Scalarane and Homoscalarane Compounds from the Nudibranchs *Glossodoris sedna* and *Glossodoris dalli*: Chemical and Biological Properties

A. Fontana,*,† E. Mollo,† J. Ortea,‡ M. Gavagnin,† and G. Cimino†

Istituto per la Chimica di Molecole di Interesse Biologico (ICMIB)[1] del CNR, via Toiano 6, 80072 Arco Felice, Napoli, Italy, and Departamento de Biologia de Organismos y Sistemas, Laboratorio de Zoologia, Universidad de Oviedo, c/Catedratico R. Uría s/n, 33071 Oviedo, Spain

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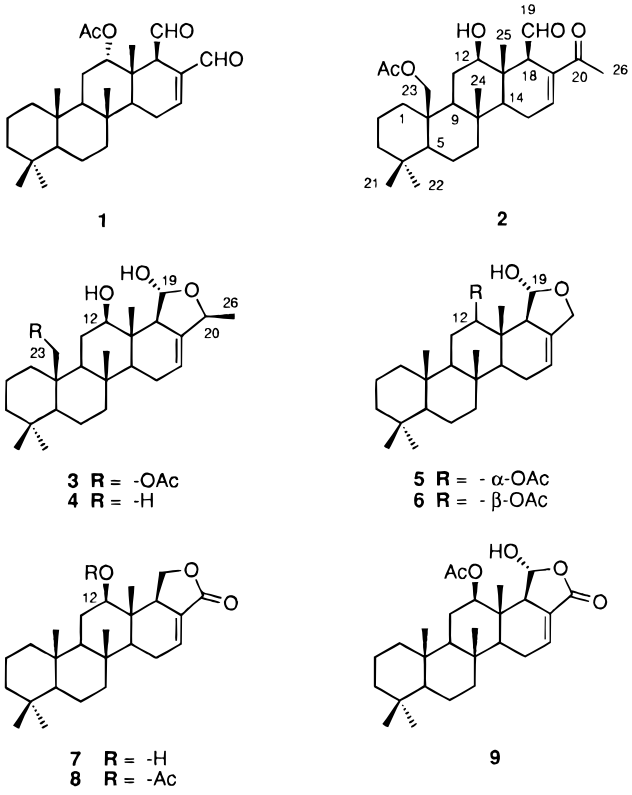
A series of homoscalarane and scalarane compounds (**2**−**7**) have been isolated from two distinct species of Pacific *Glossodoris* nudibranchs. The structure and elucidation of the relative stereochemistry of the new metabolites **2** and **3** were obtained by spectroscopic methods. Compound **2** was ichthyotoxic at 0.1 ppm against *Gambusia affinis* and showed moderate activity (IC_{50} 18 μM) to inhibit mammalian phospholipase A_2.

The organic extracts of dorid nudibranchs are well-known to contain scalarane[2−4] or homoscalarane[5] compounds, probably derived from feeding on sponges. All these compounds are related to 1,4-dicarbonyl products of which the earliest example is the biologically active scalaradial (**1**).[6,7] In analogy with other metabolites containing the vicinal dialdehyde moiety,[8] **1** tends to interact with nucleophiles to give adducts of condensation,[8,9] and such a property may be also involved to explain its antiinflammatory activity.[7] From a general point of view, scalarane and homoscalarane compounds show a series of pharmacological properties (cytotoxicity, antimicrobial, and anti-inflammatory activity and platelet aggregation inhibition), and many authors also believe these sesterterpenoids are involved in the chemical defense of mollusks and sponges. Nevertheless, the ecological studies about the defensive role of scalaradial and related compounds have given conflicting results, inasmuch as most of them were not active as deterrents against many species of potential generalist predators.[2,3]

Studying two species of Pacific nudibranchs, *Glossodoris sedna* and *Glossodoris dalli*, we have recently isolated a number of scalarane and homoscalarane products (**2**−**7**). This paper reports the structure assignment of the new compounds **2** and **3**, as well as the spectral data of the metabolite **4** partially described by other authors.[5] The keto-aldehyde **2** was active in ichthyotoxic tests and inhibited mammalian phospholipase A_2 (PLA_2). Both properties seem to be related to the chemical reactivity of the vicinal keto-aldehyde moiety toward nucleophiles. Comparison of the biological activity and the chemical reactivity between scalaradial (**1**) and compound **2** is also drawn in the paper.

