*Phytochemistry*, Vol. 27, No. 6, pp. 1735–1740, 1988.
Printed in Great Britain.

0031-9422/88 \$3.00 + 0.00


# SATURATED AND UNSATURATED STEROLS OF NITROGEN-FIXING BLUE-GREEN ALGAE (CYANOBACTERIA)

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(*Received* 20 *July* 1987.)

**Key Word Index**—blue-green algae; nitrogen-fixing blue-green algae; sterols; cyanobacteria.

**Abstract**—Five species of filamentous nitrogen-fixing blue-green algae (Cyanobacteria), (*Anabaena cylindrica, Anabaena solitaria, Anabaena viguieri, Nostoc carneum, Nodularia harveyana*) were grown in a freshwater medium containing 10% seawater (DS medium). The sterols were analysed by means of a newly developed procedure involving precipitation with digitonine, GC and GC/MS but not TLC and CC. All species were found to synthesize a great variety of sterols (11–15 compounds). The digitonin precipitable sterols made up *ca* 0.005–0.03% of the dried algal biomass. Remarkably, each species produced the saturated sterols, 5α-cholestan-3β-ol, 24-methyl-5 α-cholestan-3β-ol and 24-ethyl-5 α-cholestan-3β-ol. The latter was the main sterol in all organisms. Furthermore, the investigated microalgae were found to synthesize known $C_{27}$, $C_{28}$ and $C_{29}$ sterols.

## INTRODUCTION

In 1968, the presence of sterols in blue-green algae was reported for the first time. Before this discovery these organisms were believed not to produce sterols [1]. In that year, Reitz and Hamilton [2] found sitosterol and cholesterol in *Anacystis nidulans* and *Fremyella diplosiphon*, and De Souza and Nes [3] detected seven unsaturated sterols in *Phormidium luridum*. Thereafter, only a few papers have been published on blue-green algal sterols [4–9].

In a research programme on the axenic mass culture of microalgae carried out in our laboratory, blue-green algae (Cyanobacteria) and green algae are being investigated for pharmaceutically relevant constituents [10–13]. This publication reports on the isolation and identification of sterols in the nitrogen-fixing blue-green algae, *Anabaena cylindrica, Anabaena solitaria, Anabaena viguieri, Nostoc carneum* and *Nodularia harveyana*.

## RESULTS AND DISCUSSION

The blue-green algae used in these studies were grown in a recently described fresh-water medium (DS medium) consisting basically of 90% demineralized water and 10% seawater [14]. There were no detectable contaminations by other organisms in the cultures. The sterols were isolated from the dried blue-green algal biomass by means of a newly developed procedure involving isolation of the total lipids, liquid/liquid extraction of the saponified total lipids with petrol, precipitation of the sterols with digitonin, GC and GC/MS analyses. Sterols were identified by comparison of their $RR_t$s and of their mass spectra with the data of free and silylated reference compounds published by other authors [15–21].

To avoid loss of sterols TLC and CC were not performed during the isolation procedure. The biomass (dry weight), the amounts of total lipids and of the digitonin precipitable sterols of the blue-green algae investigated are shown in Table 1. The sterols composed *ca* 0.005–0.03% of the biomass. The sterol compositions of the individual organisms are shown in Table 2.

The blue-green algae investigated here contained 17 sterols, 13 of which could be identified. Two compounds were determined tentatively. *A. solitaria* possessed all 17 sterol compounds. The organism contained mainly 24-ethylcholestan-3β-ol (45.5% of the sterols), 24-ethylcholest-5-en-3β-ol (33%) and cholesterol (7.8%). Except for a rather high content of 24-ethylcholest-5,22-dien-3β-ol (13%), *A. cylindrica* resembled *A. solitaria* in sterol composition. *A. viguieri* had a sterol composition similar to that of *A. cylindrica* but it was characterized by a lower content of 24-ethylcholestan-3β-ol (30.6%) and larger amounts of 24-ethylcholesta-5,22-dien-3β-ol (28.5%). *Nodularia harveyana* and *Nostoc carneum* resembled one another in sterol composition. 70–80% of their sterols was attributable to 24-ethylcholestan-3β-ol and 24-ethylcholest-5-en-3β-ol.

Ergosterol (or its C-24α-epimer), which is unsaturated and to our knowledge has not been reported to occur in blue-green algae, was found in low quantities in three of the organisms (*A. viguieri, A. solitaria, N. harveyana*).

