DITERPENES FROM THE NUDIBRANCH CHROMODORIS LUTEOROSEA

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ABSTRACT.—Three new diterpenes, luteorosin [1], 12-epi-aplysillin [2], and 12-epi-12-deacetoxyaplysillin [3], have been isolated from the nudibranch mollusk Chromodoris luteorosea, along with the previously known macfarlandin A [4]. Compounds 1–4 display ichthyotoxic activity; hence, they might serve as defense allomones.

Nudibranchs of the genus Chromodoris have been studied to some extent from a chemical point of view (1–12), resulting in the isolation of secondary metabolites for which in some instances a role of defense allomones has been proposed (1, 6, 9, 11) on the basis of their biological activities. Often it has been proved (1, 6, 9–12) or suspected (2, 4, 5–8) that these nudibranchs feed on sponges from which they obtain their defensive chemicals.

We add here the case of Chromodoris luteorosea von Rapp (Nudibranchia, Chromodorididae) from which we have isolated three new diterpenes, luteorosin [1], 12-epi-aplysillin [2] and 12-epi-12-deacetoxyaplysillin [3], along with the known macfarlandin A [4], previously isolated from Chromodoris macfarlandi (7). All the above compounds were toxic in the mosquito fish bioassay (13,14).

Luteorosin [1] and macfarlandin A [4] were eluted together from the first Si gel column fractionation of the lipophilic extract of C. luteorosea. The fraction containing 1 and 4 exhibited a single spot on SiO$_2$ tlc with various eluents; however, nmr spectra of this fraction showed that it consisted of a ca. 1:1 mixture of two compounds, which were separated by reversed-phase hplc. Macfarlandin A [4] was identified by comparison of its spectral data with those previously reported (7).

The molecular formula of luteorosin [1] was determined to be the same as macfarlandin A, C$_{21}$H$_{26}$O$_5$, by hreims on the ion at m/z 298.1625 [M − HOAc]$^+$ (base peak) and by inspection of the ^{1}H- and ^{13}C-nmr data.

The comparison of the ^{1}H-nmr spectra of 1 and 4 suggested that the main differ-

1

2   R=Ac
3   R=H

4

5
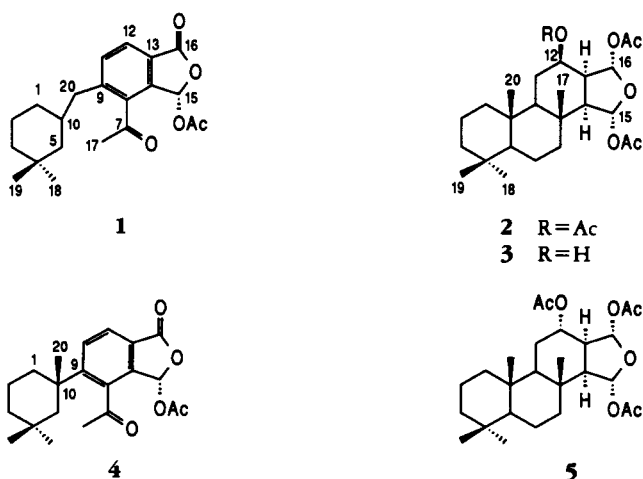
ence between the two molecules resided in the absence in **1** of a tertiary methyl signal, which was replaced by a benzylic methylene resonating as the AB part of an ABX system at δ 2.72 and 2.57 (C-20 protons). A ^{1}H-^{1}H COSY spectrum of **1** allowed the assignment of all the proton resonances on ring A (Table 1), revealing that the benzylic methylene was linked at C-10.

In the ^{13}C-nmr spectrum, 20 of the 21 expected resonances were observed, four Me, five CH_2, four CH, and seven out of eight quaternary carbons, by DEPT sequence. The signals were assigned by comparison with those of macfarlandin A (Table 1). The chemical shift values of the B- and C-ring carbons were very similar in the two compounds,

TABLE 1.   Nmr Data[a] for Compounds **1**, **2**, and **4**.