G. sedna (42 specimens) Marcus and Marcus, 1967 and *G. dalli* (18 specimens) Bergh, 1879 were collected near the Natural Park of Osa Ballena in Costa Rica. The organisms were processed following the procedure described in the Experimental Section. Fractionation of the ether soluble-material from *G. sedna* gave **2**−**4**, whereas Si gel column chromatography of *G. dalli* extracts yielded 20-deoxoscalarin (**5**), 12-*epi*-20-deoxoscalarin (**6**), and a mixture of minor components containing **7**. This last compound was purified as acetyl derivative **8** by reversed-phase HPLC. The structures of the known metabolites **4**−**6** and **8** were determined by comparison of spectral data with those reported in the literature.[5,10,11]



The keto-aldehyde **2** (12-deacetyl-23-acetoxy-20-methyl-12-*epi*-scalaradial) had the molecular formula $C_{28}H_{42}O_5$. Its ^{1}H NMR spectrum showed signals for one aldehyde (H-19, δ 9.67), one acetoxy methylene system (H_2-23, δ 4.58 and 4.13), one olefin proton (H-16, δ 7.12), one methyl ketone (CH_3-26, δ 2.31), one acetyl group (δ 2.06), and four additional methyl singlets at δ 0.83 (CH_3-21), 0.88 (CH_3-22), 1.00 (CH_3-24), and 0.87 (CH_3-25). The scalarane skeleton was totally determined by 1D and 2D NMR experiments that allowed all the resonances to be assigned (see Table 1). The ^{13}C NMR data of **2** strongly supported the presence of the acetoxymethylene group at C-23, as did

* To whom correspondence should be addressed. Tel.: +39 081 8534.156. Fax: +39 081 8041 770. E-mail: afontana@icmib.na.cnr.it.
† ICMIB−CNR.
‡ Universidad de Oviedo.

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Table 1. NMR Data ($CDCl_3$, 500 MHz) of Compounds 2−4[a]

	2	**3**	**4**
no.	δ, *m* (Hz)	δ, *m* (Hz)	δ, *m* (Hz)
1	2.09, *m*	2.12, *m*	1.72, *m*
	1.80, *m*	1.76, *m*	0.84, *m*
2	1.45, *m*	1.56, *m*	1.61, *dt* (14.0, 3.3, and 3.3)
	1.56, *m*	1.46, *m*	1.42, *m*
3	1.47, *m*	1.12, *ddd* (12.5, 5.6, and 0.5.6)	1.42, *m*
	1.20, *m*	1.44, *m*	1.14, *m*
4			
5	0.90, *m*	0.98, *m*	0.81, *m*
6	1.56, *m*	1.56, *m*	1.52, *m*
	1.41, *m*	1.44, *m*	1.46, *m*
7	1.83, *dt* (12.8, 3.0 and 3.0)	1.80, *dt* (12.9, 3.6, and 3.6)	1.72, *m*
	0.97, *m*	0.86, *m*	0.92, *m*
8			
9	1.10, *m*	0.98, *m*	0.92, *m*
10			
11	1.93, *br dd* (12.8 and 3.2)	1.91, *br dd* (13.2 and 4.2)	1.72, *m*
	1.69, *br dd* (13.4 and 3.0)	1.72, *m*	1.45, *m*
12	3.56, *br d* (11.1)	3.36, *br d* (12.1)	3.52, *br d* (11.4)
13			
14	1.15, *m*	1.06, *br d*, (12.6)	1.12, *dd* (12.1 and 5.1)
15	2.35, *m*	2.12, *br d* (15.6)	2.09, *br d* (17.6)
		2.02, *m*	1.99, *m*
16	7.12, *br s*	5.45, *m*	5.43, *m*
17			
18	3.19, *br s*	2.31, *br s*	2.32, *br s*
19	9.67, *d* (3.4)	5.37, *d* (6.3)	5.39, *br d* (6.0)
20		4.67, *m*	4.76, *m*
21	0.83, *s*	0.83, *s*	0.81, *s*
22	0.88, *s*	0.87, *s*	0.84, *s*
23	4.58, *d* (12.0)	4.58, *d* (12.2)	0.86, *s*
	4.13, *d* (12.0)	4.16, *d* (12.2)	
24	1.00, *s*	0.99, *s*	0.93, *s*
25	0.87, *s*	0.82, *s*	0.82, *s*
26	2.31, *s*	1.25, *d* (6.1)	1.24, *d* (6.3)
Ac	2.06, *s*	2.08, *s*	