In addition, all five blue-green algae appeared to contain two $C_{30}$ sterols (nos 15 and 16, see Table 2). According to the mass spectral fragmentation pattern they were 4 α-methyl-sterols. Presumably, one of them (no. 15) was 4α-methyl-24-ethylcholest-8(14)-en-3β-ol, which has already been reported by Kokke *et al.* [22] to occur in three marine dinoflagellates. Two sterols remain unidentified (nos 13 and 17).

The above sterols were precipitated by digitonin. It should be noted that this precipitation does not include 3α-and 5β-sterols. To our knowledge, however, these compounds have so far not been found in blue-green algae which of course does not exclude their existence in these organisms.

The sterols of blue-green algae have been the subject of only few investigations [1–9]. Prior to this study, the

Table 1. Biomass, total lipids and digitonin precipitable sterols of nitrogen-fixing blue-green algae grown in 8 l. batch cultures (44 days)

| Blue-green algae | Biomass (mg) | Total lipids (mg) | Total lipids % of biomass | Digitonin precipitable sterols (mg) | Digitonin precipitable sterols % of biomass |
|---|---|---|---|---|---|
| *A. cylindrica* | 4728 | 986 | 20.8 | 1.087 | 0.023 |
| *A. viguieri* | 5200 | 1014 | 19.5 | 0.520 | 0.010 |
| *A. solitaria* | 2744 | 583 | 21.2 | 0.384 | 0.014 |
| *Nostoc carneum* | 4376 | 950 | 21.7 | 1.313 | 0.030 |
| *Nodularia harveyana* | 3912 | 743 | 19.0 | 0.195 | 0.005 |

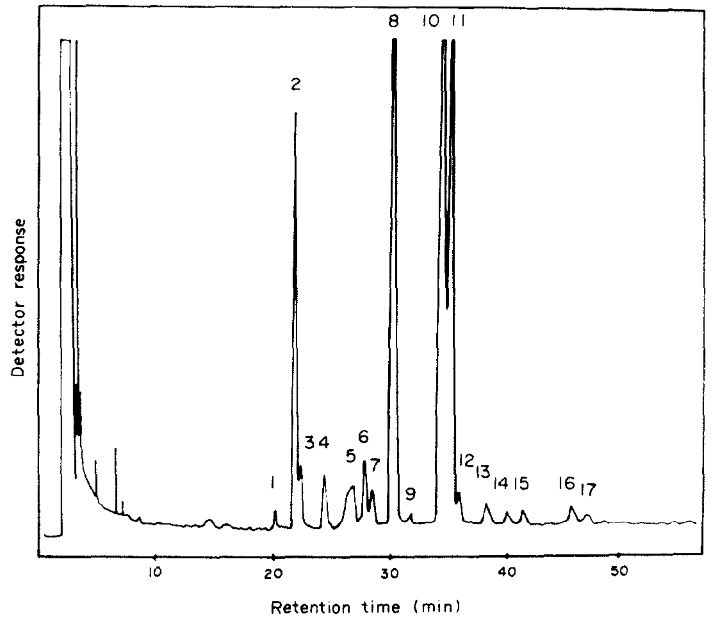


Fig. 1. GLC of the total sterols of *Anabaena viguieri* (see names of the compounds in Table 2).

following compounds have been reported, all of which are unsaturated: cholesterol, chondrillasterol, stigmasterol, sitosterol, brassicasterol, campesterol, 22-dehydrocholesterol, isofucosterol, 24-ethyl-cholest-7-enol, 24-methyl-cholest-7-enol, 24-ethylcholesta-2,5-dienol and 24-ethylcholesta-5,7,22-trienol. In the above cited studies of other authors, the individual blue-green algae were shown to contain up to ten sterols. Our investigations reveal that under the growth conditions employed in our laboratory blue-green algae are able to synthesize 13–17 sterols among them ergosterol (or its C-24α-epimer) and the above mentioned 4α-methylsterols. Most remarkably, the blue-green algae investigated by us were also found to produce saturated sterols, i.e., 5α-cholestan-3β-ol, 24-methyl-5α-cholestan-3β-ol and 24-ethyl-5α-cholestan-3β-ol. The last sterol predominated in all of the organisms.