Carbon	Compound					
	1		**4**	**2**		
	δ ^{1}H (mult; *J* Hz)	δ ^{13}C	δ ^{13}C[b]	δ ^{1}H (mult; *J* Hz)[c]	δ ^{13}C[d]	
1	a 0.80 (m)	33.1	37.8		40.0	
	b 1.61 (m)					
2	ax 1.35 (m)	22.2	19.9		18.3[e]	
	eq 1.54 (m)					
3	ax 1.06 (dt, 4.1, 13.5)	39.0	39.4		42.2	
	eq 1.54 (m)					
4		nd[g]	31.8		33.2[f]	
5	a 1.29 (m) ($J_{a,b}$ = 12.8)	46.5	52.4		56.6	
	b 0.83 (m)					
6					18.0[e]	
7		201.9	203.4		41.8	
8		141.5[e]	140.8[e]		34.6[f]	
9		145.6	152.7	0.80 (m)	55.0	
10	1.70 (m)	36.0	41.1		37.4	
11	7.49 (d, 7.9)	126.4	125.2	ax 1.47 (q, 12.4)	23.3	
				eq 1.80 (bdd, 5.9, 12.8)		
12	7.87 (d, 7.9)	134.0	132.4	5.07 (p, $J_{12-11ax}$ = 12.1, $J_{12-11eq}$ = 6.0)		
13		125.0	124.3	3.09 (q, 7.5)	41.9	
14		137.7[e]	137.6[e]	1.99 (d, 7.5)	59.5	
15	7.50 (s)	91.5	91.4	6.06 (s)	99.3	
16		168.7[f]	168.5	6.36 (d, 7.6)	98.5	
17	2.53 (s)	33.4	33.9	0.99 (s)	17.0	
18	0.83[e] (s)	24.6[h]	28.9	0.85 (s)	33.2	
19	0.88[e] (s)	31.8[i]	32.2	0.80 (s)	21.3	
20	a 2.72 (dd, 6.7, 13.5)	41.7	33.2	0.86 (s)	16.5	
	b 2.57 (dd, 7.4, 13.5)					
OCO*Me*	2.14 (s)	20.5	20.6	1.98 (s), 2.05 (s), 2.07 (s)	20.7, 21.0, 21.2	
C=O		167.0[f]	167.0		169.7, 169.9, 170.1	

[a]Bruker WM 500; $CDCl_3$. The number of protons linked to each carbon in the ^{13}C spectra was determined by DEPT sequence.
[b]50 MHz, $CDCl_3$; data in this column are from Molinski and Faulkner (7).
[c]Selected data.
[d]Assignments made by ^{1}H-^{13}C HETCOR and comparison with known compounds (see text).
[e,f]Values with identical superscripts within a column may be interchanged.
[g]Not detected.
[h]Axial.
[i]Equatorial.

while C-1 and C-5 resonated distinctly at higher fields in **1** because of the absence of the C-10 quaternary carbon in the ring A.

The substitution pattern on the aromatic ring was assumed to be the same as in macfarlandin A and aplysulphurin (15) on biogenetic grounds; moreover, luterosin displays uv and ir data very similar to those of macfarlandin A. The cd spectrum also exhibited Cotton effects of the same sign as macfarlandin A, including a positive one at 253 nm diagnostic for the 15(*S*) absolute configuration (7).

From a biogenetic point of view, luteorosin could derive from a spongian diterpene (15) through a cyclopropane intermediate[1], the opening of which generates the benzylic methylene moiety (Scheme 1).
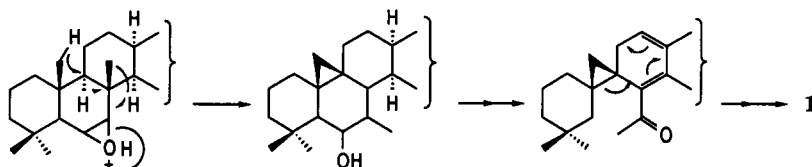
SCHEME 1

12-*epi*-Aplysillin [**2**] did not show the molecular ion in the eims spectrum, the first fragment appearing at *m/z* 344.2342 [M − 2HOAc]$^+$. Inspection of the nmr data of **2** (Table 1) suggested that it was the C-12 isomer of aplysillin [**5**], which was previously isolated from the sponge *Aplysilla rosea* (16) and whose structure was established by X-ray diffraction analysis.

