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Sterol composition of the Adriatic Sea algae *Ulva lactuca, Codium dichotomum, Cystoseira adriatica* and *Fucus virsoides*

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Abstract: The sterol composition of two green algae and two brown algae from the South Adriatic was determined. In the green alga *Ulva lactuca*, the principal sterols were cholesterol and isofucosterol. In the brown alga *Cystoseira adriatica*, the main sterols were cholesterol and stigmast-5-en-3ß-ol, while the characteristic sterol of the brown algae, fucosterol, was found only in low concentration. The sterol fractions of the green alga *Codium dichotomum* and the brown alga *Fucus virsoides* contained practically only one sterol each, comprising more than 90 % of the total sterols (clerosterol in the former and fucosterol in the latter).

Keywords: sterols, green algae, brown algae, *Ulva lactuca, Codium dichotomum, Cystoseira adriatica, Fucus virsoides*.

INTRODUCTION

Over the last decades, there have been many investigations on the chemical composition of algal lipids, including the composition of sterols. However, taxonomic classifications based on these data were not always fully substantiated, especially earlier ones, obtained by unsophisticated analytical methods.

Still, some taxonomic conclusions based on sterol composition are available.[1] Different classes of algae have a distinct sterol composition. For the evolutionary lower red algae (Rhodophyceae), cholesterol and, in some cases, its biogenetic precursor cholesta-5,24(25)-dien-3ß-ol are the major sterols, while those of the same class considered more advanced contain 24-methylenecholesterol, the C-24 alkylated sterols and sterols with Δ^{22}-double bond being present in low concentrations.

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In brown (Phaeophyceae) and green (Chlorophyceae) algae, which are regarded as evolutionary more advanced, methylation products of 24-methylenecholesterol are the major sterols – fucosterol ((*E*)-stigmasta-5,24(28)-dien-3ß-ol) in the former and isofucosterol ((*Z*)- stigmasta-5,24(28)-dien-3ß-ol) in the latter, while in advanced green algae, the $\Delta^{24(28)}$ double bond is reduced, leading to an accumulation of sitosterol ((24*R*)-stigmast-5-en-3ß-ol).

In this work, the qualitative and quantitative content of sterols, as potential taxonomic markers, were determined in four different algal species, two green and two brown algae with rare reports on their sterol composition. The green algae studied were *Ulva lactuca* L. (family Ulvaceae, order Ulvales) and *Codium dichotomum* (Huds.) Setch. (family Codiaceae, order Siphonales). The brown algae investigated were *Cystoseira adriatica* Sauvageau (family Cystoseiraceae, order Fucales), algae of the genus *Cystoseira* representing a substantial part of the Adriatic Sea benthos, and *Fucus virsoides* J. Agardh. (family Fucaceae, order Fucales), endemic for the Adriatic Sea. Except for *U. lactuca*, there are no data on the sterol composition of the studied algae.

The algae were collected in the month of July in the Bay of Kotor, Montenegro, which is a rather unique and hydrologically well-defined basin.[2] In mid-summer, the salinity at the collection site is approximately 31 ‰, while in spring it is lower, around 25 ‰.

RESULTS AND DISCUSSION

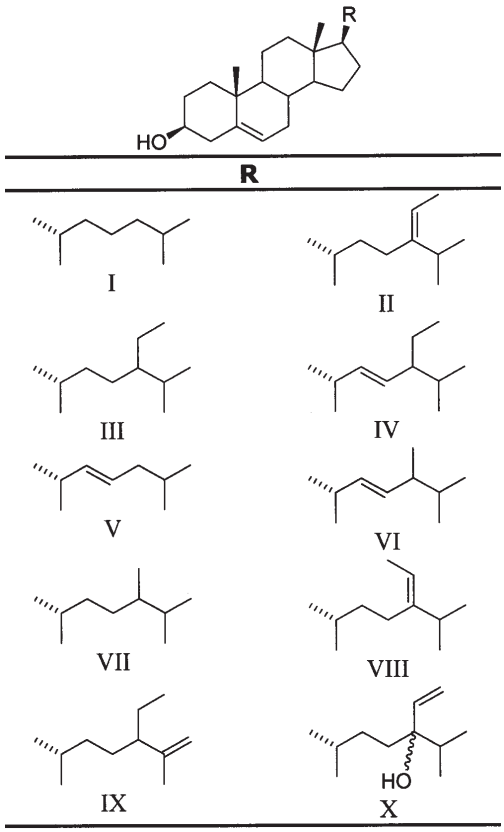
The sterol compositions of the investigated algae are given in Table I and the structures of the sterols in Scheme 1.

TABLE I. Sterol composition (%) of the investigated algae

	U. lactuca	*C. dichotomum*	*C. adriatica*	*F. virsoides*
Cholesterol **I**	34	–	40	–
(*Z*)-Stigmasta-5,24(28)-dien-3ß-ol* **II**	26	–	–	–
(24*R*)-Stigmast-5-en-3ß-ol **III**	4	–	30	–
Stigmasta-5,22-dien-3ß-ol **IV**	4	–	4	–
22-Dehydrocholesterol **V**	4	–	3	–
Ergosta-5(22)-dien-3ß-ol **VI**	2	–	6	–
Ergost-5-en-3ß-ol **VII**	1.3	–	4	–
(*E*)-Stigmasta-5,24(28)-dien-3ß-ol** **VIII**	trace	–	5	92
(24*R*)-Stigmasta-5,25-dien-3ß-ol **IX****	–	93.5	–	–
Stigmasta-5,28-dien-3ß,24-diol **X**	–	–	3	–