A similar sterol composition including saturated sterols has been reported for a blue-green alga, *Microcystis aeruginosa* [23]. Saturated sterols have also been found in various marine Dinophyceae [24–32], Rhodophyceae [33] and Bacillariophyceae [34–36]. The Dinophyceae synthesized various 4α-methylstanols and 4α-dimethylstanols, the Rhodophyceae cholestan-3β-ol, 24-methylenecholestan-3β-ol and 24-methylcholestan-3β-ol, and the Bacillariophyceae small amounts of 5α-stanols. Ballantine [37] reported a high stanol content (50% of the total sterols) in the marine brown alga, *Monochrysis lutheri*, when it was analysed during the stationary phase; stanols were not observed during the exponential growth phase.

Ergosterol (or its C-24α epimer), which was detected in small quantities in three of the blue-green algae investigated by us, has so far been found in only a few algae. It is a major sterol of some *Chlorella* species (Chlorophyceae) [38]. Anding and Ourisson [39] reported a high content of ergosterol (73%) in dark-grown *Euglena gracilis*. Two Prophyridium species (Rhodophyceae) have also been reported to produce ergosterol [40, 41]. The so-called blue-green coloured Cyanophyte *Cyanidium*

Table 2. Digitonin precipitable sterols in $N_2$-fixing blue-green algae (% of sterols)

| Peak number | $RR_t$–FS* | $RR_t$–SS* | Sterol | *Anabaena solitaria* | *Anabaena cylindrica* | *Anabaena viguieri* | *Nodularia harveyana* | *Nostoc carneum* |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.92 | 0.92 | Cholesta-5,22-dien-3β-ol (22-dehydrocholesterol) | trace | — | 0.2 | 0.1 | trace |
| 2 | 1.00 | 1.00 | Cholest-5-en-3β-ol (cholesterol) | 7.8 | 3.4 | 7.8 | 6.3 | 7.4 |
| 3 | 1.02 | 1.03 | Cholestan-3β-ol | 0.1 | 0.3 | 1.2 | 0.9 | 1.5 |
| 4 | 1.11 | 1.10 | 24-Methylcholesta-5,22-dien-3β-ol(brassicasterol) | 1.5 | 1.2 | 1.1 | 0.8 | 2.8 |
| 5 | 1.22 | 1.23 | 24-Methylcholesta-5,7,22-trien-3β-ol (ergosterol or 24α-epimer) | 0.2 | — | 1.2 | 0.2 | — |
| 6 | 1.27 | 1.27 | 24-Methylcholest-5-en-3β-ol (campesterol or (24*S*)-24-ergost-5-en-3β-ol) | 0.8 | 0.5 | 1.9 | 1.7 | 0.8 |
| 7 | 1.29 | 1.30 | 24-Methylcholestan-3β-ol (campestanol or (24*S*)-24-ergostan-3β-ol) | 1.1 | 1.2 | 0.3 | 1.9 | 0.8 |
| 8 | 1.37 | 1.36 | 24-Ethylcholesta-5,22-dien-3β-ol (stigmasterol or poriferasterol) | 0.6 | 13.0 | 28.5 | 1.5 | 1.0 |
| 9 | 1.48 | 1.47 | 24-Ethylcholesta-5,7,22-trien-3β-ol | 2.8 | — | 0.1 | 1.5 | 0.2 |
| 10 | 1.57 | 1.55 | 24-Ethylcholest-5-en-3β-ol (sitosterol or clionasterol) | 33.0 | 29.9 | 23.9 | 25.2 | 27.8 |
| 11 | 1.60 | 1.59 | 24-Ethylcholestan-3β-ol (stigmastanol or 5,6-dihydroclionasterol) | 45.5 | 44.7 | 30.6 | 44.9 | 46.9 |
| 12 | 1.63 | 1.64 | 24-Ethylcholesta-5,24(28)-dien-3β-ol (isofucosterol) | 1.4 | — | 0.7 | 1.9 | 0.7 |
| 13 | 1.74 | 1.71 | Unidentified | 0.3 | 0.5 | 0.6 | — | — |
| 14 | 1.76 | 1.73 | 24-Ethylcholest-7-en-3β-ol (stigmast-7-en-3β-ol or 22-dihydrochondrillasterol) | 0.5 | 0.2 | 0.5 | 3.3 | 1.3 |
| 15 | 1.82 | 1.77 | 4α-Methyl-24-ethylcholest-8(14)-en-3β-ol† | 0.5 | 0.7 | 0.5 | 1.7 | 0.6 |
| 16 | 2.09 | 2.02 | 4α-Methyl-24-ethyl-cholestan-3β-ol‡ | 0.8 | 0.9 | 0.5 | 1.8 | 0.6 |
| 17 | 2.13 | 2.06 | Unidentified | 0.4 | — | 0.4 | 3.4 | 4.7 |
| others | | | | 2.7 | 3.5 | — | — | 2.9 |

*$RR_t$–FS: Retention times (GLC) of free sterols relative to cholesterol (1.00). *$RR_t$–SS: Retention times (GLC) of trimethylsilylated sterols relative to cholesterol trimethylsilylether (1.00); both on $SE_{54}$ capillary column.