The proton sequences from C-9 to C-16 in **2** were established by ^{1}H-^{1}H COSY. The coupling constant values established that the relative stereochemistry at carbons 13, 14, 15, and 16 was the same as in **5** and related molecules (17,18), while the C-12 acetoxy group should be equatorial in **2**. In fact, the C-12 proton appeared as a five lines signal (W/2 24 Hz) which was transformed to a double doublet when the C-13 proton was irradiated, exhibiting axial-axial (12.1 Hz) and axial-equatorial (6.0 Hz) *J* values with the C-11 protons. Accordingly the ^{13}C resonance of C-12 in **2** was found at lower field (δ 71.1) than the corresponding one in **5**, as expected for an equatorial compared with an axial acetoxy group.

The ^{13}C chemical shift values of **2** were assigned by a ^{1}H-^{13}C heterocorrelation nmr spectrum and by comparison with those of several model compounds (19); these latter were of particular value in assigning the resonances of the A- and B-ring carbons. The values were in agreement with those of **5** [incompletely reported in Kazlauskas *et al.* (16)], taking into account the γ effect imposed by the axial OAc group in **5**.

12-*epi*-12-Deacetoxyaplysillin [**3**] was the most abundant diterpene in *C. luteorosea*; however, it turned out to be rather unstable in CDCl$_3$ solution. Its structure was deduced from the ^{1}H-nmr spectrum (see Experimental) and by acetylation affording 12-*epi*-aplysillin [**2**].

The ichthyotoxicity bioassay was done with the mosquito fish *Gambusia affinis*, and the toxicity ranking was defined according to Coll *et al.* (13). Luteorosin [**1**] was found to be very toxic at 10 μg/ml while 12-*epi*-aplysillin [**2**], 12-*epi*-12-deacetoxyaplysillin [**3**], and macfarlandin A [**4**] were toxic at the same concentration. At lower concentrations (1 and 0.1 μg/ml) the compounds were inactive.

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[1]After this manuscript was submitted for publication, a paper appeared in the literature (22) reporting a spongian-derived metabolite with a C-20–C-9–C-10 cyclopropyl ring similar to the one proposed as an intermediate in the biogenesis of luteorosin (Scheme 1). We thank a referee for having pointed out this reference.

The ichthyotoxicity of compounds **1–4** strongly suggests that they may serve as defense allomones in *C. luteorosea*. It has been reported (20) that *C. luteorosea* feeds on *Spongionella* species sponges; however, the only *Spongionella* species living in the Mediterannean sea which has been studied from a chemical point of view is *Spongionella gracilis*, from which diterpenes related to but different from **1–4** have been reported (21). It is highly probable that *C. luteorosea* obtains the above diterpenes from thin, encrusting *Spongionella* species sponges.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded on a Bruker WM 500 instrument. Mass spectra were obtained on AEI MS-30 and Kratos MS-50 instruments. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Hplc was performed on a Waters 6000A apparatus, equipped with uv and ri detectors. Cd spectra were recorded on a JASCO J-6000 spectropolarimeter. Uv spectra were recorded on a Varian DMS 90 spectrophotometer. Ir spectra were recorded on a Bio-Rad FTS-7 spectrophotometer.

ANIMAL MATERIAL.—*C. luteorosea* (87 specimens) were collected by scuba diving in the bay of Taranto in June 1988, at a depth of 1–4 m and identified by G. Villani (Istituto per la Chimica di Molecole di Interesse Biologico). Voucher specimens are available for inspection at the Istituto per la Chimica di Molecole di Interesse Biologico.

