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The blue-green algae *Microcystis aeruginosa* and *Gleothricia echinulata,* obtained from blooms in lakes of northwest Iowa, have been shown to contain at the most trace amounts of digitonin-precipitable sterols and show a low level of isotopic incorporation from 1-^{14}C-acetate into these compounds.

INDEX DESCRIPTORS: Algal Sterols, *Microcystis aeruginosa, Gleothricia echinulata*

Although sterols occur in most plants, few reports have described their presence in blue-green algae (Good and Goodwin, 1972; de Souza and Nes, 1968). In the current work an attempt was made to investigate the possible presence of one type of sterol, i.e., those precipitable by digitonin, both by direct bulk isolation and by detection of any radioactive sterols which might have been synthesized by the cells after incubation with 1-^{14}C-acetate. As source material, in lieu of *in vitro* cultures, advantage was taken of the almost pure "blooms" of blue-green algae that occur during late summer in the lakes of northwest Iowa.

MATERIALS AND METHODS

Sample collection

Collections were made in early August. The algae were collected at least 30 feet from the lake shores, from the surface to three feet down, by trailing a plankton net with bottle attached from the rear of a small boat. The algal suspensions, stored in wide-mouth glass jugs, were sampled and incubated *in vitro* within three hours of collection. The samples were examined in a light microscope for identification and evaluation of purity. Of the algae collected, *Anabaena, Aphanizamenon, Microcystis aeruginosa* and *Gleothricia echinulata,* only the latter two were obtained in sufficient purity to be used. These suspensions were apparently 90 percent pure with respect to the type of algae present. Blooms of *Microcystis aeruginosa* were collected from Center Lake and of *Gleothricia echinulata* from Lake Okoboji.

Bulk Extraction

Algal samples not used immediately for incubation experiments were preserved by the addition of sufficient acetic acid to attain a pH of 4.5. Just prior to use they were neutralized with sodium hydroxide and then lyophilized. Fifty grams of the lyophilized residue were powdered in a mortar and extracted for 24-36 hours in a Soxhlet extractor with 500 ml acetone. The extract, a clear gray green, was evaporated to dryness under nitrogen. The residue, a semi-solid waxy dark green sludge, was refluxed under nitrogen with 45 ml of 20 percent KOH in 80 percent ethanol for 24 hours. The dark green solution was mixed with 150 ml water and extracted with 7 portions (80 ml) of peroxide-free ethyl ether. The yellow-colored ether extracts were combined and washed with 40-50 ml portions of water until the aqueous layer was no longer alkaline. After filtration through cotton the ether extract was evaporated to dryness under nitrogen. The residue was extracted with 50 ml of 95 percent ethanol and 50 ml of digitonin solution (0.4 percent in 50 percent ethanol) added. After standing for eight hours, a faint cloudiness appeared in the *Microcystis aeruginosa,* and an even lesser turbidity in the *Gleothricia echinulata* extracts. No further work was done with these systems.

Incubations

Four dilutions of the algal suspensions were made with filtered lake water as follows: undiluted, diluted 3:1, 1:1 and 1:3. Five ml aliquots of each sample were transferred to 10 ml Erlenmeyer flasks. After addition of 30,000 cts/min of 1-^{14}C-Na acetate (spec. act. 1 mCi/mmole) contained in 0.2 ml of lake water, the flasks were incubated at 25°C in a Dubnoff Metabolic Shaker Bath for various times. The pH of the systems was always between 6.5-7.5. In some experiments light was excluded during the incubation. All systems were run in duplicate. At the end of the incubation time, 1 ml of absolute ethanol was added, the flask heated for 30 min at 80-90°C on a hot plate, and the contents transferred to screwtop bottles containing 2 ml of absolute ethanol and then stored.

