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# STEROLS AND VOLATILES IN *CYSTOSEIRA BARBATA* AND *CYSTOSEIRA CRINITA* FROM THE BLACK SEA

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**Key Word Index**—*Cystoseira barbata*; *C. crinita*; Phaeophyta; algae; sterols; volatiles; halogenated compounds; chemosystematics.

**Abstract**—Five sterols were identified in *Cystoseira barbata* and seven sterols in *C. crinita*. Saringasterol appeared to be an artefact. Volatiles in *C. barbata* consisted mainly of halogenated hydrocarbons, while in *C. crinita* terpenoids predominate. The results obtained can be used in chemosystematics. 

## INTRODUCTION

The algae from genera *Cystoseira* (Phaeophyta) are widespread around the world. In the Black Sea there are only two representatives of this genera, *Cystoseira barbata* (Good et Wood) Ag. and *C. crinita* Bory, which are the most widespread brown algae in this sea. They show small morphological differences and it is not easy to distinguish between them. In the Black Sea they provide a large amount of biomass and for this reason their chemical composition is of practical and ecological interest. Also, any differences in the chemical composition of both algae could be used in systematics.

The secondary metabolites of Black Sea *Cystoseira* species have not been investigated until now. In these algae from other seas a few $\Delta^5$-sterols were identified, fucosterol being the main sterol, analogously to other brown algae [1]. Terpenoids, phenols and carotenoids were found also and recently tetraprenyltoluquinols, naphthoquinones, diterpenoids and acetogenins were used in the taxonomy of this genus [2–4]. Here we report the composition of sterols and volatiles in the Black Sea *Cystoseira barbata* and *C. crinita*.

## RESULTS AND DISCUSSION

Both investigated species show very small morphological differences and there have been no comparative investigations on their secondary metabolite composition. They inhabit different locations, *C. barbata* prefers deeper waters, while *C. crinita* is located in the tidal zone. In order to compare their chemical composition these algae should be collected from an area where they are growing together to eliminate the influence of the ecological factors on their secondary metabolism. We found such a place and the collected algae were extracted consecutively with methanol and methanol–dichlorethane. The concentrated extracts were diluted with water and the lipophylic materials extracted with hexane. Preliminary TLC investigations of the total extracts and lipophylic fractions showed that their chemical composition was almost identical, with the exception of some pigments. For this reason we performed detailed analysis of two groups of secondary metabolites, sterols and volatiles. The sterol composition of other *Cystoseira* species has been reported [1].

Sterols were isolated by CC on silica gel, followed by prep. TLC on silica gel. The isolated fractions were investigated by GC and mass spectrometry and sterols identified by comparison of their data with authentic samples. The results obtained are summarized in Table 1. In both algae the main sterol was fucosterol (**6**), which is characteristic of brown algae, including previously investigated *Cystoseira* sp. The *C. barbata* sterol mixture consisted almost exclusively of this sterol, while in *C. crinita* we found significant concentrations of 24-ethyl-cholesa-5,22-dien-3$\beta$-ol (**5**), which is not a typical sterol of Phaeophyta.

In both samples investigated we found a more polar sterol, which was isolated and proved to be saringasterol (**8**). This sterol was found earlier in some brown algae which also contained fucosterol [5–7]. It

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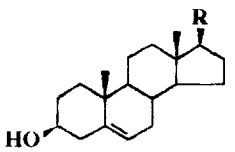


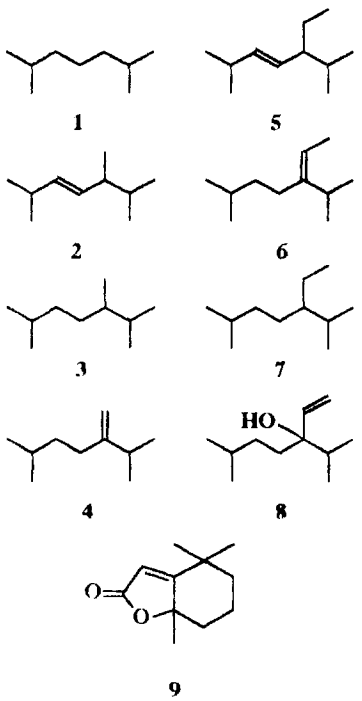


Table 1. Sterol composition of *C. barbata* and *C. crinita* (% from the total sterol mixture)

| Sterol | *C. barbata* | *C. crinita* |
|---|---|---|
| **1** | 2.1 | 2.4 |
| **2** | tr. | 2.2 |
| **3+4** | 2.4 | 3.7 |
| **5** | tr. | 25.4 |
| **6** | 95.4 | 61.2 |
| **7** | tr. | tr. |

tr: trace.

