Constituents of the digestive gland of molluscs of the genus *Aplysia*. II. Halogenated monoterpenes from *Aplysia limacina*

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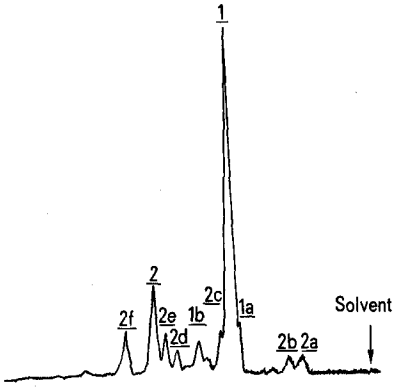
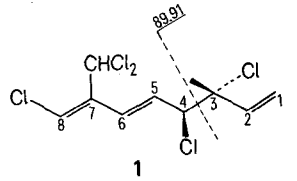
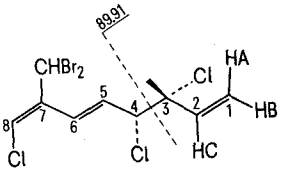
Summary. The digestive gland of the sea hare Aplysia limacina, collected in the bay of Naples, contains an array of polyhalogenated monoterpenes. The 2 major components are 3,4-erythro-7-dichloromethyl-3-methyl-3,4,8-trichloro-1,5 (E), 7 (Z)-octatriene (**1**), previously isolated from pacific red alga Plocamium cartilagineum, and the new 3,4-threo-7-dibromomethyl-3-methyl-3,4,8-trichloro-1,5 (E), 7 (E)-octatriene (**2**), whose structure has been assigned on the basis of analogy and of spectroscopic data. Examination of extracts of local red algae revealed that **1** was present in Plocamium coccineum.

In recent years the chemical constituents of the digestive gland of a number of opisthobranch molluscs, especially belonging to the genus Aplysia, have been intensively investigated, leading to the discovery of a variety of novel terpenoidic molecules, most of them containing bromine and chlorine[2–4], which are generally algal metabolites. Circumstantial evidence pointing to the existence and possible storage of algal metabolites in the digestive gland, which could, in turn, provide the components of the defensive secretion, have been reported by Faulkner and his associates[5].

We also became interested in Aplysia and have examined the more common mediterranean species. Recently we have described a series of a closely related diterpenes of a novel perhydroazulene class[6,7] from A. depilans, also found in the brown alga Dictyota dichotoma[7,8].







Gas chromatographic trace of the polyhalogenated monoterpenes mixture of Aplysia limacina (column of 1.5 m of 3% SE-30 on 100–200 mesh Chromosorb W, temp. 130–270 °C/min). Molecular formulas estimated from GC-MS[13]: **1a**, $C_{10}H_{11}Cl_5$; **1b**, $C_{10}H_{11}Cl_4Br$; **2a**, $C_{10}H_{12}Cl_4$; **2b**, $C_{10}H_{14}Cl_3Br$; **2c**, $C_{10}H_{11}Cl_5$; **2d**, $C_{10}H_{13}Cl_3Br_2$; **2e**, $C_{10}H_{11}Cl_3Br_2$; **2f**, $C_{10}H_{13}Cl_3Br_2$.

Examination of the extracts from the digestive gland of A. limacina has now led to a complex mixture of polyhalogenated monoterpenes; and in this paper we wish to describe the 2 major components, having a 3,7-dimethyl-1,5,7-octatriene skeleton. Polyhalogenated monoterpenes were first isolated by Faulkner et al. from the digestive gland of Aplysia californica[3] and also from red algae Plocamium sp.[9–11] and Chondrococcus hornemanni[12].

Materials and methods. The digestive gland from 5 adult animals collected in the bay of Naples were excised and extracted with acetone. Ether-water partition of the residue and chromatography on silica gel in chloroform of the ether extract (5.2 g) yielded in the first fractions a complex mixture of halogenated monoterpenes (1.6 g) as shown in the gas chromatographic trace (figure). Chromatography on preparative SiO_2 TLC in n-hexane at 5 °C (3 stages) gave 2 principal fractions (visible under UV-light). Rechromatography of the major less polar fraction (containing **1**–**1b**) on preparative alumina TLC in n-

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13 All peaks, except **2f** (M^+/e 396, 398, 400, 402, 404), did not show molecular ions, but they exhibited base peak at m/e *89*, *91* (3:1, $C_4H_6Cl^+$) and cluster of ions corresponding to M^+-C_4H_6Cl. On this basis their molecular formulas were estimated.

hexane gave pure **1** while the more polar fraction (containing **2**–**2f**) was subjected to preparative gas-liquid chromatography[14] to give pure sample of the trichlorodibromomonoterpene **2**.

Results and discussion. Compound **1**, which accounts for ca. 80% of the total halogenated monoterpenes in A. limacina $[\alpha]_D$ + 4.8 °C (Lit + 5.1 °C)[11], λ_{max} 242 nm (ε, 14, 550 in cyclohexane), (*Lit.* 243 nm)[11], had a MS with M^+ 306, 308, 310, 312 and 314 corresponding to $C_{10}H_{11}Cl_5$ and major peaks at m/e 217, 219, 221, 223 ($C_6H_5Cl_4^+$) and 89.91 (base peak, $C_4H_6Cl^+$). The ^{1}H-NMR of this material also fully conforms with that of 3,4-erythro-7-dichloromethyl-3-methyl-3,4,8-trichloro-1,5(E),7-octatriene (**1**) previously isolated from the pacific red alga Plocamium cartilagineum[11].

