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TERPENOIDS AND STEROLS IN *CLADOPHORA VAGABUNDA*

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Key Word Index—*Cladophora vagabunda*; Cladophoraceae; sterols; triterpenes; volatile oils; food chains.

Abstract—Ten sterols and ursolic acid were identified in *C. vagabunda*. Hexahydrofarnesylacetone, dihydroactinidiolide, benzyl alcohol, myrtenol and 2-nonanone were found in the volatile oil of this alga. The eventual participation of *C. vagabunda* in Black Sea food chains has been discussed.

INTRODUCTION

Eleven species from *Cladophora* genus (Chlorophyta, order Siphonocladales, family Cladophoraceae) inhabit the Black Sea. *C. vagabunda* is one of the most widespread algae in the Black Sea.

A few investigations on the chemical composition of *Cladophora* species have been reported and sterols, widespread in marine algae, have been identified [1–7] (Table 1). Free sterols, as well as their esters and glycosides, have been found [4]. Also, one brominated diphenyl ether [8], an unidentified polysaccharide [9], fatty acids and 5-pentyl-dihydro-2/3H/-furanone [10] have been found in *Cladophora* species. Volatile compounds, including hexahydrofarnesylacetone and dihydroactinidiolide, have been found in *C. rudolphiana* and other green algae [11]. In the Black Sea *Cladophora* species, including *C. vagabunda*, pigments, proteins and lipids [12, 13] have been identified. In this work we report the isolation and identification of sterols, triterpenoids and volatile oils from *Cladophora vagabunda*.

RESULTS AND DISCUSSION

The fresh alga was extracted with ethanol, the extract concentrated and the lipophylic components extracted with dichloroethane. The dichloroethane extract was subjected to separation on a silica gel column and the sterol fraction was purified further by column chromatography on alumina. In this way, free sterols were isolated as a mixture. The sterol fraction was silylated and investigated by GC and GC/MS. The mass spectral fragmentation and RR_t were used for the identification of the individual sterols. The comparison with authentic samples confirmed the proposed structures (Table 1). Almost all of the identified sterols have been found previously in different *Cladophora* sp., but not in *C. vagabunda*. 5α-Cholestanol is reported for the first time in Cladophoraceae, but very often low concentrations of it cannot be detected because of the similarity of its GC behaviour with that of cholesterol. The GC investigation of the total sterol mixture showed the presence of very low concentrations of sterols with short side chains. In order to identify these rare sterols we used fractional precipitation with digitonin [14]. The last fraction, containing one main short side chain sterol was investigated by mass spectrometry [M^+ 314 (12), 299 (6), 286 (14), 271 (15), 255 (8), 229 (10), 213 (7), 175 (20), 96 (64), 69 (100), 55 (72)]. This enabled us to identify it by comparison as **1**, found earlier only in a few sponges [15]. There is a hypothesis that short side chain sterols are produced by autoxidative degradation of the normal sterol side chains, with intermediate hydroperoxide formation [15]. Sterol **1** could be produced by the same way in *C. vagabunda*, but its discovery for the first time in algae is an indication that it may have a dietary origin in invertebrates. The investigation of the short side chain sterols showed that there were no C_{26}-sterols. This is in agreement with the hypothesis that these widely spread sterols are biosynthesised by phytoplankton and not by macroalgae [16].

In Table 1 we include data for the sterol composition of other investigated *Cladophora* species [1–6]. Evidently in most of these algae the concentrations of cholesterol are higher than found in other green algae and terrestrial plants. Cholesterol is a typical animal sterol and in the plant kingdom only the evolutionary lower red algae contain substantial amounts. In *C. vagabunda* there is a surprisingly high concentration of cholesterol (35.7% from the total sterols). In this alga there is also an unusually low amount of alkylated sterols, especially 24ξ-ethylcholesterol. Surprisingly, in *C. vagabunda* from Senegalese coast [6] the concentration of cholesterol is

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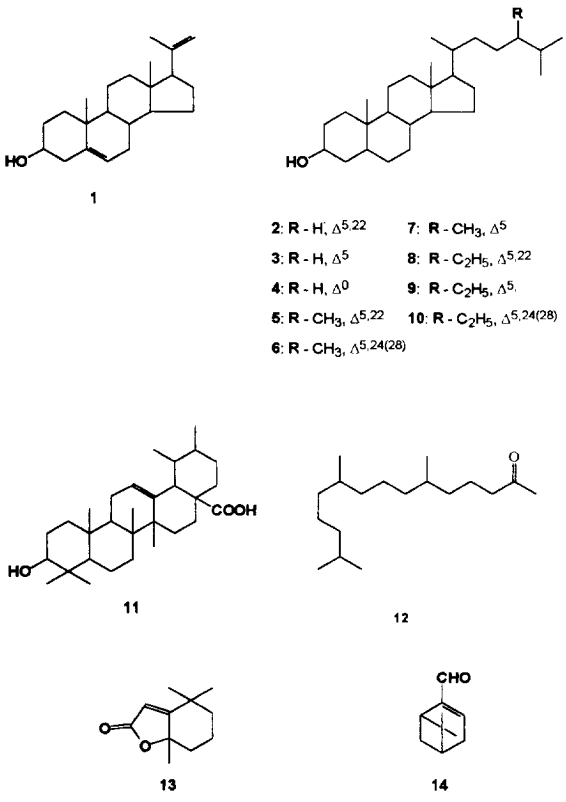
Table 1. Sterol composition of *Cladophora* species (% from the total sterol mixture)

