

# Terpenoids from a Black Sea Bryozoan *Conopeum seuratum*

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In the Bryozoan *Conopeum seuratum* six esterified monoterpene alcohols, three free monoterpene alcohols, three monoterpene ketones, two monoterpene aldehydes, four esters of diterpene acids, two diterpene acids and one triterpene acid were identified, most of them new for marine organisms. Some non-terpenoid compounds were identified too. Preliminary investigation on the terpenoids of the Black Sea sponge *Halichondria panicea* was performed and its terpenoid composition was compared with those of *C. seuratum*.

## Introduction

Bryozoa are minute, sessile, colonial animals. In spite of their wide distribution in tropical and temperate waters, marine bryozoans have until recently attracted little attention from chemists. Several interesting compounds have been isolated from them. Among them are alkaloids – physostigmine [1], methyltryptamine [2] and gramine [3] types, antineoplastic macrolides, called bryostatines [4] and others.

One of the most widespread groups of marine natural products are terpenoids. In *Flustra foliacea* (Bryozoa) five monoterpenes, characteristic for higher plants only – geraniol, nerol, citronellol, 1,2-*trans*- and *cis*-citral [5] have been identified. Recently, in another Bryozoan, *Bugula turrita*, Findley *et al.* [6] identified series of diterpenoids – manool, dehydroabietic acid, its ethyl ester, dehydroabietinal, dehydroabietinol, together with the triterpenoids serratenediol-3-methylether and 21-oxo-3-methoxy-serratenene.

All identified terpenoids are typical for higher plants, but not for marine organisms. This directed our interest towards terpenoid composition of other Bryozoa species.

The object of our research was the most widespread Black Sea Bryozoan, *Conopeum seuratum*

(class Gimnolamelata, Alman 1856, fam. Membraniporidae, Bufk 1834). Till now steroids and fatty acid methyl esters only have been identified in it [7, 8].

## Results

Chloroform soluble part from the total MeOH extract was separated into neutral and acid fractions.

### Neutral fraction

Besides steroids and fatty acid methyl esters two main groups of neutral compounds – terpenoid esters (fraction A) and free terpenoids (fraction B) were isolated and investigated by GC/MS and in some cases by GLC.

### Fraction A

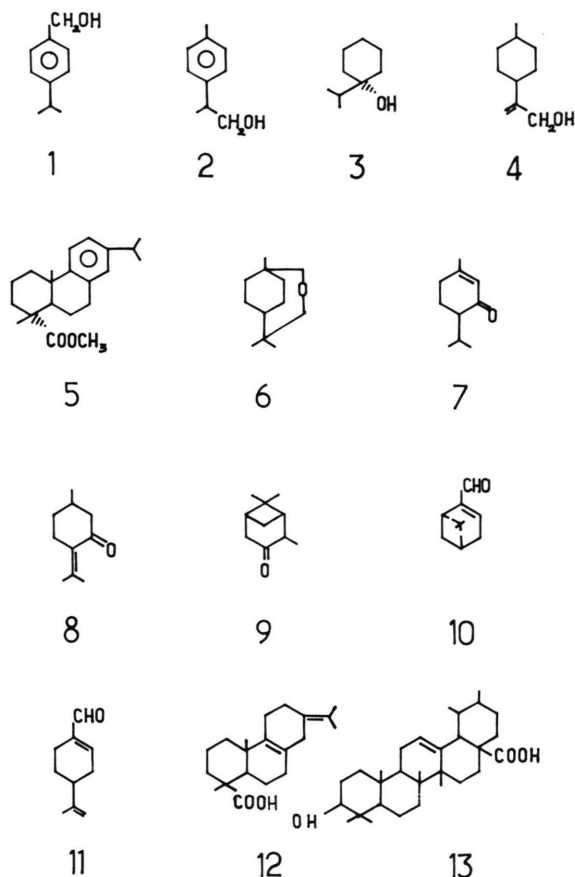
The IR spectrum proved the presence of esters in this fraction. Because of its extremely complex composition part of this fraction was hydrolyzed with base and another part was reduced with LiAlH<sub>4</sub>. The obtained alcohols were investigated by GC/MS. The results of all analyses showed the presence of monoterpenoids, sesquiterpenoids and diterpenoids in this fraction.