†Tentative; characteristic ions for a $\Delta^{8(9)}$ or $\Delta^{8(14)}$ double bond are $m/z$ 260 and $m/z$ 261. The fragmentation pattern is in full agreement with that of 4α-methyl-24-ethylcholest-8(14)-en-3β-ol found in *Glenodinium* sp. [22].

‡Tentative.

Table 3.

| Peak | |
|---|---|
| 1 | 22-*trans*-5, 22-Cholesta-dien-3β-ol-TMS.MS *m/z* (rel. int.): 456 [M]$^+$ (15), 441 (17), 351 (6), 327 (28), 282 (6), 255 (15), 253 (8), 251 (30), 207 (18), 141 (30), 129 (35), 111 (60), 83 (66), 81 (59), 69 (100), 55 (100)<br>free sterol: 384 [M]$^+$ (46), 369 (8), 366 (10), 351 (9), 301 (13), 300 (54), 285 (12), 273 (22), 271 (26), 255 (62), 253 (15), 213 (17), 207 (18), 111 (55), 81 (75), 69 (100) 55 (100) |
| 2 | 5 α-Cholesten-3β-ol-TMS.MS *m/z* (rel. int.): 458 [M]$^+$ (40), 443 (10), 368 (69), 353 (28), 329 (78), 301 (6), 275 (9), 255 (20), 247 (19), 229 (4), 213 (13)<br>free sterol: 386 [M]$^+$ (100), 371 (29), 368 (45), 353 (32), 301 (48), 275 (55), 255 (28), 213 (34) |
| 3 | 5α-Cholestan-3β-ol-TMS.MS *m/z* (rel. int.): 460 [M]$^+$ (7), 454 (7), 452 (15), 445 (7), 370 (84), 355 (6), 306 (4), 257 (13), 230 (19), 229 (81), 217 (5), 215 (10), 57 (100),<br>free sterol: 388 [M]$^+$ (95), 373 (42), 355 (15), 349 (7), 331 (6), 301 (4), 262 (17), 257 (5), 248 (15), 234 (76), 233 (100), 217 (34), 216 (43), 215 (89), 165 (44) |
| 4 | 24-Methyl-cholesta-5, 22-dien-3β-ol-TMS.MS *m/z* (rel. int.): 470 [M]$^+$ (25), 471 (4), 381 (40), 366 (10), 341 (14), 253 (32), 251 (12), 215 (6), 213 (10), 207 (6), 69 (100).<br>free sterol: 398 [M]$^+$ (82), 383 (10), 380 (12), 365 (13), 355 (7), 337 (15), 314 (10), 313 (9), 300 (49), 285 (10), 271 (50), 255 (66), 213 (22) |
| 5 | Ergosta-5, 7, 22-trien-3β-ol-TMS.MS *m/z* (rel. int.): 468 [M]$^+$ (16), 378 (25), 376 (20), 363 (48), 362 (24), 337 (23), 253 (67), 251 (42), 237 (26), 211 (27), 169 (28), 157 (37), 143 (34), 69 (100), 55 (100),<br>free sterol: 396[M]$^+$ (93), 397 (28), 378 (10), 364 (28), 363 (100), 338 (13), 337 (31), 271 (25), 253 (52), 251 (20), 213 (15), 211 (25), 157 (38), 143 (37), 69 (70), 55 (50). |
| 6 | 24-Methyl-cholest-5-en-3β-ol-TMS.MS *m/z* (rel. int.): 472 [M]$^+$ (29), 457 (11), 383 (22), 382 (65), 378 (25), 343 (72), 342 (14), 315 (5), 289 (6), 261 (14), 129 (100)<br>free sterol: 400 [M]$^+$ (100), 385 (26), 382 (46), 367 (33), 315 (53), 289 (42), 273 (25), 255 (32), 213 (36) |
| 7 | 24-Methyl-cholestan-3β-ol-TMS.MS *m/z* (rel. int.): 474 [M]$^+$ (11), 475 (5), 459 (14), 417 (5), 384 (6), 369 (7), 305 (8), 300 (7), 281 (8), 257 (4), 230 (7), 217 (12), 216 (15), 215 (25)<br>free sterol: 402 [M]$^+$ (58), 400 (26), 387 (37), 396 (12), 363 (10), 345 (4), 315 (6), 276 (14), 257 (4), 235 (19), 234 (69), 233 (100), 217 (35), 216 (24), 215 (92), 207 (15), 165 (41) |
