of cyanogenic glycosides, and work in our laboratory is exploring its feasibility.

Dithyreanitrile is one of the simplest compounds with insect antifeedant properties to have been reported. Possibly this simplicity will allow it or an analog to be exploited on a broad scale.

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Drimane sesquiterpenoids in Mediterranean *Dendrodoris* nudibranchs: Anatomical distribution and biological role

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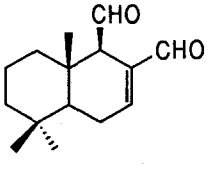
Summary. Two Mediterranean species of *Dendrodoris* nudibranchs have elaborated a very sophisticated defensive strategy against predators, involving the denovo biosynthesis of a series of drimane sesquiterpenoids, some of which are strongly ichthyodeterrent. Anatomical distribution of the drimane terpenoids in different sections and egg masses of the mollusc is reported, together with further studies aimed at finding out how the animals are protected against the noxious effects of their own allomones.
Key words. Chemical defense; *Dendrodoris* species; polygodial; drimane sesquiterpenoids; nudibranch.

Naked nudibranch molluscs exhibit a series of defensive strategies [2, 3] against potential predators, which include the use of chemicals obtained either from the diet or by de novo biosynthesis. Polygodial (**1**), already known as plant metabolite [4] strongly anorectic for insects [5], is the defensive allomone isolated from two Mediterranean *Dendrodoris* species [6, 7], *D. limbata* and *D. grandiflora*, and from some other Pacific porostome nudibranchs [8]. *Dendrodoris* molluscs are able to biosynthesize de novo [7,9,10] both the active polygodial, which can be isolated by chromatographic procedures from their mantles, and some inactive structurally related drimane esters (**2**), which are stored in the viscera.

The biological properties of **1** (antifeedant to fish, anorectic to insects, hot-tasting to humans) are most probably due to the simultaneous interaction of both the aldehydic groups with primary amine moieties [11]. Analogously, other terpenoidic dialdehydes display the same activities when particular structure-activity parameters, such as the distance between the two aldehydic groups and the molecular bulkiness, are taken into consideration [12]. Owing to the toxicity of **1** to *D. limbata* [10], the related sesquiterpenoid olepupuane (**3**) was strongly suspected [13] to be the masked form of the allomone present in the animal.



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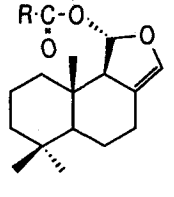

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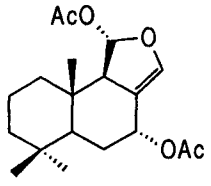
We now report the results we obtained when studying two questions which remained to be clarified:

1) Is polygodial completely absent in *Dendrodoris* species?
2) Where are the drimane sesquiterpenoids localized in *Dendrodoris* nudibranchs?



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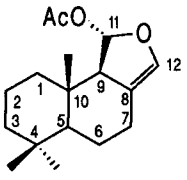

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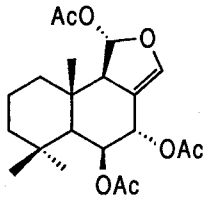

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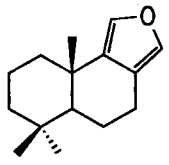

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In addition, the new drimane sesquiterpenoid, 7-deacetoxy-olepupuane (**4**), was isolated from the gills of both *D. limbata* and *D. grandiflora*.

Material and methods

General experimental procedures. ^{1}H-NMR spectra were recorded on a Bruker WM-500 spectrometer coupled to an Aspect 2000 computer system. Chemical shifts expressed in δ units are reported in ppm downfield from the internal standard TMS ($\delta = 0$) EIMS spectra were recorded on an AEI MS-30 mass spectrometer. Column chromatography was carried out using Merck Kieselgel 60 powder (70-230 mesh ASTM). Analytical TLC was carried out using precoated Merck F254 plates.

The anatomical dissections were made observing the animal through a Swift Microscope (20-40 ×).

Biological material. 24 specimens of *D. limbata* were studied (2 from Cubelles, NE Spain, and 22 from Pozzuoli, SW Italy), as well as 20 specimens of *D. grandiflora* (from Pozzuoli, SW Italy), 7 spawn masses of *D. limbata* and 3 of *D. grandiflora* (all these from Pozzuoli, SW Italy). All the samples were collected between January and May 1989, at about 0.2–5 m depth, in the places mentioned above.