[a] Numbering is in agreement with Hochlowski et al.[5]

the NOESY correlations between H-23a (δ 4.58) and CH_3-21, as well as between H-23b and CH_3-24. The relative stereochemistry of **2** was further supported by NOESY correlations, which demonstrated the equatorial orientation of the substituents at C-12 and C-18 (intense NOEs from H-12 to H-18, H-9, and H-14). It is noteworthy that, although **2** was present in the extract of both inner organs and mantle, it was rather more abundant in the latter (3.0 mg in the mantle; 0.5 mg in the inner organs). On the other hand, the structure of **2** is closely related to that of the homosesterterpenes isolated by Bowden and co-workers from a sponge from the Great Barrier Reef.[12]

Compound **3** (12-deacetyl-23-acetoxy-20-methyl-12-*epi*-deoxoscalarin) is formally the cyclic product of the dihydroderivative of **2**. Accordingly, HREIMS (*m*/*z* 442.3081) indicated the molecular formula $C_{28}H_{42}O_4$ [M − 18], and the ^{13}C NMR spectrum was featured by the hemiacetal signal at δ 98.0 (C-19). The ^1H NMR spectrum of **3** was very similar with that of **2**, except for the presence of the hemiacetal proton at δ 5.37 (H-19) and of a further methine signal at δ 4.67 (H-20) coupled to the downshifted methyl doublet at δ 1.25 (CH_3-26). All remaining resonances were fully attributed by 2D NMR experiments (Table 1). The substitution pattern and the stereochemistry of **3** were elucidated by NOESY and comparison of the spectral data with those reported in the literature for similar compounds.[5,13]

Compound **4** (12-deacetyl-20-methyl-12-*epi*-deoxoscalarin) had the same structure of the main metabolite reported from the Pacific dorid *Chromodoris sedna*[5] (= *Glossodoris*). As the ^1H NMR data of this compound have been described in CCl_4,[5] a complete assignment of **4** in $CDCl_3$ is reported in Table 1. Although **4** had a structure similar to that of **3**, the chemical shift of H-12 appeared rather different in the two compounds (δ 3.36 and 3.52 for **3** and **4**, respectively), thus suggesting some differences in their stereochemistry. However, NOEs between H-18 (δ 2.32) and both H-12 (δ 3.52) and H-20 (δ 4.76), as well as between H-19 (δ 5.39) and CH_3-25 (δ 0.82), confirmed unambiguously the structure previously proposed.[5]

