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Sterol Compositions of the Filamentous Nitrogen-Fixing Terrestrial Cyanobacterium *Scytonema* sp.

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ABSTRACT. Thirteen unsaturated sterols were identified by gas chromatography–mass spectrometry using serially-coupled capillary columns from the filamentous nitrogen-fixing terrestrial cyanobacterium *Scytonema* sp. isolated from the microbial community of cyanobacterial on ‘Black Cover’ biofilms limestone walls in Jerusalem. The dominant sterols were cholest-5-en-3β-ol (18.9 %), 3β-methoxycholest-5-ene (16.2 %) and 3β-acetoxycholest-5-ene (11.2 %).

The presence of sterols in cyanobacteria was reported for the first time by Reitz and Hamilton (1968) who found cholesterol and sitosterol in *Anacystis nidulans* and *Fremyella diplosiphon*, and De Souza and Nes (1968) who discovered seven unsaturated sterols in *Phormidium luridum*. Cyanobacterial sterols could be one of the major lipid biomarkers in hydrothermal environments and ancient sediments (Carpenter *et al.* 1997; Summons *et al.* 1999; Volkman *et al.* 1998). The sterol components of these organisms may also serve in many cases as a systematic marker of the taxon (Patterson 1994).

The filamentous nitrogen-fixing cyanobacteria belonging to the genus *Scytonema* are widespread in nature and are also found in cyanobacterial ‘Black Cover’ biofilms on rocks, stones, concrete walls, and drains in the subtropical part of Taiwan (Juttner and Wu 2000). *Scytonema* species have also been found as dwelling stone monuments in some European countries (Belgium, Greece, Germany, Italy, Spain, Sweden; Tomaselli *et al.* 2000). Two species of the genus *Scytonema* were identified as living on rocks and in soil around Jerusalem, *viz. Scytonema drilosiphon* and *S. hofmanii* (Dor 1998). The black-colored biofilms consist of euendolithic coccoid cyanobacteria and cyanophilous lichens occupying most of the surface of walls in Jerusalem (Danin and Caneva 1990); they were classified and studied for the principal biodeterioration processes in the facing of old buildings and hard limestone walls (Danin and Caneva 1990).

This report extends our previously studies of the cyanobacterial compounds (Dembitsky *et al.* 1999, 2001; Řezanka *et al.* 1986, 2003) and describes sterol composition of the filamentous nitrogen-fixing terrestrial cyanobacterium *Scytonema* sp., isolated from the microbial community of Black Cover cyanobacterial biofilms.

MATERIALS AND METHODS

Scytonema sp. (*Nostocales*, *Scytonemataceae*) was isolated from the microbial community of Jerusalem building limestones. Chasmoendolithic cyanobacteria were collected together with a small piece of rock during summer 1998; it was cultivated on dry agar and then on BG-11 medium at 27 °C (Dor 1987), under illumination of cool white fluorescent lights of 30 $\mu E\,m^{-2}\,s^{-1}$ at the surface of the flasks over 9 months. Strain GRC was additionally cultivated at 35 °C using a monochromatic (312 nm) UV B lamp T 15M 7C (*Vilber Laurmat*) at 16 W/m^2 in a special UV-protected box for 4 months. The cells were harvested by centrifugation, lyophilized, and stored in a deep freeze.

Lyophilized cells (2 g) were extracted in a Soxhlet apparatus with CH_2Cl_2–MeOH (2 : 1, *V*/*V*) for 3 h. After removal of the solvent, the dark green extract was refluxed with 1 mol/L KOH (in MeOH–H_2O, 20 : 1, *V*/*V*) for 4 h and filtered. The nonsaponifiable fraction containing the sterols was extracted with hexane, and the H_2O layer was also extracted with hexane, and combined. The sterols were converted to trimethylsilyl derivatives by *N*,*O*-bis(trimethylsilyl)trifluoroacetamide plus 1 % trimethylchlorosilane in pyridine at 80 °C for 15 min.

Gas chromatograph *Hewlett-Packard* 5890 (series II) with an HP–GC-mass selective detector (5971B MSD) was used. The trimethylsilyl derivatives of sterols were identified by mass spectral library search (*Wiley Registry* 2000, and/or home-made special sterol library).

RESULTS AND DISCUSSION

The sterol composition of *Scytonema* sp. (Table I) makes up ≈360 ppm of lyophilized cells. Thirteen unsaturated sterols with three major compounds, *viz.* cholest-5-en-3β-ol (18.9 %), 3β-methoxycholest-5-ene (16.2 %) and 3β-acetoxycholest-5-ene (11.2 %) were identified. Serially coupled capillary columns with different polarity of stationary phases were used for separation of original sterol mixture (Fig. 1) (Dembitsky *et al.* 1999).

Table I. Natural sterols isolated from the terrestrial cyanobacterium *Scytonema* sp.