Direct ^{1}H-NMR analsis of the mantle metabolites from D. limbata. A specimen of *D. limbata* was treated with C_6D_6 (5 ml) in a separatory funnel. The C_6D_6 extract was dried on Na_2SO_4 and then submitted to NMR analysis. The ^{1}H-NMR spectrum (fig. 1 b) was compared with the proton NMR spectra of authentic samples of (**3**) (fig. 1 a), and 6-β-acetoxy-olepupuane[7] (**5**) (fig. 1 c).

Anatomical dissection and extraction of the organic metabolites. 13 specimens of *D. limbata* and 14 of *D. grandiflora* were carefully dissected into the sections listed in table 1. An anatomical view of *D. limbata* is shown in figure 2. Subsequently, the different parts along with the egg ribbons, were separately extracted with acetone. The yields are reported in table 1 along with the amounts of the diethyl ether soluble fractions from the acetone extracts.

Chemical analysis. The substances soluble in diethyl ether were analyzed by silica gel TLC (benzene: diethyl ether 9:1). Drimane sesquiterpenoids were detected only in some extracts (table 2) of both *D. limbata* and *D. grandiflora*.

The diethyl ether extracts from gills, hermaphrodite glands and egg ribbons were purified on very short silica gel columns (Pasteur pipette, gradient steps light petroleum: diethyl ether from 98:2 to 50:50). The ^{1}H-NMR spectra of the main fractions from hermaphrodite glands and egg masses were identical to those already described for the esters (**2**)[14]. The ^{1}H-NMR spectrum ($CDCl_3$) of the product isolated from the gills (**4**) was almost identical to those above, differing only in the absence of the intense signal at δ 1.26 (replaced by a singlet at δ 2.08).

7-deacetoxy-olepupuane (**4**):δ^1H-NMR ($CDCl_3$), 6.31 (1H, d, J = 2 Hz, H-11); 6.05 (1H, bt, J = 2 Hz, H-12); 2.47 (1H, ddd, J = 13.8, 5.2, 1.7, H-7eq.); 2.28 (1H, bs, H-9); 2.08 (3H, s, CH_3CO); 2.00 (1H, ddddd, J = 13.8, 13.8, 5.7, 1.8, 2 Hz, H-7ax.); 1.71 (1-H, bdd, J = 13.1, 5.7 Hz, H-6eq.); 1.26 (1H, m, H-6ax.); 1.00 (1H, dd, J = 12.5, 2.5, H-5); 0.89 (3H, s); 0.83 (3H, s); 0.81 (3H, s), other resonances between δ 1.70 and δ 1.00; δ^1H-NMR (C_6D_6): 6.70 (1H, d, J = 2 Hz); 5.91 (1H, bt, J = 2 HZ); 2.24 (1H, bs); 2.17 (1H, bdd, J = 4, 13.5 Hz);

Table 1. Organic fractions recovered from the dissected sections and egg ribbons of *D. limbata* and *D. grandiflora*. (All the data are mean values given for animal or spawn mass).

Dissected section	*Dendrodoris limbata*			*Dendrodoris grandiflora*		
	Dry weight[a] (mg)	Acetone extract (mg)	Diethyl ether extract (mg)	Dry weight[a] (mg)	Acetone extract (mg)	Diethyl ether extract (mg)
Gills	10.2	4.6	2.3	23.0	5.4	1.7
Border of the mantle	32.2	4.0	3.3	141.0	48.9	2.3
Rest of the mantle	390.8	107.5	1.0	781.1	152.6	2.6
Ptyaline gland	5.9	1.7	-	12.5	3.3	-
Hermaphrodite gland	31.7	10.2	6.0	54.3	14.3	6.0
Digestive gland	62.1	18.7	4.0	293.1	80.0	58.9
Reproductive organs and rest of viscera	82.1	32.7	14.0	224.7	95.0	27.7
Egg ribbons	126.7	13.5	1.7	220.9	41.0	5.0

[a] After extraction with acetone.

