon crystallization from MeOH at 5° yielded white crystals of **4**, 10 mg. (Yield: 1.4×10^{-4}% of wet sponge weight).

Compound **3**.—mp 269° (dec) Lit. (3) 275-277°; ir (KBr) 3450, 3220, 3080, 1662, 1650, 1638, and 1478 cm^{-1}; uv (MeOH) max 295 (ε=6770), 245 (8370) and 216 nm (19,100); ^1H nmr and ^{13}C nmr, see Table 1; hrms: (composition interpret., calcd. millimass) 164.05706 ($C_8H_8N_2O_2$, M^+, 164.05857), 136.06521 ($C_7H_8N_2O$, M^+CO, 136.06366); 136.02981 ($C_6N_4N_2O_2$, M^+-C_2H_4, 136.02728); 135.03128 (C_6H_5NO, M^+-CH_2NH, 135.03202); 108.03269 ($C_5H_4N_2O$, M^+-C_2H_2CO, 108.03236); 107.03609 (C_6H_5NO, M^+-CH_2NHCO, 107.03711); 93.02179 (C_5H_3NO, M^+-C_3H_5NO, 93.02146); ms 70 eV, low resolution, *m/z* (relative intensity) 164, (M^+, 60), 136 (34), 135 (11), 120 (10), 108 (14), 107 (56), 106 (10), 94 (25), 93 (100), 92 (40), 79 (44), 67 (28), 66 (28), and 65 (33).

[1]Addition of a few drops of 2 N HCl to DMSO-d_6 solutions also sharpens the ^{13}C-nmr spectrum. Personal communication from Prof. I. Kitagawa who notes that the ^{13}C-nmr spectra of **1** (free base) and **2** reported by Kitagawa *et al.* (5) were in fact taken in DMSO-d_6 (1.5 ml) with 2 drops of 2 N HCl.

Compound **4**.—mp 243°, ir (KBr) 3500, 3290, 3210, 3070, 1663, 1652, 1550, 1460, 1365, 908, 828, and 682 cm^{-1}; uv (MeOH) λ max 303 (ε 8130), 244 sh (12,100) and 220 nm (29,600); ^1H nmr and ^{13}C nmr, see Tables 1 and 2; hrms [composition, interpretation, calcd. millimass] 241.97048 (M^+, $C_8H_7N_2O_2{}^{79}Br$; 241.96908), 243.97019 (M^+, $C_8H_7N_2O_2{}^{81}Br$; 243.97215); 215.97012 ($C_7H_7N_2O^{81}Br$, M^+-CO, 215.97213); 213.97234 ($C_7H_7N_2O^{79}Br$, M^+-CO, 213.97417; 214.93917 ($C_7H_4NO_2{}^{81}Br$, M^+-CH_2NH, 214.94049); 212.94059 ($C_7H_4NO_2{}^{79}Br$, M^+-CH_2NH, 212.94253); 186.94548 ($C_6H_4NO^{81}Br$, M^+-CH_2NHCO, 186.94558); 184.94792 ($C_6H_4NO^{79}Br$, M^+-CH_2NHCO, 184.94762); 172.92906 ($C_5H_2NO^{81}Br$, M^+-CH_2CH_2NHCO, 172.92993), 170.93194 ($C_5H_2NO^{79}Br$, M^+-CH_2CH_2NHCO, 170.93197); 164.05731 ($C_8H_8N_2O_2$, M^+-Br, 164.05857). Ms 70 eV, low resolution: *m/z* (rel. int.) 244, 242 (M^+ 84, 100), 216 (46), 215 (14), 214 (48), 213 (12), 200 (8), 198 (14), 187 (57), 186 (16), 185 (63), 174 (26), 173 (49), 172 (57), 170 (35), 159 (38), 158 (12), 157 (42), 144 (18), 117 (12), 108 (10), 100 (12), 92 (42), 91 (15), 78 (22), 65 (24), and 64 (39).