In contrast to *G. sedna*, the nudibranch *G. dalli* contained exclusively scalarane products. Besides the known metabolites **5**[11] and **6**,[10] the mollusk extracts contained a mixture of more polar compounds that, after acetylation, was fractionated by HPLC to give 0.3 mg of **8**. Although scarce, the structure of the product was inferred by taking advantage of the concomitant finding of the other sesterterpenoids described above. In the ^1H NMR spectrum of **8**, the presence of the γ-lactone ring was supported by the presence of the AB system at δ 4.23 and 4.11 (H_2-19), coupled with the broad singlet at δ 2.86 (H-18). This last signal was weakly correlated to the olefin proton at δ 6.87 (H-16), thus supporting the presence of a conjugated α,β-unsaturated ketone. Finally, the ^1H NMR spectrum also showed a downshifted double doublet (H-12) at δ 4.67, an acetyl group at δ 2.05, and five methyl singlets at δ 0.96, 0.86, 0.85 (6H), and 0.81. These data were in good agreement with those reported for a semisynthetic compound obtained from 12-*epi*-scalarin (**9**) by treatment with $NaBH_4$.[10] Comparison of the ^1H NMR data of **8** with those reported in the literature[10] supported this hypothesis. Degradation of the product prevented any further analysis. However, because no acetyl signals were present in the ^1H NMR spectrum of the natural mixture before purification, it is

conceivable to conclude that the natural compound occurring in the living mollusk has the structure **7**. It is noteworthy that **7** has been isolated from the sponge *Hyrtios erecta* by bioassay-guided separation during the search for new antineoplastic agents.[14]

Compound **2** was as strong as scalaradial (**1**) in the ichthyotoxicity assay against *Gambusia affinis* (toxic at 0.1 ppm).[15] Furthermore, **2** inhibited mammalian cytosolic PLA_2 (IC_{50} 18.0 μM),[16] although it occurred at a higher concentration than scalaradial (IC_{50} 0.6 μM). The analogies in the biological properties of **1** and **2** may be due to a similar chemical reactivity toward nucleophiles. In fact, compound **2** reacted with ethylamine under biomimetic conditions (pH = 7.4)[8,9] to give a mixture of condensation products, the study of which is still in progress. Nevertheless, the NMR data strongly suggested that the reaction proceeded, in more than one aspect, in a different manner from that reported for 1,4-dialdehyde compounds. In fact, five minutes after the addition of ethylamine, the ^1H NMR (CD_3CN) showed the disappeareance of the signals due to H-19 (δ 9.82), H-16 (δ 7.19), and CH_3-26 (δ 2.25). At this time, the NMR revealed the presence of a transient intermediate that was featured by two downshifted signals at δ 6.88 and 5.69.[9] After 15 minutes, the reaction reached equilibrium. A sharp signal at δ 6.58 led us to suggest the formation of a pyrrole-like structure,[9] but the absence of other downshifted signals ruled out any further analogies with the chemical behavior of dialdehyde compounds (e.g., scalaradial, **1**).[9] This may also explain the differences shown by **1** and **2** in the potency to inhibit PLA_2. Relating to the biological properties of scalarane products, it is noteworthy that the other products **3**−**6** were totally inactive in both assays.

In conclusion, this paper reports the isolation of scalarane and homoscalarane compounds from *Glossodoris* nudibranchs. The presence of the same metabolites in the mantle and in the gland of each animal supported the dietary origin of the defensive allomones. As **2**−**7** are typical sponge metabolites, the behavior of *G. sedna* and *G. dalli* is in agreement with previous studies of *Glossodoris* mollusks from different geographical sites.[17] In fact, most of the chemical investigations about this genus of nudibranchs (*G. cincta*, *G. pallida*, *G. hikeurensis*, *G. atromarginata*)[17,18] support the idea of a dietary relationship with scalarane-containing sponges. From an ecological point of view, the chemical patterns of *G. dalli* and *G. sedna*, moreover, reinforce the conjecture of a high feeding specialization among the three main genera (*Hypselodoris*, *Chromodoris* and *Glossodoris*) of the family Chromodoridae. In fact, each group of animal is able to eat sponges displaying specific secondary metabolites: furanosesquiterpenes for *Hypselodoris*, diterpenes for *Chromodoris*, and sesterterpenes for *Glossodoris*.[17] The analogies of the chemical patterns of *G. sedna* and *G. dalli* with those of other *Glossodoris* species raise a question about the taxonomic classification of nudibranchs assigned to other genera (*Hypselodoris orsini*, *Hypselodoris capensis*, *Chromodoris funerea*, *Chromodoris inornata*) but containing scalarane products.[17] The fact that two species of *Glossodoris* from the same marine habitat contained chemically different compounds, scalarane and homoscalarane, may have some taxonomic significance within this group of mollusks.