| 8 | 24-Ethylcholesta-5,22-dien-3β-ol-TMS.MS *m/z* (rel. int.): 484 [M]$^+$ (28), 485 (11), 470 (5), 469 (5), 395 (10), 394 (33), 379 (11), 355 (12), 351 (14), 256 (7), 255 (34), 253 (11), 213 (11), 129 (47), 83 (100),<br>free sterol: 412 [M]$^+$ (100), 413 (33), 397 (8), 394 (9), 379 (11), 369 (18), 365 (8), 351 (30), 301 (15), 300 (43), 272 (29), 271 (54), 255 (66), 213 (25), 83 (84), 55 (100) |
| 9 | 24-Ethylcholesta-5,7,22-trien-3β-ol-TMS.MS *m/z* (rel. int.): 482 [M]$^+$ (36), 394 (17), 392 (25), 378 (20), 377 (93), 352 (14), 351 (50), 343 (5), 281 (4), 271 (4), 253 (35), 213 (15), 211 (25), 129 (52), 55 (100) |
| 10 | 24-Ethylcholest-5-en-3β-ol-TMS.MS *m/z* (rel. int.): 486 [M]$^+$ (43), 489 (19), 471 (12), 397 (25), 381 (28), 357 (75), 356 (19), 329 (6), 297 (11), 275 (18), 255 (23), 129 (100)<br>free sterol: 414 [M]$^+$ (100), 399 (21), 396 (22), 381 (18), 329 (26), 273 (19), 255 (14), 303 (25), 231 (22), 229 (35), 213 (25) |
| 11 | 24-ethylcholestan-3β-ol-TMS.MS *m/z* (rel. int.): 488 [M]$^+$ (74), 489 (30), 474 (23), 473 (59), 431 (14), 398 (35), 384 (37), 383 (12), 306 (27), 305 (31), 290 (12), 257 (8), 231 (18), 230 (22), 217 (46), 216 (60), 215 (98), 75 (100)<br>free sterol: 416 [M]$^+$ (100), 417 (34), 401 (33), 383 (15), 359 (6), 313 (6), 290 (13), 248 (12), 234 (63), 233 (86), 217 (28), 216 (35), 215 (78), 165 (35) |
| 12 | 24-Ethylcholesta-5, 24(28)-dien-3β-ol-TMS.MS *m/z* (rel. int.): 484 [M]$^+$ (16), 386 (100), 379 (11), 371 (12), 355 (11), 253 (10), 296 (71), 281 (38), 257 (23), 255 (11), 213 (11), 211 (13), 159 (21), 129 (70)<br>free sterol: 412 [M]$^+$ (63), 397 (20), 394 (8), 379 (21), 314 (17), 299 (5), 296 (2), 281 (8), 271 (40)(with concomitant peaks of stigmastanol) |
| 14 | 24-Ethylcholest-7-en-3β-ol-TMS.MS *m/z* (rel. int.): 486 [M]$^+$ (100), 488 (73) 472 (11), 471 (25), 396 (12), 381 (21), 345 (12), 303 (7), 255 (97), 256 (21), 229 (36), 213 (45)<br>free sterol: 414 [M]$^+$ (100), 399 (25), 381 (6), 273 (18), 255 (54), 254 (12), 246 (8), 231 (17), 229 (15), 213 (17), 147 (14), 119 (13), 107 (19) |
| 15 | 4α-Methyl-24-ethylcholest-8(14)-en-3β-ol(tentative) free sterol: 428 [M]$^+$ (100), 413 (25), 410 (10), 395 (10), 287 (16), 269 (7), 261 (5), 260 (6), 245 (12), 243 (14), 227 (16) |
| 16 | 4α-Methyl-24-ethyl = $C_{30}$ sterol (tentative) free sterol: 430 [M]$^+$ (54), 412 (100), 397 (52), 394 (43), 383 (24), 289 (21), 275 (26), 271 (24), 253 (15), 247 (21), 243 (14), 229 (31), 211 (29), 149 (47), 135 (73) 95 (100) |