ACETYLATION OF **3** TO FORM **5** AND **6**.—Pyrrole **3** (6 mg) was treated with 2 ml of Ac_2O-pyridine (1:1) and heated at 70° for 16 h. The product was diluted with H_2O (3 ml) and extracted with $CHCl_3$. Resolution of the product mixture by hplc using silica gel (5 μ) with 1% MeOH-$CHCl_3$ afforded triacetate **5** and diacetate **6**.

Triacetate **5**.—3.4 mg, ir ($CHCl_3$) 1765 (sh), 1750 (s), 1690, 1680, and 1300 cm^{-1}; uv (EtOH) λ max 293 (ε=11,420), 222 (18,470), 200 nm (24,650); ^1H nmr and ^{13}C nmr, see Tables 1 and 2; ms 70 eV, *m/z* (relative intensity) 290 (M^+, 5), 248 (15), 106 (12), 105 (19), 164 (30), 163 (100), 135 (12), 120 (18), 119 (15), 94 (24), 80 (5), 70 (17), and 65 (8).

Diacetate **6**.—1.3 mg, ir ($CHCl_3$) 3450, 1764, 1697, 1660, 1410, and 1375 cm^{-1}; uv (EtOH) λ max 300 (ε=9460), 295 sh (8990), 223 (22 700) and 200 nm (20 930); ^1H nmr, see Table 1; ms 70 eV, low resolution, *m/z* (rel. int.) 248 (M^+, 12), 205 (20), 168 (2), 163 (100), 135 (10), 120 (12), 119 (10), 94 (16), 85 (4), 73 (6), and 70 (7).

HYDROGENOLYSIS OF **4**.—Pyrrole **4** (1.5 mg) was dissolved in 5 ml of ethanolic KOH (0.3 M KOH) and hydrogenated in the presence of a trace of Raney Nickel at room temperature and atmospheric pressure for 18 h. Separation of the resulting mixture of products using hplc (SiO_2, 5 μ) with $CHCl_3$-MeOH (19:1) afforded one fraction, ≈0.2 mg, whose Rf value on tlc, and ^1H-nmr spectrum were identical with that of **3**.

ISOLATION OF **1a, 3, 4** FROM AN UNIDENTIFIED SPONGE FROM KOROLEVU, FIJI.—Fresh sponge specimens, collected March 7, 1973, at −10 M, were immersed in i-PrOH alcohol and stored in this solvent in sealed meal pails until workup. The filtrate from the stored specimens was concentrated to a small volume and this was freeze-dried for storage. A portion of the freeze-dried residue (10 g) was later extracted with MeOH in a Soxhlet apparatus, and the MeOH solution was filtered and concentrated in vacuo to give 5 g of a syrupy mass which was chromatographed on Sephadex LH-20 using $CHCl_3$-MeOH (1:1) to give 11 fractions. Fractions 7-11, which exhibited similar tlc behavior, were combined and chromatographed on silica gel using a gradient elution of $CHCl_3$ with increasing amounts of MeOH (max 10%). Fractions exhibiting similar tlc patterns were rechromatographed in like manner and, where necessary, further purification was achieved by preparative tlc on silica gel plates using $CHCl_3$-MeOH (90:10). In this manner, compounds **1a, 3,** and **4** were obtained [listed in order of elution from silica gel using $CHCl_3$-MeOH (90:10) elution].

1a *(salt)*.—100 mg, yellow crystals from slow evaporation of solvent; mp >360° (dec) [Lit. (3) 220-225° (dec) for hydrochloride]; ir (KBr) 3400, 3220, 3050, 1690, 1658, 1604, 1470, 1430, 1350, 1335, 1270, 1170, 1125, 1105, 1023, 1010, 890, 785, 770, 668, and 645 cm^{-1}; uv (MeOH) max 217 (20 500), 251 (13 700), 282 (15 700), and 360 nm (43, 650); nmr, see Table 3; ms (12 eV, low resolution) *m/z* (rel. int.) 245.1 (M^+, 100); 243.2 (3.4), 229 (2.7), 228 (10.8), 217.9 (2.8), 217.0 (4.1); 216.1 (14.9), 203.1 (9.6), 202.1 (73.8), 201.0 (6.0), 175.0 (3.2), 174.0 (10.3), 173.2 (1.0), 147.0 (1.0), 146.0 (2.5).