Finally, although we could not reach any conclusion on the mechanism of the reaction of **2** with nucleophiles, our experiments suggested some parallels between the chemical reactivity of dialdehyde and the keto-aldehyde functions of scalaranes. This seems to be further supported by the similar behavior of **1** and **2** in the biological assays described above.

Experimental Section

General Methods. 1D and 2D NMR spectra were recorded on Bruker AMX-500. The $CHCl_3$ resonances at δ 7.26 and 77.0 were used as internal references. MS spectra were obtained on a Kratos MS 50 spectrometer operating at 70 eV. IR data were recorded by BIO-RAD FTS-7 FT/IR spectrophotometer. Optical rotations were determined by JASCO DIP-370 polarimeter. HPLC was performed by Waters Liquid Chromatography apparatus equipped with two 510 pump units and a JASCO Uvidec 100 III spectrophotometer.

Animal Material. The nudibranchs (42 specimens of *G. sedna* and 18 specimens of *G. dalli*) were collected off the Natural Park of Osa Ballena (Costa Rica) during the summer of 1996. Voucher specimens are kept at University of Oviedo (Spain). The animals were immediately frozen and transferred to Italy.

Extraction. The nudibranchs were carefully thawed, immersed in acetone, and sonicated for 2 min. The acetone (mantle extract) was removed, and the animals were exhaustively extracted with acetone again (viscera extract). After removing the volatile solvent, the residues were diluted with fresh water and separately partitioned with Et_2O. The organic layers were dried on Na_2SO_4, filtered on paper, and evaporated to small volume. The ether-soluble material was analyzed by TLC in different eluent systems [light ether/Et_2O (1:1), light ether/Et_2O (2:8); $CHCl_3$/MeOH (95:5)].

Biological Assays. Ichthyotoxicity was tested by the method of Gunthorpe and Cameron.[15] Inhibition of PLA_2 was tested on RBL-2H3 cells stimulated with ionomycin in agreement with Bisogno et al.[16]

Fractionation of *G. sedna* Extracts. As the metabolite pattern of mantle and viscera were identical, the Et_2O extract of *G. sedna* viscera (32.2 mg) was fractionated by Si gel column. The Ehrlich-positive fractions of this column were further purified by reversed-phase HPLC (Spherisorb ODS-2 column, 10 × 250 mm; 1 mL/min; detector UV 208 nm) to give **2** [0.5 mg, eluted with MeOH/H_2O (85:15)], **3** [2.2 mg, eluted with MeOH/H_2O (95:5)], and **4** [0.5 mg, eluted with MeOH/H_2O (85:15)]. Fractionation of the mantle extract (11.1 mg) by the above-described procedure led to **2** (3 mg), **3** (1.6 mg), and **4** (0.6 mg).

Fractionation of *G. dalli* Extracts. After the TLC analysis, the mantle and the viscera extract of *G dalli* were combined and fractionated by Si gel column to give **5** (2.1 mg), **6** (1.9 mg), and a mixture of Ehrlich-positive compounds. This last fraction (2.6 mg) was acetylated in dry pyridine (room temperature, overnight) and further purified by reversed-phase HPLC (Spherisorb ODS-2 column, 10 × 250 mm; 1 mL/min; detector UV 208 nm) to yield **8** [0.3 mg, MeOH/H_2O (85:15)], together with other uncharacterized compounds.