ICHTHYOTOXICITY BIOASSAY.—Ichthyotoxicity assays were conducted using a mosquito fish, *G. affinis* (Baird and Girard), as described by Gunthorpe and Cameron (14). Compounds **1–4** were assayed at 10, 1, and 0.1 μg/ml by dissolving the appropriate amount in 0.5 ml of Me_2CO. Two replicate controls, one containing fish and freshwater only and the other fish, freshwater, and 0.5 ml of Me_2CO, were observed in conjunction with each test run. The toxicity ranking was defined as in Coll *et al.* (13).

ISOLATION OF COMPOUNDS **1–4**.—*C. luteorosea* (85 specimens) were soaked in Me_2CO (500 ml $\times$ 3). The combined extracts were evaporated, and the aqueous residue was extracted with Et_2O (50 ml $\times$ 3). The combined Et_2O extracts were combined and evaporated to obtain an oil (97 mg) which was chromatographed on a Si gel column using $CHCl_3$ as eluent. Compounds **1–4** were collected into three main fractions, A, B, and C.

Fraction A (4 mg) contained a mixture of luteorosin [**1**] and macfarlandin A [**4**]. Fraction B (40 mg) was a mixture of the above products, 12-*epi*-aplysillin [**2**], and sterols. This fraction was chromatographed on a Si gel column using C_6H_6-Et_2O (99:1) as eluent, affording fractions B_1 (8 mg), containing a mixture of **1** and **4**, and B_2 (5 mg) consisting of pure **2**. Fraction C afforded, after evaporation of the $CHCl_3$, 10 mg of crystalline 12-*epi*-12-deacetoxyaplysillin [**3**]. Fractions A and B_1 were combined and purified by hplc on an RP-18 column, using $MeCN$-H_2O (7:3) as eluent, to afford, in order of increasing retention time, 2 mg of **4** and 2 mg of **1**.

LUTEOROSIN [**1**].—Ir (liquid film) ν max 2923, 1789, 1701, 1203 cm^{-1}; uv λ max (MeOH) 215 (ϵ 19,000), 243 (ϵ 11,200); ^{1}H and ^{13}C nmr see Table 1; cd (MeOH), 220 ($\Delta\epsilon$ −6.3), 253 ($\Delta\epsilon$ +1.1), 312 ($\Delta\epsilon$ +1.3); eims *m/z* (rel. int.) 298 (100), 283 (15), 188 (38); hreims *m/z* 298.1625, $C_{19}H_{22}O_3$ [M − HOAc]$^+$ requires 298.1569.

12-*epi*-APLYSILLIN [**2**].—[α]^{25}D +8.2 (*c* = 0.4, $CHCl_3$); ir ($CHCl_3$) ν max 2970, 1740, 1370 cm^{-1}; ^{1}H and ^{13}C nmr see Table 1; eims *m/z* (rel. int.) 344 (100), 302 (48), 191 (21); hreims *m/z* 344.2342, $C_{22}H_{32}O_3$ [M − 2HOAc]$^+$ requires 344.2351.

12-*epi*-12-DEACETOXYAPLYSILLIN [**3**].—Mp 192–197°; [α]^{25}D +2.5 (*c* = 0.4, $CHCl_3$); ir ($CHCl_3$) ν max 2970, 1740, 1370 cm^{-1}; eims *m/z* (rel. int.) [M − 2HOAc]$^+$ 302 (100), 287 (22), 284 (28), 269 (60), 191 (32); ^{1}H nmr ($CDCl_3$) δ 6.37 (d, *J* = 7.5 Hz, H-16), 6.07 (s, H-15), 4.01 (m, W/2 = 24 Hz, H-12), 2.93 (q, *J* = 7.4 Hz, H-13), 2.10 and 2.07 (3H singlets, acetyl methyls), 1.83 (bdd, *J* = 12.7 and 5.4 Hz, H-11eq), 0.80, 0.84, 0.87, and 0.97 (3H singlets, C-4, C-8, and C-10 methyls).

Compound **3** (3 mg) was acetylated with Ac_2O (0.1 ml) in pyridine (1 ml) overnight at room temperature. Removal of the solvents and chromatography on Si gel pasteur pipette (eluent $CHCl_3$) yielded 2.5 mg of **2**, identified by tlc and ^{1}H nmr.

ACKNOWLEDGMENTS

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