FREE BASE **1b**.—Compound **1a** (5 mg) in DMSO (0.5 ml) was mixed with 5% Na_2CO_3 solution (5 ml) and stirred for 1 h. Pale yellow crystals (4 mg) were deposited upon leaving the solution stand overnight. Recrystallization from H_2O gave pale yellow crystals, mp 240-245° (dec) [Lit. mp 220-225° (dec) (3), 171-180° (dec) (5)]; uv (MeOH) λ max 223 (ε=13 520), 263 (10 050) and 338 nm (19 000); nmr, see Table 3; ms: same as for **1a**.

ISOLATION OF **4, 7** AND **8** FROM A *LISSODENDORYX* SP. —A room temperature MeOH extract of fresh wet sponge, *Lissodendoryx sp* (Myxillidae), collected in Sri Lanka, was concentrated in vacuo and the residue was diluted with a little H_2O and extracted sequentially with CH_2Cl_2 and *n*-BuOH. The CH_2Cl_2 solubles (1.51 g) were chromatographed on Sephadex LH-20 (450 g; 2′ x 3½″ column) eluting with $CHCl_3$-MeOH (1:1). Two fractions, *A* (69 mg) and *B* (11 mg), which eluted immediately after those containing sterols (tlc analysis), contained compounds **4, 7** and **8**. Resolution of 47 mg of fraction A by hplc

using a C_{18} reverse phase column and eluting with H_2O-MeOH (35:65) gave, in order of elution, **4** (9.5 mg) and **8** (12.6 mg).

Compound **4**.—^{1}H nmr (300 MHz; CD_3OD) δ 2.81 (2H, m, H-5), 3.5 (2H, m, H-6), 6.65 (1H, s, H-3); ^{13}C nmr (75 MHz; CD_3OD) δ 38.0, 44.6, 104.2, 113.2, 126.8, 130.6, 164.2, 196.2; ms *m/z* (rel. int.) 244 (83.2), 242 (100), 216 (42.5), 214 (49.5), 187 (53.9), 185 (56.3), 173 (44.4), 172 (51.1), 171 (42.9).

Compound **8**.—mp 161.0° [Lit. (7) 158-159°]; ir ($CHCl_3$) 3430, 3250, 1700, 1555, 1440, 1395; ^{1}H nmr (300 MHz; $CDCl_3$) δ 3.84 (3H, s), 6.87 (1H, d, J=3.6 Hz; singlet after addition of CD_3OD, H-3); ^{13}C nmr (75 MHz; $CDCl_3$) δ 53.2 (OMe), 101.7 (C-4), 108.3 (C-5), 119.1 (C-3), 125.0 (C-2), 161.3 (CO); ms *m/z* (rel. int.) 285, 283, 281 (M^+, 28.5, 54, 26.6), 254 (11.3), 253 (54.5), 251 (100), 250 (12.4), 249 (59.4), 199 (8.9), 197 (21.2), 195 (10.3).

Hplc of fraction B (10 mg thereof) on a C_{18} reversed phase column using H_2O-MeOH (20:80) gave, in order of elution, **7** (0.9 mg) and **8** (1.8 mg).

Compound **7**.—^{1}H nmr (300 MHz; $CDCl_3$) δ 3.86 (3H, s), 6.88 (1H, br s), 6.94 (1H, br s); signals at 6.88 and 6.94 sharpen on addition of CD_3OD; ms *m/z* (rel. int.) 205, 203 (M^+, 71.4, 66.3), 174 (46.8), 173 (100), 172 (47.9), 171 (90.7), 146 (46.3), 145 (15), 144 (50.5), 143 (10.1).

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