Common names: *isofucosterol, **fucosterol, ***clerosterol

The principal sterols in the extract of the green alga *Ulva lactuca* were cholesterol (34%) and isofucosterol (26 %). Stigmast-5-en-3ß-ol, stigmasta,5,22-dien-3ß-ol, 22-dehydrocholesterol, ergosta-5(22)-dien-3ß-ol and ergost-5-en-3ß-ol were found



Scheme 1.

in lower concentrations, while fucosterol appeared in trace concentrations. Generally, in Chlorophyceae the dominant sterol varies within the order, or for the same order, within a family.[3] However, in the algae of the family Ulvaceae, the main sterol is almost always isofucosterol.[4–8] In the samples of *U. lactuca* studied to date, isofucosterol was always the major sterol.[4,9,10] Our results obviously differ from the literature data by the finding that the most abundant sterol in *U. lactuca* from the Bay of Kotor was cholesterol, followed by isofucosterol. It seems reasonable to assume that it is a consequence of the specific ecological conditions in the Bay of Kotor, where both temperature and salinity vary greatly over the year.[2] The data obtained with *Ulva rigida* from the Black Sea support this assumption.[11] Namely, this alga, collected in the sea of permanently low salinity, had a sterol composition completely different from other Ulvaceae, the main sterol being fucosterol (63 %), with lower concentrations of isofucosterol and cholesterol.

The next green alga studied in this work was *Codium dichotomum*. Only one sterol, clerosterol ((24*R*)-stigmasta-5,25-dien-3ß-ol) could be identified by gas chromatography and ^{1}H-NMR spectroscopy[12] in the free sterol fraction, compris-

ing 93.5 % of the mixture. This sterol is characteristic for the algae of the genus *Codium,*[3,4,13–21] while in some cases the lower homologue, ergosta-5,25-dien-3ß-ol, is also present in significant amounts. Therefore, sterols containing Δ^{25}-double bond appear to be the chemotaxonomic marker of the genus *Codium*.

The sterol composition of the brown alga *Cystoseira adriatica* was relatively complex. Eight sterols were identified, the main ones being cholesterol (40 %) and stigmast-5-en-3ß-ol (30 %). The most interesting finding was the low content of fucosterol (5 %), the sterol characteristic for brown algae, and usually the principal sterol of the algae belonging to the genus *Cystoseira*.[5,22–27] It should be mentioned that there is some ambiguity regarding the taxonomic classification of *C. adriatica*. Some authors suggested that this alga is a sub-species of *Cystoseira compressa*. However, our results show that the sterol composition of *C. adriatica* from the Bay of Kotor is fairly different from that of *C. compressa* from the Saronikos gulf of Greece,[28] in which, for instance, sigmast-5-en-3ß-ol was not detected. Based on the results of the sterol analysis it seems that *C. adriatica* should at present be regarded as a separate species.

In the sterol fraction of the brown alga *Fucus virsoides* only one sterol, fucosterol, comprising 92 % of the total sterols, was identified. Such a high amount of fucosterol is characteristic for many species of brown algae, and especially for the genus *Fucus*, where the percentage of fucosterol in the sterol fraction ranges from 78 % in *Fucus evanescens*[29] to 99 % and 100 % in *Fucus vesiculosus*[6] and *Fucus serratus,*[30] respectively. However, as fucosterol is a common constituent in brown algae, a high concentration of fucosterol can not be used as a taxonomic marker for the genus *Fucus*.

EXPERIMENTAL

Collection of the samples

The algae were collected in the Bay of Kotor, southern Adriatic Sea in mid-summer. The algae were rinsed with water and dried carefully in the shade in a stream of air for 15 days. The dried algae were ground in a mill.

Extraction and isolation of sterols

The dried alga was first washed with petroleum ether (50 °C – 70 °C) and then extracted with benzene in a Soxhlet apparatus.

The benzene extract was subjected to column chromatography on silica gel 60 (0.063–0.200 mm), using a benzene – petroleum ether (4 : 1) mixture as the eluent, and the fraction containing sterols was rechromatographed on a silica gel column 100 (0.040 – 0.063 mm), using the same eluent. The homogenity of the fractions was tested by TLC on silica gel GF_{254} plates with benzene–petroleum ether (3:1).

Analysis of sterols

The total sterol mixture was analysed by gas chromatography. A GC Hewlett Packard 5890, equipped with a flame ionisation detector and a quartz capillary column with OV-17 (12 m x 0.25 mm) was used. The temperature programme was 260 °C – 300 °C at 6 °C min^{-1}, and a 10-min hold. The injector temperature was 300 °C, and the detector temperature 320 °C. The carrier gas was nitrogen. Retention times were compared with those of authentic sterols.

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И З В О Д

САСТАВ СТЕРОЛНЕ СМЕСЕ АЛГИ ЈАДРАНСКОГ МОРА *Ulva lactuca, Codium dichotomum, Cystoseira adriatica* и *Fucus virsoides*

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Одређен је састав стеролне смесе две зелене и две мрке алге из јужног Јадрана. У зеленој алги *Ulva lactuca* главни стероли су холестерол и изофукостерол. У мркој алги *Cystoseira adriatica* главни стероли су холестерол и стигмаст-5-ен-3ß-ол, док је фукостерол, стерол карактеристичан за мрке алге, нађен само у малој количини. Стеролне смесе зелене алге *Codium dichotomum* и мрке алге *Fucus virsoides* састоје се практично само од по једног стерола, клеростерола у првој и фукостерола у другој, који сачињавају више од 90 % стеролних фракција.

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