Peak no.	Compound	Retention time min	Molar mass Da	Abundance %[a]
1	*unidentified*	107.35	428	3.1
2	*unidentified*	107.39	428	5.5
3	3β-acetoxycholest-5-ene	107.44	426	11.2
4	3β-methoxycholest-5-ene	112.22	400	16.2
5	(24*R*)-methylergost-5-en-3β-ol	112.64	400	3.6
6	5α-ergost-7-en-3β-ol	113.22	400	2.9
7	ergosta-5,7-dien-3β-ol	114.09	398	6.3
8	ergosta-5,22(*E*)-dien-3β-ol	115.20	398	2.6
9	cholest-5-en-3β-ol	115.28	386	18.9
10	5α-ergosta-7,22(*Z*)-dien-3β-ol	115.39	398	5.3
11	ergosta-7,22(*E*)-dien-3β-ol	115.45	398	9.4
12	ergosta-4,7,22-trien-3β-ol	117.07	396	4.1
13	ergosta-5,7,22(*E*)-trien-3β-ol	117.84	396	4.9
14	ergosta-5,8,22(*E*)-trien-3β-ol	118.74	396	6.3
15	ergosta-5,7,22,24(28)-tetraen-3β-ol	120.38	394	2.7

[a]Percentage of total sterols.

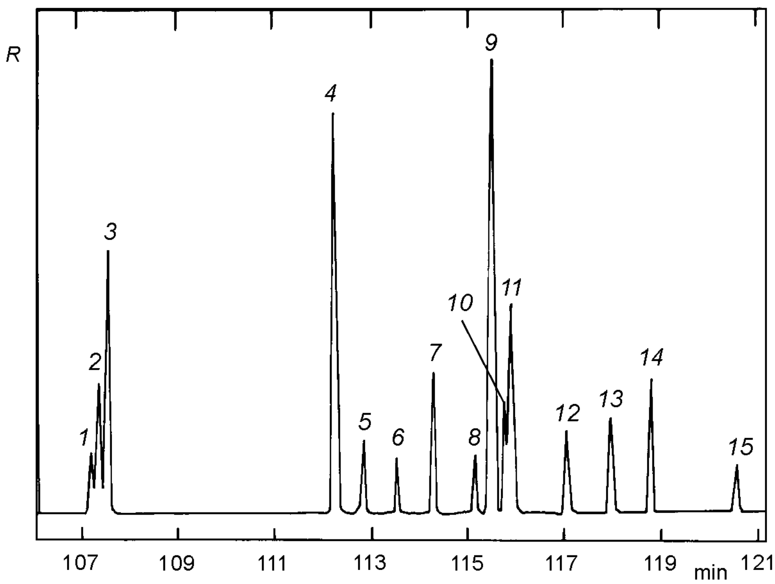

Fig. 1. GC–MS chromatogram of natural sterols isolated from *Scytonema* sp. (part 107–121 min); *R* – detector response; for numbering of peaks *see* Table I.

Previous investigation of cyanobacterial sterols showed that the major part is constituted by unsaturated compounds (*e.g.*, cholesterol, campesterol, sitosterol or clionasterol), and by other minor components. These sterols were identified in *Anabaena solitaria*, *A. cylindrica*, *A. viguieri*, *Nodularia harveyana*, and *Nostoc carneum* (Kohlhase and Pohl 1988). Δ^5- or Δ^7-sterols have been isolated from *Spirulina platensis*, *Calothrix* sp., and *Nostoc commune* (Paoletti *et al*. 1976). Five Δ^5- and Δ^7-sterols were isolated from *Scytonema* sp.: cholesterol and their methoxy and acetoxy derivatives, and also (24*R*)-methylergost-5-en-3β-ol and 5α-ergost-7-en-3β-ol (Table I).

Brassicasterol is well known among dienoic sterols occurring in cyanobacterial species, being found in *Anabaena solitaria*, *A. cylindrica*, *A. viguieri*, *Nodularia harveyana* and *Nostoc carneum* in a range of 0.8–2.8 % of total sterols (Kohlhase and Pohl 1988). Stigmasterol (or his diastereoisomer poriferasterol) was also found in *Anabaena solitaria*, *A. cylindrica*, *A. viguieri*, *Nodularia harveyana* and *Nostoc carneum* with high percentage (up to 28.5 %) in *A. viguieri* (Kohlhase and Pohl 1988). Further dienoic sterols were detected in *Spirulina platensis*, *Calothrix* sp. and *Nostoc commune* (Paoletti *et al.* 1976). We found 6 dienoic sterols, predominantly ergosta-7,22(*E*)-dien-3β-ol (9.4 %).

Sterols with 3 or 4 double bonds are mainly derivatives of ergostatrienols and ergostatetraenols. Ergosterol is the principal sterol of the cyanobacteria *Anabaena solitaria*, *A. viguieri*, *Nodularia harveyana* and *Nostoc carneum* (Kohlhase and Pohl 1988). Three sterols having 3 double bonds were also found in our *Scytonema* sp.: ergosta-4,7,22-trien-3β-ol, ergosta-5,7,22(*E*)-trien-3β-ol, ergosta-5,8,22(*E*)-trien-3β-ol (Table I).

Only one tetraenoic sterol was identified in *Scytonema* sp.; it was determined on the basis of its chromatographic data and the mass spectrum as ergosta-5,7,22,24(28)-tetraen-3β-ol. It is a rare sterol. Its first detection was described in the acidophilic hot springs algae *Cyanidium caldarium* and *Galdieria sulphuraria* (Seckbach *et al*. 1993). Previously this sterol has been found in the protozoan *Tetrahymena pyroformis* (Nes and Nes 1980).

Sterol content determination in the cyanobacteria seems to be the first step to its liquidation. As mentioned above, cyanobacteria contribute to a decay of the exterior of ancient buildings. Knowledge of the content of sterols, which are important building blocks of membranes, makes possible a goal-directed intervention in their biosynthesis. Patterson (1994) stated that the use of specific herbicides leads to cessation of sterol biosynthesis and inhibition of cyanobacterial growth.

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