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FURTHER CHEMICAL STUDIES OF MEDITERRANEAN AND ATLANTIC *HYPSELODORIS* NUDIBRANCHS: A NEW FURANOSESQUITERPENOID FROM *HYPSELODORIS WEBBI*

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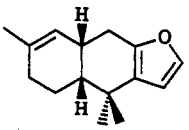
ABSTRACT.—Chemical studies of three *Hypselodoris* species from distinct geographical areas are reported. A new furanosesquiterpenoid, (+)-(6*R*,11*R*)-9,10-dehydrofurodysinin [**6**], was isolated from *H. webbi* from Spanish coasts and its structure was elucidated by spectroscopic data and confirmed by chemical reduction to (−)-(6*R*,11*R*)-furodysinin.

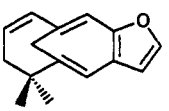
Many chemical studies (1–3) have recently proved that nudibranchs in the genus *Hypselodoris* are protected by defensive chemicals, generally furanosesquiterpenoids that are selectively stored in specific organules, called Mantle Dermal Formations (MDFs) (4). Among the nudibranchs of the *Hypselodoris* genus, only *H. orsini* [=*Glossodoris tricolor* (5)] contains a different chemical pattern characterized by scalarane sesterterpenoids (3).

Continuing this line of study, we have chemically investigated populations of *H. webbi* (D'Orbigny, 1839) from Spain, *H. bayeri* (Marcus & Marcus, 1967) from Cuba, and *H. tricolor* (Bouchet & Tardy, 1976) from Italy. In this paper we report the isolation and structure elucidation of the secondary metabolites potentially involved in the defensive strategy of these mollusks.

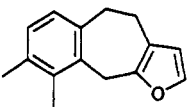
All nudibranchs were characterized by furanosesquiterpenoids that are probably sequestered from dietary sponges. In fact, the sponge metabolite (−)-(6*R*,11*R*)-furodysinin [**1**] (2, 6–9) was found in *H. bayeri*, whereas the known metabolites of *Pleraplysilla spinifera*, spiniferin-1 [**2**] (10) and spiniferin-2 [**3**] (11), were present in *H. tricolor*. Finally, pallescensin-A [**4**] (12) and (+)-furodysin [**5**] (13) co-occur with a new natural product, (+)-(6*R*,11*R*)-9,10-dehydrofurodysinin [**6**], in *H. webbi*. The products **1–5** were identified by comparison of their spectroscopic data with those reported in the literature. The ^{1}H-nmr spectrum (C_6D_6) of **6** displayed characteristic signals attributable to the protons (H-1, δ 7.07; H-2, δ 6.13) of an α,β-disubstituted furan ring, along with a vinyl methyl (δ 1.65), two methyl singlets (1.26 and 1.13 ppm), an olefin proton (H-7, δ 5.58), and a well-resolved AB system (J=12 Hz) assigned to the protons (H-9, δ 5.78; H-10, δ 5.81) of a trisubstituted conjugated diene further coupled with a proton (H-11) resonating as a broad singlet at δ 2.30. The presence of two uv maxima at 216 and 257 nm confirmed, in addition to the furan ring, the presence of a homoannular diene. The ^{1}H-nmr spectrum was completed by a complex three-proton signal at δ 2.51. A definitive assignment was performed by recording a ^{1}H-nmr spectrum of **6** in $CDCl_3$ where the resonances of protons at carbons 5, 6, and 11 were well-resolved. In particular, irradiation at δ 2.67 (H-6) simplified the resonances at δ 2.34 (H-11), δ 2.41 and



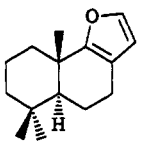
1
(from *H. bayeri*)



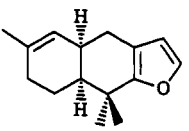
2
(from *H. tricolor*)



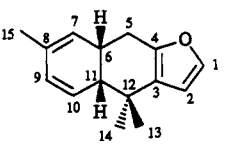
3
(from *H. tricolor*)



4
(from *H. webbi*)



5
(from *H. webbi*)



6
(from *H. webbi*)

δ 2.47 (H-5a and H-5b), and 5.76 (H-7). These data, along with the molecular ion peak at *m/z* 214, were in good agreement with the structure of a 9,10-dehydro-derivative, either of furodysinin [**1**] or of furodysin [**5**]. Various 2D nmr (^{1}H-^{1}H COSY, ^{1}H-^{13}C HETCOR, ^{1}H-^{13}C COLOC) experiments were used to assign all the ^{1}H- and ^{13}C-nmr resonances (Table 1). In particular, the ^{13}C-nmr resonance of C-3 (δ 150.5) was in agreement with previous data for both enantiomers of furodysinin (2,13). The long-range ^{1}H-^{13}C heterocorrelation between C-3 (δ 125.76) and H_3-13 (δ 1.13) and H_3-14 (δ 1.26) confirmed the localization of C-3 near the quaternary C-12. In order to define the stereochemistry of the carbons 6 and 11, the reduction of **6** was undertaken. Treatment with H_2/Pd reduced **6** to yield a mixture of products with (−)-furodysinin [**1**] as one of the main products. All of the spectral data of the dihydroderivative of **6** were identical to those of **1**, including the positive cd curve at ca. 220 nm.