Saponification, Extraction and Digitonin Precipitation

The contents of each flask were transferred to 125 ml Erlenmeyer flasks to which were added 38 ml absolute ethanol and 10 gms KOH to give a final mixture of 20 percent KOH in 80 percent ethanol. Nitrogen was bubbled into the mixture in a fine continuous stream, a "cold-finger" inserted loosely in the neck of the flask and the system heated for 24 hrs. After hydrolysis the contents of each flask were diluted 1:1 with water and extracted in a separatory funnel with five 10 ml portions of peroxide-free ether. The combined ether extracts were filtered through a cotton plug and then dried by the addition of anhydrous sodium sulfate and filtered. The extract was then evaporated to dryness under a stream of nitrogen, the residue dissolved in 5 ml of 95 percent ethanol transferred to a 15 ml centrifuge tube and 5 ml of a 0.4 percent solution of digitonin in 50 percent ethanol added. After mixing, the system stood for eight hours and was then centrifuged at 2000 x g for one hour. The supernatant fluid was poured off, the trace amount of precipitate

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washed with 1 to 2 ml of ethanol, the washings discarded, and the digitonide pellet suspended in 1 ml of ethanol.

Assay of Radioactivity

The digitonide suspension was poured into a tared planchet, dried in air and then in an oven at 90°C, weighed and finally counted in a Tracerlab flow-gas counter at a 5 percent error level. Self-absorption corrections were applied to the background-corrected counting rates, although in most instances the residue weight was small enough (< 1 mg) for the sample to be considered "infinitely thin." The corrected figure was multiplied by three since sterols constitute 1/3 the mass of their digitonide complexes. The results are reported as cts/min mg sterol.

Results and Discussion

Bulk extraction of lyophilized samples of either *Microcystis aeruginosa* or *Gleothricia echinulata* did not yield significant amounts of digitonin precipitable material, although a trace amount was present in the former alga. To test the effectiveness of the method used, 25 mg of ergosterol was added to another 50 gm sample of dried *Gleothricia echinulata* and the mixture processed as before. Approximately 50 mg of digitonide was recovered, or about 70 percent of added sterol.

TABLE 1. INCORPORATION OF RADIOACTIVITY FORM 1-^{14}C-ACETATE INTO DIGITONIN-PRECIPITABLE FRACTIONS OF IOWA LAKE BLUE-GREEN ALGAE

Organism	Dry Weight of Alga (mg)	Incubation Time (min.)	Sample Dilution	Specific Activity (cts. min^{-1} mg^{-1} sterol)
Microcystis aeruginosa	21	60	0	51
	16	60	3:1	33
	11	60	1:1	15
	5	60	1:3	18
	21	17	0	30
		30	0	24
		60	0	84
		60	0	71
Gleothricia echinulata	19	60	0	9
	14	60	3:1	9
	5	60	1:3	9
	19	15	0	0
		30	0	3
		45	0	5
		60	0	12

The data obtained from incubations of algae with 1-^{14}C-acetate are consistent with the bulk extraction results in that a low level of isotope incorporation is suggested for *Microcystis aeruginosa* but not for *Gleothricia echinulata*. With whole cell incubations, the question of permeability to the acetate ion must be raised although acetate is utilizable by many blue-green algae (Pearce and Carr, 1967). The slightly increased specific activity observed with the more concentrated algal systems (Table 1) may be ascribed to a more complete precipitability of the digitonides of the radioactive compounds when they are present in higher concentration, although this suggestion is speculative. There was apparently a slightly greater isotope incorporation in the light as compared to dark, but the difference is not significant. Since samples were approximately 90 percent unialgal, the trace amounts of digitonides obtained could have been due to contaminating organisms, possibly protozoan, but not bacterial, since the latter reportedly contain no steroids.

It should be emphasized that only those sterols which form relatively insoluble digitonides would be detectable by our procedures, i.e., those containing a 3β-hydroxy group and a $\Delta^{5(6)}$ unsaturation. The sterol intermediate, cycloartenol, would, for example, not be expected to precipitate, although most of the final sterol products such as fucosterol or ergosterol would be detected.

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