Compound 2: 12-deacetyl-23-acetoxy-20-methyl-12-*epi*-scalaradial, $C_{28}H_{42}O_5$, colorless oil (3.5 mg), $[\alpha]_D$ −12.2° (*c* 0.1, $CHCl_3$); IR (film) ν_{max} 3468, 2963, 2865, 1734, 1721, 1668, 1360, 1243, 1034 cm^{-1}; UV (EtOH) 273, 229, 204 nm; ^1H NMR data, see Table 1; ^{13}C NMR data, 204.8 (d, C-19), 198.2 (s, C-20), 170.2 (s, Ac), 143.4 (d, C-16), 138.2 (s, C-17), 82.0 (d, C-12), 64.6 (t, C-23), 61.2 (d, C-18), 58.2 (d, C-9), 56.8 (d, C-5), 53.0 (d, C-14), 43.6 (s, C-13), 42.0 (t, C-7), 41.5 (t, C-3), 40.4 (s, C-10), 37.6 (s, C-8), 34.8 (t, C-1), 33.7 (q, C-22), 32.9 (s, C-4), 30.3 (d, C-11), 25.1 (q, C-26), 23.3 (t, C-15), 21.8 (q, C-21), 21.3 (q, Ac), 18.3 (t, C-6 or C-2), 17.9 (t, C-2 or C-6), 16.6 (q, C-24), 9.6 (q, C-25); EIMS (*m/z*) 440 (100, M−H_2O), 428 (25, M−CH_2O), 381 (25, M−H_2O−59), 191 (20), 177 (20), 147 (20); HREIMS *m/z* 440.2931 [C_{28} H_{40} O_4, $(M-18)^+$ Δ + 0.5 mmu].

Compound 3: 12-deacetyl-23-acetoxy-20-methyl-12-*epi*-deoxoscalarin, $C_{28}H_{44}O_5$, colorless oil (3.8 mg), $[\alpha]_D$ −35.5° (*c* 0.1, $CHCl_3$); IR (film) ν_{max} 3405, 2926, 2857, 1738, 1243 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, 170.3 (s, Ac), 140.9 (s, C-17), 116.1 (d, C-16), 98.0 (d, C-19), 81.8 (d, C-12), 74.5

(d, C-20), 64.8 (t, C-23), 61.7 (d, C-18), 59.0 (d, C-9), 56.9 (d, C-5), 53.4 (d, C-14), 42.0 (t, C-7), 41.6 (t, C-3), 40.6 (s, C-10), 37.5 (s, C-8 and C-13), 34.8 (t, C-1), 33.7 (q, C-22), 31.2 (s, C-4), 28.5 (d, C-11), 22.1 (q, C-21), 21.9 (t, C-15), 21.3 (q, Ac), 19.1 (t, C-26), 18.3 (t, C-6 or C-2), 18.0 (t, C-2 or C-6), 16.4 (q, C-24), 8.5 (q, C-25); EIMS (*m*/*z*) 442 (100, M−H_2O), 367 (10), 267 (10); HREIMS *m*/*z* 442.3081 [C_{28} H_{42} O_4, $(M-18)^+$ Δ − 0.2 mmu].

Compound 4: 12-deacetyl-20-methyl-12-*epi*-deoxoscalarin, the spectroscopic data for **4** were identical with those reported in the literature;[5] ^{1}H NMR data, see Table 1; ^{13}C NMR data, 140.4 (s, C-17), 116.3 (d, C-16), 98.0 (d, C-19), 81.2 (d, C-12), 74.5 (d, C-20), 62.0 (d, C-18), 58.9 (d, C-9), 56.5 (d, C-5), 53.3 (d, C-14), 42.1 (t, C-7), 41.6 (t, C-3), 39.9 (t, C-1), 39.7 (s, C-10), 37.4 (s, C8 and C13), 33.3 (s, C-4), 33.3 (q, C-22), 26.1 (t, C-11), 22.0 (t, C-15), 21.3 (q, C-21), 19.1 (t, C-26), 18.6 (t, C-2 or C-6), 18.1 (t, C-6 or C-2), 17.0 (q, C-24), 16.6 (q, C-23), 8.7 (q, C-25).

Compounds 5, 6, and 8: spectroscopic data for **5**, **6**, and **8** were identical to those reported in the literature.[5,10,11]

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