As furanosesquiterpenes are probably involved in the defensive strategy of nudibranchs belonging to *Hypselodoris* genus, the biological activity of **6** was tested. The compound exhibited antifeedant activity at 100 μg/cm^2 against

TABLE 1. ^{1}H- and ^{13}C-Nmr Data of 9,10-Dehydrofurodysinin [**6**] [Chemical Shifts δ (ppm), multiplicity, *J* Values in Hz] in C_6D_6.

Carbon	^{1}H	^{13}C
C-1	7.07, br s (*J*=1.9)	140.21, d
C-2	6.13, br d (*J*=1.9)	108.15, d
C-3	—	125.76, s
C-4	—	150.50, s
C-5	2.51, m	24.95, t
C-6	2.51, m	30.41, d
C-7	5.58, d (*J*=5.7)	126.40, d
C-8	—	131.96, s
C-9	5.78, br d (*J*=12.0)	129.15, d
C-10	5.81, br d (*J*=12.0)	128.12, d
C-11	2.30, br s	46.05, d
C-12	—	32.85, s
C-13	1.13, s	32.11, q
C-14	1.26, s	25.95, q
C-15	1.65, s	20.85, q

the freshwater fish *Carassius auratus* (5,14). Moreover, **6** showed considerable toxicity (LD_{50}= 1.52 ppm) as determined by the brine shrimp test (15), whereas **6** was ichthyotoxic in an assay against *Gambusia affinis* (16,17) at 10 ppm. Finally, anatomical dissection confirmed the localization of the sesquiterpenoids in the Mantle Dermal Formations (MDFs) of the molluscs.

Previous studies on *H. webbi* and *H. tricolor* from the Mediterranean and Atlantic coasts have led to the characterization of a series of terpenoids related to **1**–**6** (1,2,5). This paper has confirmed, by studying molluscs from distinct geographical areas, that *Hypselodoris* species are able to selectively prey upon sponges which contain furanosesquiterpenoids. It is worth noting that spiniferin-2 [**3**] (18), pallescensin-A [**4**] (14) and (+)-furodysin [**5**] (14, 19–21) have already been isolated from Chromodorididae nudibranchs from the Pacific Ocean.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a Bio Rad FTS-7 spectrophotometer, and optical rotations were measured on a Jasco Dip-370 polarimeter. Cd curves were performed on a Jasco J-710 spectropolarimeter in *n*-hexane or EtOH. Uv spectra were obtained on a Varian DMS 90 spectrophotometer. 1D and 2D ^{1}H- and ^{13}C-nmr experiments were recorded at room temperature on either Bruker WM 500 (1D, ^{1}H- and ^{13}C-nmr spectra) or AMX-500 (2D, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HETCOR, ^{1}H-^{13}C COLOC) nmr spectrometers which were locked onto the major deuterium signal of the solvent, C_6D_6 or $CDCl_3$. Chemical shifts are given in ppm relative to signal of solvent, and coupling constants are reported in Hz. All mass spectra were taken on an AEI MS 30 instrument. Analytical tlc was carried out both on Merck precoated Si-Gel 60 F_{254} and Merck precoated Kieselgel 60 F_{254} treated with a solution of $AgNO_3$ (8% in Me_2CO-H_2O, 8:2) visualized by Ehrlich's reagent. Merck Kieselgel 60 (70–230 mesh) was used for Si gel chromatography and precoated Kieselgel 60 F_{254} plates were used for prep. tlc.

BIOLOGICAL MATERIALS.—*H. webbi* (8 specimens) was collected in the autumn of 1990 at a depth of 20 m off Blanes (northeast Spain). A voucher specimen is deposited at CEAB-Blanes (CEAB#190-1990).

H. bayeri (2 specimens) was collected in Cuba during the summer of 1992. A voucher sample is deposited at CBOS-Oviedo (CBOS#78.92).

H. tricolor (6 specimens) was collected by scuba diving near Sorrento (southwest Italy) during the summer of 1993. A voucher sample is deposited at CBOS-Oviedo (CBOS#45.93).

The taxonomic identification of *H. webbi* from Blanes was carried out by C. Avila, whereas *H. bayeri* and *H. tricolor* were identified by J. Ortea. All frozen specimens of *Hypselodoris* were dissected in four parts: mantle dermal formations (MDFs), digestive gland, rest of viscera, and rest of mantle.

COMPOUND ISOLATION.—The frozen sections (2.8 g dry wt) of *H. webbi* (6 specimens) were separately extracted three times with Me_2CO. The concentrated extracts were partitioned between Et_2O and H_2O. The Et_2O-soluble fractions were compared by tlc. Such analysis revealed the presence of the same pattern of products sensitive to Ehrlich's reagent in the MDFs and in the digestive gland. The Et_2O extract of the digestive gland was fractionated on a Si gel column by using an increasing polarity gradient from petroleum ether to Et_2O to give pallescensin-A [**4**, 10 mg; $[\alpha]^{25}D$ +50.7° ($CHCl_3$, c=0.03)], (+)-furodysin [**5**, 15.5 mg; $[\alpha]^{25}D$ +26.1° ($CHCl_3$, c=0.1)], and ten other fractions. Chromatography of the complex fraction 4 (110 mg) on Si gel treated with 7.5% $AgNO_3$ led to the isolation of a third component subsequently characterized as (+)-(6*R*,11*R*)-9,10-dehydrofurodysinin (**6**, 11 mg).