a) *Monoterpenoids*. By comparison with the mass spectra of known compounds we identified citronellol, cuminol (**1**), its isomer with structure **2**, menth-4-ol (**3**) and menth-8-en-10-ol (**4**). Geraniol and nerol were identified by GC/MS, without separation of their GLC peaks. Compounds **1–4** were identified

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for the first time in marine organisms. All these alcohols are esterified, according to their chromatographic behaviour, which is the first such identification in Bryozoans.

GC/MS investigation of the ester fraction showed the presence of citronellol and menth-4-ol acetates. In the mass spectra of four chromatographic peaks we obtained ions, characteristic for benzyl alcohol and in other two — ions for 2-phenethyl alcohol. This was confirmed after the investigation of the alcohols, obtained by base hydrolysis. Such esters are identified for the first time in marine organisms.

*b) Sesquiterpenoids.* Few sesquiterpene alcohols were found in this fraction. According to their mass spectra they have skeletons characteristic for the groups of  $\gamma$ -ylangene and cubebene.

*c) Diterpenoids.* Few of the components of fraction A have molecular masses and mass spectral fragmentation, characteristic for diterpene acid esters. One of them was identified as dehydroabietic acid methyl

ester (5). It could be an artifact, due to the extraction of the animal with MeOH, but the isolation of ethyl ester of the same acid from *Bugula turrata*, extracted also with MeOH shows, that this esters have natural origin. This was also in agreement with the presence in fraction A of the propyl(isopropyl) ester of dehydroabietic acid. These esters were distinguished from the esters of the epimeric calictristic acid by the intensities of the  $(M-15)^+$  peaks, which form the base peak in the spectra of calictristic acid and its derivatives. Two more methyl esters were found: of the isomers of dihydroabietic acid and abietic acid, but it was not possible to establish the exact locations of the double bonds by mass spectrometry only.

#### Fraction B

Free terpenoids are in smaller amount. By GC/MS and GLC we identified citronellol, 1,8-cineol (6) and geraniol.

In the same fraction we identified by GC/MS few monoterpene ketones (piperitone (7), pulegone (8) and isopinocampheol (9)) and aldehydes (mirtanal (10) and perilaldehyde (11)). The first two ketones were identified also by GLC.

During the course of our investigation we isolated some non-terpenoid compounds, which were investigated by GC/MS and in some cases by GLC.

From the total extract a hydrocarbon fraction was isolated and analyzed by GC/MS. Terpenes were not found. The main constituents were saturated normal hydrocarbons from  $C_{14}$  to  $C_{32}$  with  $C_{27}$  and  $C_{29}$  hydrocarbons predominating. There were small differences in the concentrations of odd and even numbered compounds, which could be explained with the participation of blue-green algae or bacteria in the diet of *C. seuratum*.

In fraction B one non-terpene alcohol, 1,1-dimethyl-2-phenylalcohol was identified, by comparison with an authentic sample.

After the reduction of fraction A a mixture of polar compounds was isolated and identified as glycerol ethers. These compounds were not found in the total extract of *C. seuratum*, which shows that they are produced by the reduction of diacyl alkylglycerols, which are often found in marine invertebrates, but still have not been isolated from Bryozoans.

#### Acid fraction

After prep. TLC we isolated two fractions with chromatographic mobilities similar to these of abietic

and ursolic acids. The first fraction contained few compounds similar to abietic acid (MS data). After methylation this fraction was investigated by GC/MS and neoabietic acid (**12**) was identified (for the first time in marine organisms) as well as dehydroabietic acid. In the second fraction there was only one compound. Its mass spectrum was identical with this of ursolic acid (**13**). It was distinguished from the isomeric oleanolic acid on the basis of the intensities of the  $m/z$  203 and 133 peaks [9]. This is the first isolation of pentacyclic triterpene with five six-membered rings from marine organisms.

