Ichthyotoxic Diterpenoids from the Cantabrian Nudibranch Chromodoris luteorosea

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ICHTHYOTOXIC DITERPENOIDS FROM THE CANTABRIAN NUDIBRANCH *CHROMODORIS LUTEOROSEA*

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ABSTRACT.—Five spongian diterpenoids **2**, **3**, **6–8**, previously found in sponges and nudibranchs from very distinct geographical areas, have been isolated from the Cantabrian nudibranch *Chromodoris luteorosea*. The terpenoids are mainly localized along the border of the mantle of the mollusk. All the diterpenoids are toxic to *Gambusia affinis*.

Chromodorididae nudibranchs of the four genera *Chromodoris*, *Glossodoris*, *Hypselodoris*, and *Cadlina* have elaborated a very effective defensive strategy against predators. They are protected by dietary allomones (1–3), generally sequestered from sponges and transferred either into selected mantle formations or into mucous secretions. In particular, many *Chromodoris* mollusks possess a well-assorted arsenal of degraded and rearranged diterpenoids deriving from precursors with the spongian (4) carbon skeleton **1**. The dietary origin of the diterpenoids from *Chromodoris* nudibranchs was strongly suggested by structural analogies with typical sponge metabolites from the genera *Dysidea* (5), *Aplysilla* (6,7), *Dendrilla* (8), and *Spongionella* (9).

The recent finding of four diterpenoids, macfarlandin A [**2**], luteorosin [**3**], 12-*epi*-aplysillin [**4**], and 12-*epi*-12-deacetylaplysillin [**5**] (incorrectly named as 12-*epi*-12-deacetoxyaplysillin), in the Mediterranean *Chromodoris luteorosea* Rapp (Nudibranchia, Chromodorididae) (10), has prompted further research on specimens belonging to the same species but living in the Atlantic Ocean (Cantabrian Sea, North Spain). The present study has led to the chemical characterization of five diterpenoids: **2** and **3** again, the previously described polyrhaphin C [**6**] and norrisolide [**7**] and, finally, chelonaplysin C [**8**], recently found in an encrusting Pacific sponge *Chelonaplysilla* sp. (11). Norrisolide was previously found in the nudibranch *Chromodoris norrisi* (12), in sponges *Dendrilla* sp. (13), *Dysidea* sp. (14), *Chelonaplysilla* sp. (11), and *Aplysilla polyrhaphis* (6). Polyrhaphin C was isolated from the sponge *A. polyrhaphis* (6).

The Et_2O-soluble fraction (124 mg) from the Me_2CO extract of 50 specimens of *Chr. luteorosea* was analyzed on Si gel tlc [petroleum ether-Et_2O (4:6)], revealing, in order of increasing polarity, three relevant components: A, R_f 0.5; B, R_f 0.3; and C, R_f 0.28. After a series of chromatographic steps, A was recovered and separated into two components, identical in every chemical and spectral aspect to **2** and **3**. Analogously, B yielded **6** and **7**, whereas C resulted in a single component that, after an exhaustive spectral study, was shown to be identical to chelonaplysin C [**8**]. Nmr data were in agreement with those previously published (11), while $[\alpha]_D$ ($CHCl_3$) $-55°$ and mp 180°–182° differ considerably from reported values. We are unable to explain this discrepancy. All the ^1H- and ^{13}C-nmr resonances of **8** were assigned by 2D methods and confirmed by monodimensional ^1H-^1H de-

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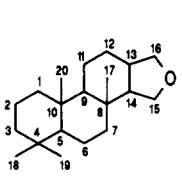

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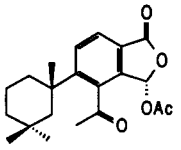

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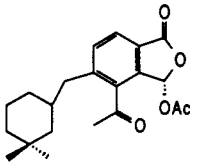

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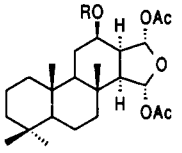
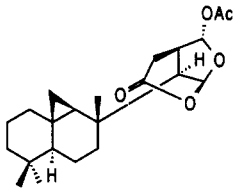


4 R=Ac
5 R=H



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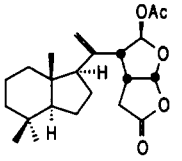

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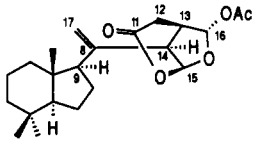

8

coupling and nOeds experiments (Experimental).

Studies on *Chr. luteorosea* from Spanish coasts confirm the ability of *Chromodoris* nudibranchs to prey selectively on sponges possessing rearranged spongian diterpenoids. Even though these metabolites are typical of many sponges widespread in different geographical areas, the *Chromodoris* mollusks have been observed grazing on sponges only rarely. On the basis of the recent finding of norrisolide [**7**] and chelonaplysin C [**8**] in a Pacific sponge *Chelonaplysilla* sp. (11), it is possible that the Atlantic *Chr. luteorosa* preys on unidentified aplysillid sponges.