The frozen *H. bayeri* (1 specimen; 1.1 g dry wt) was examined as described above. After tlc controls, the combined Et_2O fraction of each section (55 mg) was purified on a Si gel column to yield (−)-furodysinin [**1**, 12 mg; $[\alpha]^{25}D$ −55.2° ($CHCl_3$, c=0.01)].

The extracts of the sections (0.8 g dry wt) from *H. tricolor* (5 specimens) were compared by tlc in petroleum ether. The Et_2O-soluble fractions of the MDF and the digestive gland, which showed the same metabolic pattern, were combined to afford 32 mg of yellow oil. Prep. tlc purification yielded spiniferin-2 (**3**, 1 mg) and 0.5 mg of another product positive to Ehrlich's reagent identified as spiniferin-1 (**2**) by tlc comparison with an authentic extract of *P. spinifera*. The data of **1**–**5** were in agreement with previous reports: (−)-**1** (2, 6–9), **2** (10), **3**, (11), **4** (12), and (+)-**5** (8,13).

(+)-(6R,11R)-9,10-Dehydrofurodysinin [**6**].—$[\alpha]^{25}D$ +259.0° (c=0.22, $CHCl_3$); hreims m/z 214.1305 (calcd for $C_{15}H_{18}O$, 214.1357); eims m/z 214 (M^+, 100), 199 (M^+-CH_3, 70), 197 (40) uv (MeOH) λ max 257, 216 nm; ^{1}H nmr ($CDCl_3$) δ 1.21 (3H, s, H_3-13 or H_3-14), 1.34 (3H, s, H_3-14 or H_3-13), 1.76 (3H, s, H_3-15), 2.34 (1H, bd, J=3.8 Hz, H-11), 2.41 (1H, dd, J=8.8 and 16.3 Hz, H-5a), 2.47 (1H, dd, J=6.2 and 16.3 Hz, H-5b), 2.67 (1H, m, H-6), 5.76 (1H, d, J=5.7 Hz,

H-7), 5.85 (2H, bs, H-9 and H-10), 6.25 (1H, d, J=1.6 Hz, H-2), 7.17 (1H, bd, J=1.6 Hz, H-1). The nmr data in C_6D_6 are reported in Table 1. Selected $^{13}C \rightarrow {}^{1}H$ long range correlations in C_6D_6: $C_3 \rightarrow H_3$-13 and H_3-14; $C_4 \rightarrow H_2$-5; $C_6 \rightarrow$H-7 and H_2-5; $C_8 \rightarrow H_3$-15 and H-10; $C_9 \rightarrow$H-7 and H_3-15; $C_{10} \rightarrow$H-11; $C_{11} \rightarrow H_3$-13, H_3-14 and H-10; $C_{12} \rightarrow H_3$-13, H_3-14 and H-11.

CATALYTIC HYDROGENATION OF **6**.—A suspension of 5% Pt/C (16 mg) in $CHCl_3$ (2 ml) was stirred vigorously with **6** (4 mg, 0.0187 mmol) under H_2 gas at 20° for 40 min. The reaction mixture was filtered and dried at reduced pressure. The residue, dissolved in *n*-hexane, was fractionated by Si gel/8% $AgNO_3$ prep. tlc by eluting with *n*-hexane-C_6H_6 (95:5) to yield 1 mg of (−)-furodysinin [**1**] together with a mixture of other products. The stereochemistry of the hydrogenated product was demonstrated by comparison of optical activity [$[\alpha]^{25}D$ −45.9° (c=0.04, $CHCl_3$)] and the cd curve [$[\theta]_{220}$ +2640] with an authentic sample of **1**.

BIOLOGICAL TESTING.—An ichthyotoxicity assay was performed using the mosquito fish, *Gambusia affinis* (16,17). Compound **6** in pure Me_2CO was added to 50 ml fresh H_2O at three different concentrations: 0.1, 1, and 10 ppm; pure Me_2CO was used as control. Toxicity was defined as death of fish within 24 h.

Antifeedant activity was evaluated by the methods of Cimino *et al.* (5) and Thompson *et al.* (14). The test was carried out in triplicate by observing the feeding response of goldfish, *Carassius auratus*, toward food pieces treated with pure **6**, applied to the pellets with Et_2O. Feeding deterrence was evaluated in a range of 50–200 $\mu g/cm^2$ of fish food. Blanks were performed using pellets treated with pure Et_2O before and after the experiments with treated food.

The brine shrimp test was carried out as previously described (15). Activity, manifested as toxicity against *Artemia salina*, is expressed as LD_{50}, values in μg/ml (ppm) at 95% confidence intervals.

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