## Discussion

In our investigation we observed the presence of more than hundred compounds in *C. seuratum*, most of them terpenoids. Only a small part of them were identified, but the mass spectra showed the absence of halogen atoms in all compounds while it is known, that most of the marine terpenoids contain halogens. In different Bryozoans alkaloids [1–3], monoterpenoids [5], di- and triterpenoids [6] unusual for marine organisms but characteristic for higher plants have been isolated. Another new 13 compounds of this type are reported in this paper. From all these data it is evident, that Bryozoans have unusual terpenoid (and alkaloid) composition, characteristic for higher plants, but not for marine organisms. The origin of these unusual terpenoids can be the diet or

their biosynthesis in Bryozoans. To check these possibilities we performed a preliminary investigation on the terpenoid composition of the sponge *Halichondria panicea*, collected at the same location. This animal is also a filter-feeder and probably has a diet similar to this of Bryozoans. By GLC and GC/MS we identified few terpenoids in it – *p*-menthen,  $\gamma$ -terpinen, dihydroabietic acid and an isomer of the abietic acid. These data showed, that the obtained unusual terpenoids are produced from some marine organisms which are included in the diet of the filter-feeders. In agreement with this suggestion is the recent isolation of ursolic and oleanolic acids from the Black Sea algae from fam. Cladophoraceae [10].

## Experimental

GC/MS analysis were carried out on JEOL JGC-20K gas chromatograph directly coupled to JEOL JMS-D300 mass spectrometer under 70 eV electron impact mode. Helium was used as carrier gas. All GC conditions are given in Table I.

*Conopeum seuratum* was collected in July 1984 near cape Kaliakra and immediately soaked in methanol. After two extractions with methanol three chloroform extractions followed. MeOH extracts were concentrated and extracted twice with  $\text{CHCl}_3$ . The combined  $\text{CHCl}_3$  extracts were concentrated and extracted consecutively with 5%  $\text{NaHCO}_3$  and 5% KOH solutions. The basic extracts were com-

Table I.

Sample	Capillary column	Injector temperature	Temperature programme
Fraction A	OV-101 FSCC	25 m 280 °C	50–280 °C at 6 °C/min
Reduced fraction A	DEGS glass cap. column	50 m 200 °C	50–190 °C at 3 °C/min
Hydrolyzed fraction A (neutral part)	OV-101 FSCC	25 m 280 °C	50–280 °C at 5 °C/min
Fraction B	DEGS glass cap. column	50 m 200 °C	50–190 °C at 3 °C/min
Hydrocarbon fraction	OV-101 FSCC	25 m 300 °C	50–280 °C at 5 °C/min
Acid fraction (methylated)	OV-101 FSCC	25 m 280 °C	50–280 °C at 4 °C/min

bined, acidified and extracted twice with  $\text{CHCl}_3$ . After evaporation acid fraction was obtained (0.4 g). The neutral fraction (2 g) was obtained after the evaporation of the  $\text{CHCl}_3$  solution, remaining after the extraction of the acids.

#### Neutral fraction

Two grams of this fraction were chromatographed on silica gel column. Hydrocarbons (100 mg) were eluted with hexane and the gradient elution continued with hexane–acetone. Fraction A (730 mg) was eluted with hexane–40% acetone, and further purified by prep. TLC on silica gel G with benzene–chloroform 4:6. Part of the obtained esters (100 mg) were hydrolyzed with 2 N ethanolic KOH (2 h reflux) and the obtained alcohols were extracted with ether. Another part of these esters (400 mg) was

reduced with  $\text{LiAlH}_4$  and alcohols also extracted with ether. All obtained alcohols were purified by preparative TLC (silica gel G; hexane–40% acetone).

Fraction B (300 mg) was eluted with hexane–40% acetone.

#### Acid fraction

Part of this fraction (200 mg) was subjected to prep. TLC (silica gel G;  $\text{CHCl}_3$ :EtOAc:EtOH – 100:7:3). The less polar fraction was methylated by  $\text{CH}_2\text{N}_2$ .

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