*caldarium* synthesizes cholesterol, ergosterol, campesterol, sitosterol, 5,6-dehydroergosterol and 7-dehydrositosterol [6] and thus resembles the blue-green algae investigated by us. 22-Dehydrocholesterol has also been found in the Prochlorophyte *Prochloron* [9], in several Rhodophyceae [33, 42–45] and Bacillariophyceae [46].

With few exceptions the chirality at C-24 of blue-green algal sterols has not been specified in publications to date. In contrast, green algal sterols are reported to have the 24*S*-configuration [47–49]. Investigations are now being made in our laboratory to determine the C-24 chirality for the blue-green algal sterols.

Open questions still remain with regard to the saturated sterols (stanols) described here. With the exception of one report (*Microcystis aeruginosa*) [23] investigations by other authors do not reveal the presence of saturated sterols in blue-green algae [1–9]. On the other hand, saturated sterols have been found in several marine algae [24–33]. For example, *Monochrysis lutheri* (marine Chrysophyceae) has been reported to synthesize saturated sterols during the stationary but not during the exponential growth phase [37]. Recently, Orcutt *et al.* [50] have described the occurrence of several sterols (nos 1–4, 6–8, 10, 11 in Table 2) in antarctic 'blue-green algal—(diatomaceous)—microbial mats and cores' without, however, attributing the sterols to individual species.

We do not know at present whether the formation of saturated sterols reported here was influenced by the age of the cultures and/or by the medium employed in our studies. The latter is composed of 90% demineralized water and 10% seawater with trace elements and phosphate added [14]. Despite the seawater component it is fresh-water-like because of its low total salt concentrations. Our control experiments showed that the sterols were not derived from the seawater which is reported to contain low quantities of sterols [51, 52]. Furthermore, the blue-green algae investigated here were grown to the stationary growth phase (44 days). We are presently investigating whether certain ions of the seawater and the age of the cultures have an influence on sterol formation of the blue-green algae.

## EXPERIMENTAL

*Blue-green algae. Anabaena cylindrica* Lemmermann B 1611 was obtained from the Collection of Algae at Indiana University, Bloomington, USA. *Anabaena viguieri* (Denis) Frémy, *Nostoc carneum* (Lyngbye) Agardh ex Bornet & Flahault and *Nodularia harveyana* (Thwaites) Thuret were received as gifts from the Max-Planck-Institut für Limnologie, Plön. *Anabaena solitaria* Klebahn was isolated from an urban fountain.

The organisms were grown at 23° under axenic conditions and continuous aeration in 10 l flasks containing 8 l of a newly developed inorganic medium (DS medium) consisting mainly of 90% demineralized water and 10% seawater with added phosphate and trace elements. [14]. The cultures were illuminated with two fluorescent tubes Philips TL 65 W/25 (white) and one fluorescent tube Osram L 58 W/77 Fluora (red) at a photon fluence rate of 17–20 $\mu mol/s/m^2$. The algae were harvested by centrifugation and immediately freeze-dried. The biomass ranged from 350 mg to 650 mg (dry wt) per l.

*Isolation of the sterols.* The freeze-dried biomass (2–5 g) was extracted in a Soxhlet apparatus with $CHCl_3$–MeOH (2:1) for 12 hr. After removal of the solvent the remaining total lipids were weighed and then saponified with 3 ml of 8% KOH in MeOH–$H_2O$ (2+1) for 3 hr. The unsaponifiable fraction was obtained by liquid–liquid extraction with petrol for 12 hr. The petrol extract was evapd to dryness and the residue dissolved in 15 ml of $Me_2CO$, 5 ml of a digitonin soln (1% in 60% EtOH) were added and the mixture kept at 70° for 20 min. The flasks were then stored at 4° over night. The red coloured soln with the digitonide ppt. was filtered through a glass microfibre filter (Whatman GF/C; 5.5 cm diameter) and washed thoroughly with $Me_2CO$ (50 ml) and $Et_2O$ (50 ml). The filter with the digitonides was cut into small pieces and immersed in 5 ml of DMSO; the soln was heated to 100° for 10 min on a water-bath according to ref. [53]. After cooling to room temp. 5 ml of hexane were added and the mixture shaken on a whirly-mix for 3 min. The supernatant was transferred to a test tube. This extraction procedure was repeated twice. The combined hexane extracts were evapd to dryness under a stream of $N_2$. The residue was dissolved in 0.1 ml THF and submitted directly to GLC.

