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SHORT COMMUNICATION

STEROLS OF *SPIRULINA MAXIMA*

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Ah&act-Cholesterol and β-sitosterol have been isolated and identified in the blue-green alga, ***Spirulina maxima.*** The presence of sterols is related to the antimicrobial activity of this alga.

INTRODUCTION

UNTIL recently, it has been assumed that blue-green algae lack sterols.[1–3] However, in 1967, Reitz and Hamilton[4] reported the presence of cholesterol and β-sitosterol in two species of blue-green algae, ***Anacystis nidulans*** and ***Tremyella diplosiphon,*** and in 1968 de Souza and Nes[5] isolated sterols from the blue-green alga ***Phormidium luridum.***

We have now separated, isolated and identified cholesterol and β-sitosterol in the blue-green alga, ***Spirulina maxima,*** the sterol content being closely related to its antimicrobial activities.[6]

RESULTS AND DISCUSSION

Ether extracts of water macerated algae without prior saponification, on concentration and addition of ethanol, gave crystals (m.p. 145") which produced a strong blue-green colour λ_{max} 635 nm with the Liebermann-Burchard test, typical of sterols containing a Δ^5 bond.' It was purified by TLC on silica gel and three sterol spots were obtained, one of which coincided with cholesterol (R_f 0.99) and another with β-sitosterol (R_f 0·82). The crystalline material was then extracted with chloroform, and on concentration crystals were obtained which were recrystallized from chloroform-methanol giving m.p. 144". These caused no depression of m.p. with pure colesterol. Its acetate had m.p. 115".

Crystalline material which did not readily dissolve in chloroform, was dissolved in benzene and chromatographed on an alumina column which was consecutively eluted with benzene, benzene-chloroform (1: 1), and chloroform. Sterols were detected after separation on alumina TLC plates. Fractions containing sterols were combined, and on addition of methanol and refrigeration, a small quantity of β-sitosterol (m.p. 137") was obtained.[8] Since

[1] P. W. CARTER, I. M. HEILBRON and B. LYTHGOE, *Proc. Roy. Soc.* B128, 82 (1939).
[2] W. BERGMANN, in *Comparative Biochemistry* (edited by M. FLORKIN and H. S. MASSON), Vol. 3, p. 103, Academic Press, New York (1962).
[3] E. Y. LEVIN and K. BLOCH, *Nature* 202, 90 *(1964).*
[4] R. C. REITZ and J. G. HAMILTON, *Comp. Biochem. Physiol. 25,401 (1968).*
[5] *N.* J. DE SOUZA and W. R. NES, Science 162,363 (1968).
[6] N. G. MARTINEZ NADAL, Antimicrobial activity of *Spirulina maxima.* Xth International Congress of Microbiology, Mexico (1970).
[7] G. F. GIBBONS, L. J. GOAD and T. W. GOODWIN, *Phytochem. 6,677 (1967).*
[8] K. SHETH, P. CATALFOMO, L. A. SCIUCHETTI and D. H. FRENCH, *Lloydia* 30, 78 *(1967).*

there was insufficient for the preparation of derivatives, its identity was validated by co-chromatography with authentic β-sitosterol and by its mixed m.p.

Extraction of the unsaponifiable fraction with light petroleum and subsequent freezing in some cases precipitated sterol fractions (m.p. 15.5-l 60") which on separation by chromatography on silica gel and alumina plates gave spots which were detected by UV, eluted and purified. Cholesterol, m.p. 143-144" was obtained by this method. Sterol-positive fractions were precipitated by digitonin and cholesterol was regenerated in most cases. The sterol-positive unsaponifiable fractions were chromatographed on alumina columns, progress of elution being traced by TLC. Three steroid spots, two of which seemed to be cholesterol and β-sitosterol were obtained and it is probable that there is at least one other sterol present in this alga. Recent work in these laboratories[6] has shown that *Spirulina maxima* contains three antimicrobial substances, one of which appears to be a polyene, and is closely related to the presence of sterols.

EXPERIMENTAL

Extraction of unsaponifiable fractions. Samples of algae (So-100 g) were Soxhlet extracted with organic solvents (petroleum, diethyl ether, hexane, acetone or ethanol) for at least 3 hr. Other samples were homogenized with solvents in a Waring blendor. The solvents were removed *in vacuo* and the total lipid fraction was refluxed with either methanolic 8% KOH[10] or with 15 % KOH in 85 % ethanol.' After removal of the alcohol, the aqueous residues were extracted with ether, washed again with water and dried with anhydrous Na_2SO_4. The ether portion on concentration gave crude fractions which were tested for the presence of sterols and triterpenoids utilizing the following reagents: Liebermann-Burchard, Tortelli-Jaffei,[10] Rosenheim and Drummond.[8]

Isolation of sterols. Method 1—Unsaponifiable fraction was extracted with petroleum (b.p. 20–40°) and kept overnight at -10". Sterols were precipitated out."

Method 2—Sterols were precipitated with 2% solution of digitonin in 90% ethanol. The suspension was cooled, centrifuged and the ppt. washed free from pigments with pre-cooled diethyl ether. The sterols were then regenerated by heating the digitonides with dry pyridine followed by ether extraction.[12]

Method 3-Unsaponifiable fraction dissolved in petroleum was column chromatographed through activated alumina. Elution was carried out with petroleum, benzene, benzene–$CHCl_3$ (1 : 1), $CHCl_3$, $CHCl_3$–ether (1: 1), ether and methanol.

Purification of sterols. Sterol-positive fractions separated by alumina columns and other methods described, were spotted on pre-coated silica gel and alumina plates (QuantaGram TLC) and separated by a solvent system composed of $CHCl_3$–acetone (10:90 v/v). Zones and spots were visualized by spraying with 50% H_2SO_4 and heating, and with (10 % w/w $SbCl_3$ in $CHCL_3$). Authentic cholesterol and β-sitosterol were chromatographed as markers. The sterol bands were detected under UV light, and the zones were scraped off and eluted with dry ether.[13]

Characterization of sterols. Sterols were purified and characterized by co-chromatography with reference sterols in several solvents. They were recrystallized to constant m.p. from $CHCl_3$–methanol. Steryl acetates were prepared by acetylation of sterols with pyridine-acetic anhydride.

The alga was supplied in the dried state (5–6% residual moisture) by the Institut Français du Petróle where it was cultured in synthetic media.[9]

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[9] G. CLÉMENT *et al.*, A new type of food alga. ***Institut Français du Pétrole***. Ref. 14237A. (1967).
[10] R.W. KRAUSS and W. J. MCALEER, *Algal culture (Burlew), 316.* Carnegie Inst. of Washington. Publication 600 (1964).
[11] B. H. DAVIES, ***Chemistry and biochemistry of plant pigments*** (edited by T. W. GOODWIN), p. 489, Academic Press, New York (1965).
[12] D. R. IDLER and C. BAUMANN. ***J. Biol. Chem. 195, 623 (1952).***
[13] W. M. SPERRY, ***J. Lipid Res. 4, 221 (1963).***