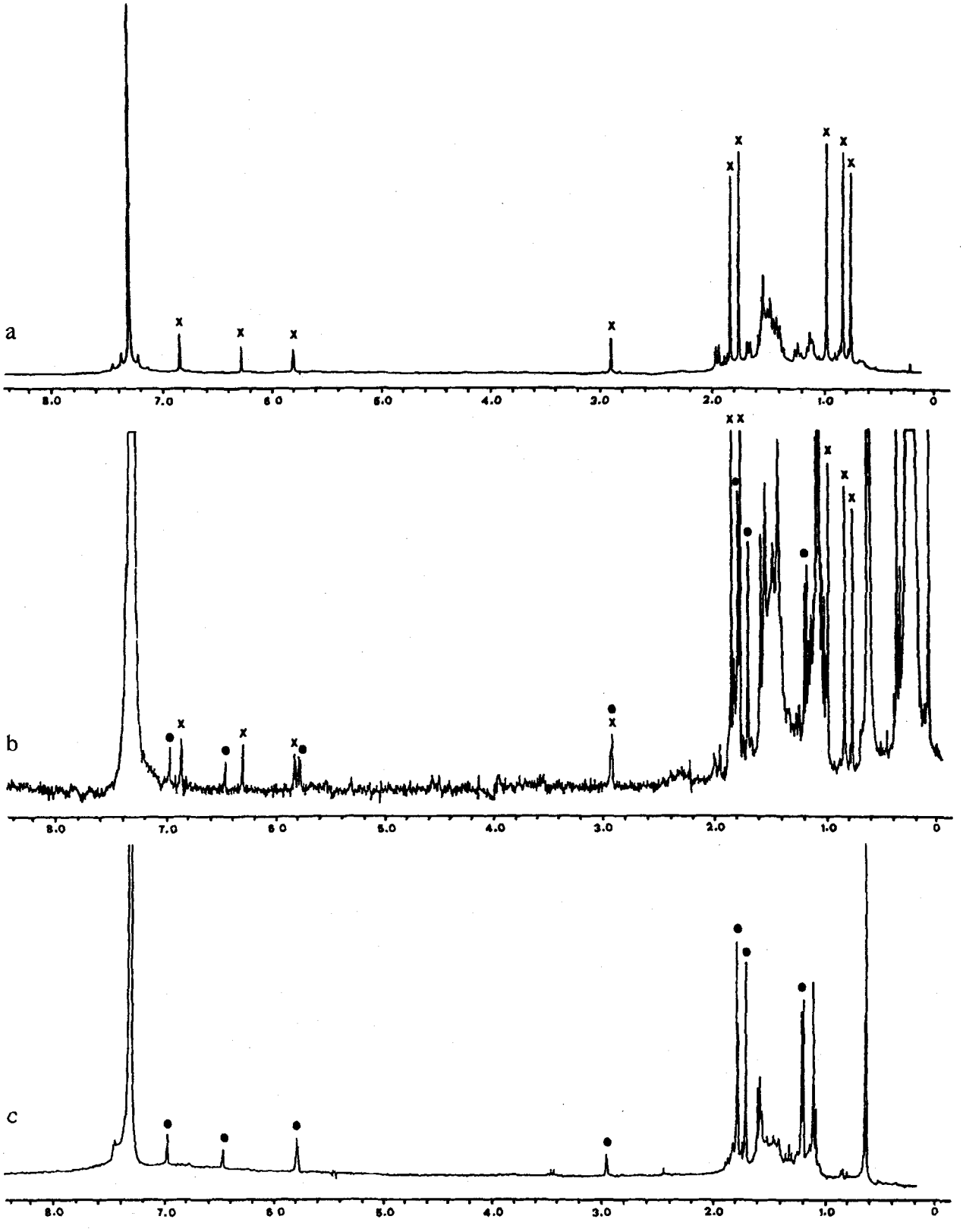

Figure 1. ^{1}H-NMR spectra of a) olepupuane (**3**); b) the C_6D_6 extract of *D. limbata*, c) 6-β-acetoxy-olepupuane (**5**).

Table 2. Localization of drimane sesquiterpenoids[a] in anatomical sections of *D. limbata* and *D. grandiflora*.

	Polygodial[b] (1)	Olepupuane (3)	7-deacetoxy-olepupuane (4)	Drimane esters (2)	Euryfuran (6)
Border of mantle	++	+			
Gills			+		
Hermaphrodite gland				+++	+
Egg masses				+++	+

[a] Detected by TLC analysis and identified by NMR analysis. [b] Deriving from olepupuane during chromatographic treatment.

1.67 (3H, s, CH_3CO); 0.76 (3H, s); 0.69 (3H, s); 0.68 (3H, s), other resonances between δ 1.80 and δ 0.80; EIMS, m/z (%): 278 (M^+, 5); 218 (30); 203 (50); 59 (100). Thermolysis (on the TLC plate) easily transformed (**4**) into euryfuran (**6**).

Experiments on D. grandiflora. The chemical analysis yielded data substantially identical to those obtained studying *D. limbata*. A second unidentified terpenoid was observed in the extract of the gills (R_f 0.4 in light petroleum: diethyl ether 95:5), while many terpenoids of dietary origin[7] were found in the digestive gland.

Results and discussion

The direct ^{1}H-NMR analysis of the mantle metabolites of *D. limbata* (fig. 1 b) revealed the absence of downshifted signals attributable to the aldehydic protons of polygodial (**1**).