Species	Sterol (per cent composition)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
C. flexuosa [1]	—	—	22.0	—	3.0	21.0	—	2.0	6.0	45.0
C. echinus [2]	—	tr.	18.5	—	tr.	8.2		—	73.0	—
C. laetevirens [3]	—	—	24.7	—	23.6	30.9	—	—	20.7	—
C. rupestris [4]	—	tr.	4.0	—	—	42.0	—	—	54.0	—
C. densa [5]	—	—	7.0	—	—	—	2.0	—	91.0	—
C. vagabunda [6]	—	0.3	5.7	—	0.3	13.5	2.5	—	55.8	18.5
*C. vagabunda**	tr.	1.6	35.7	4.1	4.3	27.5	4.6	2.5	13.4	6.3

**Cladophora vagabunda* discussed in this paper.



unusually low. This might be an indicator for the influence of ecologo-geographic factors and for the risk to use such data for chemotaxonomic studies.

In some fractions isolated by silica gel column chromatography triterpenoids were present. We isolated and identified ursolic acid (**11**), by comparison of its chromatographic behaviour and mass spectral fragmentation with an authentic sample. Marine organisms contain a big number of diverse terpenoids, but triterpenoids are very rare, contrary to terrestrial plants. Only one triterpenoid, containing five condensed six membered rings, has been found in a marine organism (Bryozoan, *Conopeum seuratum*) and identified as ursolic acid [17].

Contrary to terrestrial plants, almost nothing is known about the volatile oils of marine algae. The dichloroethane extract from *C. vagabunda* appeared to be an extremely complex mixture and we subjected it to a distillation with water vapour. The volatile oil obtained was investigated by GC/MS. The identification of its constituents was based on comparison with the mass spectra of authentic samples. By this method, hexahydrofarnesylacetone (**12**), dihydroactinidiolide (**13**), benzyl alcohol, myrtenol (**14**) and 2-nonanone were identified. All these compounds were found for the first time in *C. vagabunda* and **14** for the first time in a marine organism.

The results can be used for an investigation on the food chains in the Black Sea. Our earlier investigations on the chemical composition of the Black Sea Bryozoan, *Conopeum seuratum*, showed that it is very different from that of other marine organisms. It contained a complex mixture of terpenoids, but there were no halogenated terpenoids, characteristic for marine organisms. Instead, the animal contained monoterpenoids, diterpenoids and triterpenoids, characteristic for terrestrial plants [17]. It will be interesting to determine if these compounds are biosynthesized by *C. seuratum* or if they have a dietary origin. In the region where the Bryozoan samples were collected there are only two macroalgae, *Enteromorpha linza* and *C. vagabunda*. Our investigation on the terpenoid composition of *E. linza* [18] showed the presence of monoterpenoids, characteristic for terrestrial plants and three of them were identical with those found in *Conopeum seuratum* (*p*-menth-8-en-10-ol, pulegone and myrtenal). We find now, in *C. vagabunda*, ursolic acid, found earlier in *Conopeum seuratum*, as well as myrtenol that could be oxidized to myrtenal in this animal. The benzyl alcohol from *C. vagabunda* could be a precursor of benzylic esters of monoterpenoids, found in *Conopeum seuratum*. The content of cholesterol in *C. vagabunda* is equal to the sum of cholesterol and 7-dehydrocholesterol in *Conopeum seuratum* [19]. On the basis of all these data we suggest that detritus, produced by *E. linza* and *C. vagabunda*, is one of the sources of unusual terpenoids in *Conopeum seuratum*.

EXPERIMENTAL

The fresh algae (380 g dry wt) were collected near Cape Kaliakra (depth 1–4 m) in August 1991 (a voucher specimen deposited in the Pharmacy Faculty, Sofia) and were dipped in EtOH. After filtration, the alcoholic extract was

evapd *in vacuo*, diluted with an equal vol. of H_2O and extracted 2× with $(CH_2)_2Cl_2$. The organic layer was dried and after evapn yielded 12 g lipophylic substances. Part of the extract was subjected to sepn on a silica gel column with petrol and mixts of petrol with increasing amounts of Me_2CO. The obtained sterol mixt. was purified by CC on neutral alumina with petrol, and mixts of petrol and increasing amounts of Et_2O. Part of it was silanized with N,O-BSTFA and analysed with GLC and GC-MS (SPB-1 gas capillary column: 30 m × 0.25 mm, oven temp.: 280°). The other part of the sterols was subjected to fractional pptn with digitonin as described in ref. [13].

Ursolic acid was isolated as described in ref. [17].

Part of the $(CH_2)_2Cl_2$ extract was subjected to distillation with H_2O vapour. The obtained yellowish oil was analysed by GLC and GC/MS as described above, oven temp. 50–280° at 5° min^{-1}.

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