*Silylation of sterols.* The sterols were dissolved in 10 $\mu l$ THF After addition of 20 $\mu l$ MSFBA (*N*-methyl-*N*-trimethylsilyl-heptafluoro-butyramide) they were heated to 60° for 10 min.

The above procedure allows the analysis of digitonin pptble sterols derived from as little as 500 mg of blue-green algal biomass without using TLC and/or CC. According to our experiments the use of TLC and CC leads to a loss of sterols due to adsorption of these compounds on the silica gel.

*Gas–liquid-chromatography*: GLC was carried out with a WCOT fused silica capillary columm (25 m/0,22 mm) coated with SE-54. Columm temp.: 250°. $RR_t$s of the free sterols and of their trimethylsilylethers were expressed against cholesterol or its trimethylsilylether respectively (see Table 2). Areas below the peaks of individual sterol were calculated with a Shimadzu C-R3A-integrator. Detector: FID.

*Gas-liquid-chromatography/mass spectrometry*: This was carried out with a WCOT fused silica capillary columm (20 m/0, 22 m) coated wih SE-54. Columm temp.: 250°C. Mass spectrometry: Finnigan MAT 8230.

*Acknowledgements*—The authors express their thanks to Elmar Schneider (Institut für Organische Chemie, Kiel) for providing the mass spectra, to Dr G. Remberg (Göttingen) for identification of some of the sterol mass spectra, to Thomas Noji and Dr W. Eichenberger for reading and correcting the manuscript.

## REFERENCES

1. Levin, E. Y. and Bloch, K. (1964) *Nature* **202**, 90.
2. Reitz, R. C. and Hamilton, J. G. (1968) *Comp. Biochem. Physiol.* **25**, 401.
3. De Souza, N. J. and Nes, W. R. (1968) *Science* **162**, 3636.
4. Nadal, N. G. M. (1971) *Phytochemistry* **10**, 2537.
5. Teshima, S. and Kanazawa, A. (1972) *Bull. Soc. Sci. Fish.* **38**, 1197.
6. Seckbach, J. and Ikan, R. (1972) *Plant Physiol.* **49**, 457.
7. Forin, M.-C., Maume, B. and Baron, C. (1972) *C. R. Acad. Sci. Ser. D*, 133.
8. Paoletti, C., Pushparaj, B., Florenzano, G., Capella, P. and Lercker, G. (1976) *Lipids* **11**, 266.
9. Perry, J., Gillan, F. T. and Johns, R. B. (1978) *J. Phycol.* **14**, 371.
10. Piorreck, M., Baasch, K.-H. and Pohl, P. (1984) *Phytochem istry* **23**, 207.
11. Piorreck, M. and Pohl, P. (1984) *Phytochemistry* **23**, 217.
12. Pohl, P., Kohlhase, M. and Martin, M. (1986) *Chem. Ind.* **109**, 529.
13. Pohl, P. and Kohlhase, M. (1986) *Planta Med.* **5**, 417.
14. Pohl, P., Kohlhase, M., Krautwurst, S. and Baasch, K.-H. (1987) *Phytochemistry* **26**, 1657.
15. Patterson, G. W. (1971) *Anal. Chem.* **43**, 1165.
16. Itoh, T., Tani, H., Fukushima, K., Tamura, T. and Matsumoto, T. (1982) *J. Chromatography* **65**, 234.
17. Budzikiewicz, H., Djerassi, C. and Williams, D. H. (1964) *Structure Elucidation of Natural Products by Mass Spectrometry*, Vols I and II. Holden Day, San Francisco.
18. Knights, B. A. (1967) *J. Gas Chromatogr.* **5**, 273.
19. Brooks, C. J. W., Horning, E. C. and Youngs, J. S. (1968) *Lipids* **3**, 391.
20. Brooks, C. J. W., Henderson, W. and Steel, G. (1973) *Biochem. Biophys. Acta* **296**, 431.