Recently, the identity of two related genera, *Chromodoris* and *Hypselodoris*, both belonging to the Chromodorididae family, has been clarified on the basis of their anatomical differences (15).

Mediterranean and Atlantic *Hypselodoris* nudibranchs, that are also rarely observed near their prey, are strongly suspected to be predators of *Dysidea* sponges on the basis of chemical evidence. In fact, they possess (16) large quantities of furanosesquiterpenoids typical *Dysidea* metabolites. However, it seems that the two studied Mediterranean Chromodorididae genera are selectively attracted to closely related sponges possessing either spongian diterpenoids (*Chromodoris*) or furanosesquiterpenoids (*Hypselodoris*). The potential defensive role of the diterpenoids **2**, **3**, **6–8** from the Spanish *Chr. luteorosea* is substantiated by their ichthyotoxicity to *Gambusia affinis*. All the metabolites were toxic in the mosquito fish assay (17,18) at a concentration of 10 ppm. An additional support to the defensive role of the diterpenoids was inferred from the chemical analysis (Table 1) of dissected parts of the mollusks.

TABLE 1. Distribution[a] of Spongian Diterpenoids and Sterols in Dissected Parts of *Chromodoris luteorosea*.

Compound	Border of mantle	Foot and rest of mantle	Gills	Digestive gland
Macfarlandin A [2] + luteorosin [3]	+++	+	−	+
Polyrhaphyn C [6] + norrisolide [7]	+++	traces	−	+
Chelonaplysin C [8]	+++	traces	−	+
Sterols	+	++	+	++

[a]As estimated by tlc. +++, large, ++, significant, +, poor, −, not detected.

The diterpenoids were found concentrated only along the border of the mantle; minor amounts of the compounds were found also in the digestive gland according to their dietary origin. It seems reasonable that *Chromodoris* nudibranchs are able to sequester from selected sponges ichthyotoxic molecules that are accumulated in specific sections of the mantle, more exactly in that part (the border) more exposed to predators.

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 Jasco DIP 370 polarimeter. Ir spectra were recorded on a Nicolet FT 5DXB spectrophotometer. Si gel chromatography was performed using pre-coated Merck F_{254} plates and Merck Kieselgel 60 powder. Hplc purifications were carried out on a Waters 6000A apparatus equipped with uv and ir detectors.

BIOLOGICAL MATERIAL.—*Chr. luteorosea* (50 specimens) were collected by scuba diving in Las Llanas, Asturias, northern Spain (43°28′N, 6°05′W), during spring 1990, at depths of 2–7 meters. Animals were identified by J. Ortea. Voucher specimens are available for inspection at Departamento de Biologia de Organismos y Sistemas of the University of Oviedo.

ISOLATION OF THE DITERPENOIDS.—Fifty *Chr. luteorosea* specimens were immersed in Me_2CO (300 ml × 3) at room temperature. The combined extracts were concentrated at reduced pressure, and the residual H_2O was extracted with Et_2O (50 ml × 3). The ethereal extracts were combined and evaporated to give 124 mg of crude material, which was chromatographed on a Si gel column using C_6H_6 with increasing amounts of Et_2O as eluent. Three main fractions containing spongian diterpenoids were obtained: A, B, and C. Fraction A (4 mg), which was a mixture of macfarlandin A [**2**] and luteorosin [**3**], was purified by hplc according to previously reported conditions (10), to give in order of increasing retention time 1 mg of **2** and 1 mg of **3**, identified by their spectral data. Fraction B (9 mg) was rechromatographed on a Si gel column [petroleum ether-Et_2O (7:3)], affording in order of increasing polarity polyrhaphin C [**6**] (1 mg) and norrisolide [**7**] (3 mg), identified by comparison of their spectral data with those reported in literature. Fraction C (12 mg) was subjected to preparative Si gel tlc [C_6H_6-Et_2O (7:3)] to give 9 mg of chelonaplysin C [**8**].

Chelonaplysin C [**8**].—$[\alpha]^{25}D$ −55° (c = 0.24, $CHCl_3$); mp (*n*-hexane/Et_2O) 180°–182°; ir ($CHCl_3$) ν max 1755 cm^{-1}; ^1H and ^{13}C nmr ($CDCl_3$) carbon position (δ ^{13}C; δ ^1H): C-1 or -3 (39.7; 1.18, 1.71), C-2 (20.1; 1.52, 1.67), C-3 or -1 (41.4; 1.08, 1.46), C-4 (33.3), C-5 (58.8; 1.30), C-6 (20.5; 1.42, 1.65), C-7 (24.9, 1.73), C-8 (140.3), C-9 (56.0; 2.15), C-10 (43.4), C-11 (166.7), C-12 (32.5; 2.62, 3.14), C-13 (38.2; 2.81), C-14 (46.9; 3.20), C-15 (100.6; 5.88), C-16 (101.2; 6.26), C-17 (116.3; 5.16, 5.24), C-18 (20.6; 0.87), C-19 (33.1; 0.86), C-20 (13.8; 0.71), *C*OMe (169.4), CO*Me* (21.1; 2.11); ^1H nmr (C_6D_6) δ 6.12 (s, H-16), 5.79 (dd, J = 3.4, 0.7, H-15), 4.93 (bs, H_b-17), 4.74 (d, J = 2.2, H_a-17), 3.08 (m, H-14), 2.68 (dd, J = 19.2, 6.3, H_b-12), 2.03 (m, H-13), 2.00 (d, J = 19.2, H_a-12), 1.96 (bt, J = 9.5, H-9), 1.51 (3H singlet, Ac), 0.80, 0.78, 0.50 (3H singlets, Me-18, Me-19, Me-20); ^{13}C nmr (C_6D_6) δ 168.4, 165.5, 141.1, 116.0, 101.3, 100.5, 58.5, 56.1, 47.4, 43.4, 41.5, 39.7, 38.5, 33.2, 32.8, 25.1, 20.7, 20.4, 20.3, 20.0, 13.9; nOed's ($CDCl_3$)