However, the spectrum displayed two series of signals easily assignable, by comparison with the ^{1}H-NMR spectra (fig. 1 a, 1 c) of authentic samples, to olepupuane (**3**) and to 6-β-acetoxy-olepupuane (**5**). The results of this experiment ruled out the presence of polygodial (**1**) in living *D. limbata*, whereas they confirmed that olepupuane (**3**) is its masked form. Most probably the allomone is delivered only when there is the contact with predators, inducing repulsive reactions.

To investigate further the biological role of drimane sesquiterpenoids in the genus *Dendrodoris*, we have tried to detect in the molluscs the specific sites where the terpenoids are localized. After anatomical dissection, the organic material from the single sections, and also from mucus and egg masses of *D. limbata*, was subjected to chemical analysis (table 1). Drimane sesquiterpenoids were found only in the yellow mantle border, in the gills, in the hermaphrodite glands and in the egg ribbons (fig. 2).

TLC analysis of the mantle border revealed the presence of polygodial (**1**), clearly deriving from **3**, as the main metabolite. **1** and **3** were completely absent in other organs. The drimane esters (**2**) were found only in the hermaphrodite glands and in the egg ribbons and do not exhibit ichthyodeterrent properties. This localization of the esters (**2**), previously[10] suspected to be either precursors of **1** or its detoxification products, seems to suggest a possible biological role in the reproductive and developmental cycle of the animal. Finally, the gills possess a drimane sesquiterpenoid (**4**) closely related to the esters (**2**). In fact, the ^{1}H-NMR spectrum, compared with that of **2**, differed only in the absence of the signals due to the acyl residues and in the presence of a singlet (δ 1.67 in C_6D_6 and δ 2.08 in $CDCl_3$) assigned to an acetyl residue. The proposed structure was definitively ascertained by thermolysis of **4**, which yielded euryfuran (**6**). This latter sesquiterpenoid is always present in the extracts from both the hermaphrodite glands and the egg ribbons, but its authenticity as a metabolite of *D. limbata* is questionable, as the esters are very easily transformed into **6** during the usual work-up. No drimane sesquiterpenoids were detected in the mucous secretion of *D. limbata*. Probably this secretion has a mechanical function and, furthermore, it could favour the distribution of polygodial (**1**) over the mantle when the animal is molested.

Analogous studies on *D. grandiflora* led to substantially identical results. The differences were due to the presence of a second unidentified terpenoid in the gills and, as already reported[7], of a series of terpenoids of dietary origin in the digestive gland.

In conclusion, it seems that *Dendrodoris* molluscs have elaborated a very effective strategy to secure their own survival. A dominant biogenetic pathway leads to related compounds which are localized in different organs of the mollusc, and perform different biological roles. In particular, Mediterranean *Dendrodoris* species are able to synthesize closely related drimane esters (**2** and **4**) de novo through the mevalonic pathway. 7-Deacetoxy-olepupuane (**4**) could be the precursor of the defensive allomones olepupuane (**3**) and polygodial (**1**), whereas the esters (**2**) might play a role during the reproductive cycle.

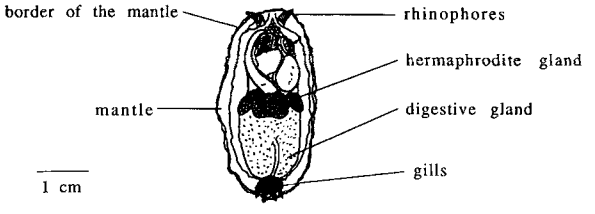

Figure 2. Anatomical view of *Dendrodoris limbata*.

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Announcements

IVth International Symposium on Quantitative Luminescence Spectrometry in Biomedical Sciences

State University of Ghent, Belgium, May 27–31, 1991

Organizing Committee

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Laboratory of Pharmaceutical Chemistry and Drug Quality Control, Pharmaceutical Institute, Harelbekestraat 72, B-9000 Ghent

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Scientific Program

– London Diagnostics Chair on 'Education in Luminescence'
May 27, 1991

The purpose of the Chair is to emphasize a particular aspect of luminescence by means of a one-day top level course, to be presented by an internationally recognized expert in the field; this year the course will be held by Prof. Dr. Frank McCapra, University of Sussex. The theme for this year will be "The Application of Chemiluminescence to Diagnostics" and should be particularly promising for immediate application in biomedical and clinical practice.

– Symposium on 'Quantitative Luminescence Spectrometry in Biomedical Sciences'
May 28–31, 1991

Invited plenary and keynote lectures, oral contributions and posters will cover the most important fields of fundamental and applied luminescence spectrometry. The Conference envisages a report on the current status and future developments in the field of quantitative luminescence techniques applied in drug quality control, clinical analysis, biochemical, toxicological and environmental analysis, and in related areas.