Table 2. Composition of volatiles in *C. barbata* and *C. crinita* (% from the total ion current*)

| *C. barbata* | | *C. crinita* | |
|---|---|---|---|
| 1-chloro-2-bromoethane | 1.0 | dihydroactinidiolide | 6.0 |
| 1,1,2-trichloroethane | 46.0 | $C_{10}H_{20}O$ | 8.0 |
| dimethylformamide | 1.5 | $C_{10}H_{20}O$ | 4.0 |
| 1,1,2,2,-tetrachloroethane | 25.0 | $C_{10}H_{20}O$ | 3.5 |
| 1-chloro-2-bromoethanol | 1.5 | | |
| hexachlorobutadiene | 2.5 | | |

* The total ion current generated depends on the characteristics of the compounds concerned and is not a true quantitation.

is not clear if this sterol is a natural compound or an artefact, perhaps produced by oxidation of fucosterol. We found saringasterol only after the purification of the lipophylic fraction, but not in the total extracts from fresh algae investigated immediately after the collection by chromatography, which proved that this sterol was an artefact.

While the volatile chemical constituents of terrestrial plants have been investigated widely, only recently have analogous investigations with marine organisms begun. Marine volatiles contain different types of organic compounds and are of significance for chemosystematics and ecology [8]. We obtained volatiles from both algal samples by hydrodistillation of the lipophylic extracts and investigated distillates obtained by GC and GC/MS. Part of their components were identified by comparison with the mass spectra of authentic samples and/or library files. Only a complete 'match' of the spectra indicates the identity of the samples. The results obtained are summarized in Table 2.

It is evident that both investigated algae contain different types of volatile compounds. In *C. barbata* the main components are halogenated hydrocarbons. Hexachlorobutadiene was discovered for the first time in nature. It is known that marine biota produce a variety of halogen-containing compounds possessing from one (halomethanes) to 30 carbon atoms. Halogenated compounds in marine organisms are mainly terpenoids and polyphenols. Most often halogenated compounds have been found in red algae (Rhodophyta). Only in the family Bonnemaisoniaceae have halogenated acetogenins been found, containing 1–30 carbon atoms together with hydrocarbons and their oxidized derivatives (alcohols, aldehydes, acids, etc.)

[9, 10]. In a few cases halogenated terpenoids [11] and polyphenols [12, 13] have been found in green algae and oxylipins [14] and polyphenols [15] in brown algae. We have now discovered for the first time halogenated hydrocarbons in Phaeophyta. Our observation of antibacterial and fungicidic activity of extracts from *C. barbata*, as well as its toxicity against some Crustaceans (unpublished data), could be connected with these halogenated hydrocarbons.

Volatiles from *C. crinita* possess different chemical constituents. Only traces of halogenated compounds with relatively high molecular masses were found. The main components appeared to be monoterpenoids, although we identified only one of them as dihydroactinidiolide (**9**). This terpenoid has been found in low concentration in some essential oils from terrestrial plants, as well as in volatiles from some green algae [16, 17]. Terpenoids also can have some defensive functions in plants.

From these data it is evident that the biologically similar species *Cystoseira barbata* and *C. crinita* show some differences in their secondary metabolite composition. *Cystoseira barbata* contained in the sterol fraction almost pure fucosterol and mainly halogenated hydrocarbons in its volatiles, while *C. crinita* contained an unusually high concentration of 24-ethylcholesta-5,22-dien-3$\beta$-ol and in volatiles we found mainly monoterpenoids instead of halogenated hydrocarbons. Both algae grew together, which excludes the influence of environmental factors and seasonal variations on their chemical composition. This is an indication that sterols and volatiles could be used for taxonomic investigations of algae from genera *Cystoseira*, but more investigations in the chemical composition of samples from different locations and different times of year must be performed.