Table 1. ^{13}C-NMR chemical shifts for **1** and **2**[a]

	1	**2**
C-1	116.4	116.5
C-2[b]	139.3	139.5
C-3	71.7	71.7
C-4[c]	68.7	69.4
C-5[d]	119.0	123.2
C-6[d]	126.6	124.1
C-7	137.6	137.0
C-8[d]	130.3	132.3
CHX_2[c]	65.5	36.8
CH_3	25.1	25.1

[a] Spectra were determined in [^{2}H] chloroform at 25.20 MHz with a Varian XL-100 Fourier Transform spectrometer operating at both proton-noise decoupling and off-resonance modes; chemical shifts are given in ppm with respect to internal Me_4Si. [b] Assignment based on selective decoupling. [c] The 2 carbons were differentiated by selective decoupling. [d] Assignments may be reversed.

Table 2. 100 MHz ^{1}H-NMR (CCl_4) data for **2**

δ (ppm)/J (Hz)								
CH_3	HA	HB	HC	H-4	H-5	H-6	CHX_2	H-8
1.78 (s)	5.37	5.24	6.02	4.48	6.54	6.58	6.76 (s)	6.30 (s)
	JAC = 16.5			J4,5 ≈ 8[a]				
	JBC = 10.5			J5,6 = 16[a]				

[a] Coupling constants as measured from the spectrum run in C_6D_6, in which the AMX system formed by H-4, H-5 and H-6 was susceptible to first order analysis with signals at 4.14 (H-4), 6.02 (H-6) and 6.16 (H-5) ppm.

The ^{13}C-NMR-data of **1**, collected in table 1, well support this assignment. The second major halogenated monoterpene, **2**, $[\alpha]_D$ −9.7 °C (c, 0.4 in $CHCl_3$), λ_{max} 252 (ε, 0.040 in cyclohexane) did not show a molecular ion, but the presence of fragments at m/e 305, 307, 309 and 311 ($C_6H_5Br_2Cl_2^+$) and m/e 89, 91 (base peak) ($C_4H_6Cl^+$) suggested the molecular formula $C_{10}H_{11}Cl_3Br_2$ from analogy with the fragmentation of the related compound **1**. The ^{1}H-NMR (table 2), also showed a striking resemblance to those of **1** and allowed only 2 gross structures, **2** or the alternative one having a bromochloromethyl group instead of the dibromomethyl group and the bromine instead of the chlorine at C-8, to be assigned to the new monoterpene. Specific location for the halogen atoms followed from ^{13}C-NMR-spectrum (table 1), which was very similar to that of **1** with the only notable exception that the chemical shift of the dihalomethyl carbon is upfield shifted by 28.7 ppm. This is only consistent with a dibromomethyl carbon[15] and in addition replacement of the chlorine by a bromine at C-8 should produce a marked upfield shift (ca. 11–12 ppm) at the α-carbon and a downfield shift (4–5 ppm) at the β-carbon[15]. The stereochemistry of **2** was tentatively assigned by using the ^{1}H-NMR empirical rules developed by Mynderse and Faulkner[11] for the assignments of stereochemistry for Plocamium cartilagineum metabolites. The chemical shift of the methyl signal suggested the threo configuration at carbons 3 and 4 and the chemical shift of proton H-6 (δ 6.58) indicated that the 7,8 double bond has the E geometry.

An examination of the gut contents of A. limacina revealed that it had been eating mainly the red alga Gracilaria verrucosa. Examination of hexane extracts of sun-dried Gracilaria verrucosa and G. compressa did not reveal the presence of halogenated monoterpenes, but **1** accompained by **1a** has been detected by GC-MS in 2 different specimens of Plocamium coccineum collected from different habitats (Naples and Catania). This may indicate that the animals have stored in the digestive gland the metabolites from Plocamium, probably an occasional component of the sea hare's diet, and confirms the endearing ability of the sea hares to concentrate the more interesting compounds from their diet[16]. The algal source of the minor halogenated monoterpenes remain to be discovered, although the failure to locate them in 2 Plocamium specimens might indicate transformations within the digestive gland.

14 2 m × 0.5 cm packed with 10% SE. 30 on silanized chromosorb P 100–200 mesh, operated at 180°; N_2 flowed at 105 ml min^{-1}.

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Anticoccidial riboflavine antagonists

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Summary. 4 types of riboflavine antagonists have broad-spectrum activity in poultry coccidiosis. 5-Deazariboflavine is most effective. 10-Benzyl analogs of riboflavine control intestinal species of coccidia.

Many B-complex vitamin antagonists are effective for the prevention of coccidiosis in poultry and other species. Sulfa-antifol combinations[1], a non-sulfa PABA antagonist[2] and anti-thiamines[3] are used as feed additives for this purpose. Likewise, anticoccidial action has been observed with antagonists of nicotinic acid[4], choline[5] and pyridoxine[6].