21. Ballantine, J. A. and Roberts, J. C. (1975) *J. Chromatogr.* **103**, 289.
22. Kokke, W. C. M. C., Fenical, W. and Djerassi, C. (1981) *Phytochemistry* **20**, 127.
23. Nishimura, M. and Koyama, T. (1977) *Geochim. Cosmochim. Acta* **41**, 379.
24. Withers, N. W., Tuttle, R. C., Holz, G. G., Beach, D. H., Goad, L. J. and Goodwin, T. W. (1978) *Phytochemistry* **17**, 1987.
25. Alam, M., Sansing, T. B., Busby, E. L., Martinez, D. R. and Ray, S. M. (1979) *Steroids* **33**, 197.
26. Alam, M., Martin, G. E. and Ray, S. M. (1979) *J. Org. Chem.* **44**, 4466.
27. Withers, N. W., Kokke, W. C. M. C., Rohmer, M., Fenical, W. H. and Djerassi, C. (1979) *Tetrahedron Letters* **38**, 3605.
28. Alam, M., Sansing, T. B., Guera, J. R. and Harmon, A. D. (1981) *Steroids* **38**, 375.
29. Kokke, W. C. M. C., Fenical, W. and Djerassi, C. (1982) *Steroids* **40**, 307.
30. Alam, M. and Sanduja, R. (1984) *J. Phycol.* **20**, 331.
31. Volkmann, J. K., Gagosian, R. B. and Wakeham, S. G. (1984) *Lipids* **6**, 457.
32. Nichols, P. D., Jones, G. J., De Leeuw, J. W. and Johns, R. B. (1984) *Phytochemistry* **5**, 1043.
33. Chardon-Loriaux, Morisaki, M. and Ikekawa, N. (1976) *Phytochemistry* **15**, 723.
34. Nishimura, M. and Koyama, T. (1977) *Geochim. Cosmochim. Acta* **41**, 379.
35. Wardroper, A. M. K. and Maxwell, J. R. (1978) *Steroids* **32**, 203.
36. Volkmann, J. K., Gillan, F. T. and Johns, R. B. (1981) *Geochim. Cosmochim. Acta* **45**, 1817.
37. Ballantine, J. A., Lavis, A. and Morris, R. J. (1979) *Phytochemistry* **18**, 1459.
38. Patterson, G. W. (1982) in *CRS Handbook of Biosolar Resources* Vol. I, Part 1 (Mitsui, A. and Black, C. C. eds), p. 433. CRC Press, Boca Raton, Florida.
39. Anding, C. and Ourisson, G. (1973) *Eur. J. Biochem.* **34**, 345.
40. Beastall, G. H., Rees, H. H. and Goodwin, T. W. (1971) *Tetrahedron Letters* **52**, 4935.
41. Beastall, G. H., Tyndall, M., Rees, H. H. and Goodwin, T. W. (1974) *Eur. J. Biochem.* **41**, 301.
42. Tsuda, K., Sakai, K., Tanabe, K. and Kishida, Y. (1960) *J. Am. Chem. Soc.* **82**, 1442.
43. Meunier, H., Zelenski, S. and Warthen, L., (1969) in *Proc. Food-Drugs* from the Sea (Youngken, H. W., Jr, ed.) Vol. 319. Marine Technology Society, Washington, D. C.
44. Cargile, N. L., Edwards, H. N. and Mc Chesney, J. D. (1975) *J. Physiol.* **11**, 457.
45. Fattorusso, E., Magno, S., Santacroce, C., Sica, D., Impellizzeri, G., Mangiofico, S., Piatelli, M. and Scinto, S. (1976) *Biochem. Syst.* **4**, 135.
46. Patterson, C. W. (1971) *Lipids* **6**, 120.
47. Orcutt, D. M. and Patterson, G. W. (1975) *Comp. Biochem. Physiol.* **50B**, 579.
48. Nes, W. R. (1977) *Adv. Lipid Res.* **15**, 233.
49. Goodwin, T. W. (1979) *Annu. Rev. Plant Physiol.* **30**, 369.
50. Orcutt, D. M., Parker, B. C. and Lusby, W. R. (1986) *J. Phycol.* **22**, 523.
51. Saliot, A. and Barbier, M. (1973) *Deep-Sea Res.* **20**, 1077.
52. Gagosian, R. B. and Heinzer, F. V. (1978) *Geochim. Cosmochim. Acta* **43**, 47.
53. Issidorides, C. H., Kitagawa, J. and Moseltig, E. (1962) *J. Org. Chem.* **27**, 4693.