## EXPERIMENTAL

*Plant material. Cystoseira barbata* and *C. crinita* were collected in May 1994 near the city of Ahtopol. The plant was identified by Dr St. Dimitrova-Konaklieva and voucher specimens are deposited in the Faculty of Pharmacy, Higher Medical School, Sofia. The sample were washed and immediately dipped into MeOH and transported to the laboratory.

*Isolation of compounds.* Fresh algae were extracted with MeOH, followed by MeOH–$Ch_2Cl_2$ (1:1). After the concentration of the combined extracts *in vacuo* the residue was diluted with $H_2O$ and extracted with hexane (x 2).

Portions of the hexane extracts (0.164 mg from *C. barbata* and 0.123 mg from *C. crinita*) were subjected to hydrodistillation with 20 ml $H_2O$ for 4 h. Volatiles were extracted with $Et_2O$ (27 mg from *C. barbata* and 32 mg from *C. crinita*) and investigated by GC/MS on a 30 m fused silica SPB-1 capillary column, in a JEOL JGC-20 K gas chromatograph, directly coupled to a JEOL JMS D-300 mass spectrometer, temp. programme 6–280° rising at 6° $min^{-1}$.

The remaining parts of the hexane extracts were subjected to CC on silica gel. Elution with hexane $Me_2CO$ with an increasing concentration of $Me_2CO$ afforded free sterols, followed by saringasterol. Sterol fractions were analysed on a Pye Unicam 304 gas chromatograph with 5 m fused silica capillary column, coated with OV-1.

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## REFERENCES

1. Francisco, C., Combault, G., Teste J. and Maume, B., *Biochem. Biophys. Acta*, 1977, **487**, 115.
2. Amico, V., *Phytochemistry*, 1995, **39**, 1257.
3. Amico, V., Piattelli, M., Cunsolo, F., Recupero, M. and Ruberto, G., *Gazz. Chim. Ital.* 1990, **120**, 9.
4. Amico, V., Giaccone, G., Piattelli, M. and Ruberto, G., *Phytochemistry*, 1988, **270**, 1069.
5. Ikekawa, N., Morisaki, N., Tsuda, K. and Yoshida, T., *Steroids*, 1968, **12**, 41.
6. Knights, B. A., *Phytochemistry*, 1970, **9**, 903.
7. Virtue, P. and Nichols, P., *Phytochemistry*, 1994, **37**, 673.
8. Roussis, V., Vayias, C., Caberi, H., Harvala, C. and Fenical, W., in *Eighth International Symposium on Marine Natural Products*, Canary Islands, 1995, p. 182.
9. Moore, R. E., in *Marine Natural Products*, Vol. 1, ed. P. J. Scheuer. Academic Press, New York, 1978, p. 44.
10. Barrow, K. D., in *Marine Natural Products*, Vol. 5, ed. P. J. Scheuer. Academic Press, New York, 1983, p. 51.
11. Paul, V. J., Cronan, J. M. and Cardellina II, J. H., *J. Chem. Ecol.*, 1993, **19**, 1847.
12. Carte, B., Troupe, N., Chan, J. A., Westley, J. W. and Faulkner, D. J., *Phytochemistry*, 1989, **28**, 2917.
13. Park, M., Fenical, W. and Hay, M. E., *Phytochemistry*, 1992, **31**, 4115.
14. Todd, J. S., Proteau, P. J. and Gerwick, W. H., *Tetrahedron Letters*, 1993, **34**, 7689.
15. Green, D., Kashman, Y. and Miroz, A., *Journal of Natural Products*, 1993, **56**, 1201.
16. Sakagami, H., Iseda, J., Fujimori, T., Hara, Y. and Chichara, M., *Bull. Jap. Soc. Sci. Fish.* 1991, **57**, 527.
17. Elenkov, I., Georgieva, T., Hadjieva, P., Dimitrova-Konaklieva, St. and Popov, S., *Phytochemistry*, 1995, **38**, 457.