irradiated (observed) H-9 (H-15 and H-5), H-13 (H-16, H_a-17, H_b-12, and H-14), H-14 (overlapped with H_b-12) (H-15, H_a-17, H-13, and H_a-12), H-15 (H-14 and H-9), H-16 (H_a-12 and H-13), H_a-17 (H_b-17, H_b-12, H-13), H_b-17 (H_a-17, H-7, Me-20); nOed's (C_6D_6) irradiated (observed) H-14 (H-15 and H-13), H_b-12 (H_a-12 and H_a-17); eims *m/z* (rel. int.) 376 (2), 361 (4), 316 (15), 301 (10), 283 (7), 192 (20), 137 (75), 123 (100); hreims *m/z* 376.2269, $C_{22}H_{32}O_5$ requires 376.2250.

ICHTHYOTOXICITY BIOASSAY.—Ichthyotoxicity assays were conducted using a mosquito fish, *Ga. affinis* (Baird and Girard), as described by Gunthorpe and Cameron (18). Compounds **6**, **7**, and **8**, analogously to 2 and 3 (10), were assayed at 10 and 1 ppm by dissolving the appropriate amount in 0.5 ml of Me_2CO. Control tests were carried out in conjunction with each test run. The toxicity ranking was defined according to Coll *et al.* (17).

DISSECTION OF *CHR. LUTEOROSEA*.—Frozen animals were usually dissected in four parts: border of mantle, rest of mantle and foot, gills, and digestive gland. The amount of terpenoids in the dissected tissues was evaluated by comparative tlc analysis.

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LITERATURE CITED

1. D.J. Faulkner, *Nat. Prod. Rep.*, **8**, 97 (1991), and references cited therein.
2. P. Karuso, in: "Bioorganic Marine Chemistry." Ed. by P.J. Scheuer, Springer-Verlag, Berlin, 1987, Vol. 1, pp. 32–60.
3. G. Cimino and G. Sodano, *Chem. Scr.*, **29**, 389 (1989).
4. R. Kazlauskas, P.T. Murphy, R.J. Wells, K. Noack, W.E. Oberhansli, and P. Schonholzer, *Aust. J. Chem.*, **32**, 867 (1979).
5. S. Carmely, M. Cojocaru, Y. Loya, and Y. Kashman, *J. Org. Chem.*, **53**, 4801 (1988), and references cited therein.
6. S.C. Bobzin and D.J. Faulkner, *J. Org. Chem.*, **54**, 3902 (1989), and references cited therein.
7. M. Tischler, R.J. Andersen, M. Iqbal Choudhary, and J. Clardy, *J. Org. Chem.*, **56**, 42 (1991).
8. S.C. Bobzin and D.J. Faulkner, *J. Org. Chem.*, **54**, 5727 (1989), and references cited therein.
9. L. Mayol, V. Piccialli, and D. Sica, *Gazz. Chim. Ital.*, **118**, 559 (1988), and references cited therein.
10. G. Cimino, A. Crispino, M. Gavagnin, and G. Sodano, *J. Nat. Prod.*, **53**, 102 (1990).
11. S.C. Bobzin and D.J. Faulkner, *J. Nat. Prod.*, **54**, 225 (1991).
12. J.E. Hochlowski, D.J. Faulkner, G.K. Matsumoto, and J. Clardy, *J. Org. Chem.*, **48**, 1141 (1983).
13. B. Sullivan and D.J. Faulkner, *J. Org. Chem.*, **49**, 3204 (1984).
14. A. Rudi and Y. Kashman, *Tetrahedron*, **46**, 4019 (1990).
15. W.B. Rudman, *Zool. J. Linn. Soc.*, **81**, 115 (1984).
16. C. Avila, G. Cimino, A. Fontana, M. Gavagnin, J. Ortea, and E. Trivellone, *J. Chem. Ecol.*, **17**, 625 (1991).
17. J.C. Coll, S. La Barre, P.W. Sammarco, W.T. Williams, and G.J. Bakus, *Mar. Ecol: Progr. Ser.*, **8**, 271 (1982).
18. L. Gunthorpe and A.M. Cameron, *Mar. Biol.*, **94**, 39 (1987).

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