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THE IDENTIFICATION OF 28-ISOFUCOSTEROL IN THE MARINE GREEN ALGAE *ENTEROMORPHA INTESTINALIS* AND *ULVA LACTUCA*

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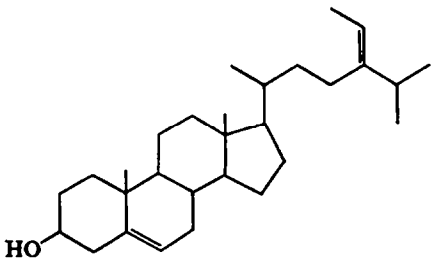
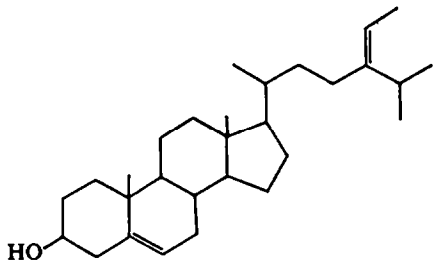
Abstract—The major sterol of *Enteromorpha intestinalis* and *Ulva lactuca* has been identified as 28-isofucosterol. In addition, gas–liquid chromatography has indicated the presence of cycloartenol and 24-methylene cycloartanol in both algae and 24-ethylidene lophenol and 24-methylene lophenol in *E. intestinalis*.

INTRODUCTION

DURING early studies on the sterols of marine algae, Heilbron and co-workers[1–3] reported that the green algae *Enteromorpha compressa* and *Ulva lactuca* contained sitosterol. More recently the sterol from *E. linza* was isolated[4] and found to be identical to Δ^5-avenasterol previously obtained from oat seeds[5] but which had not been assigned a definite structure. Evidence now available[6] has demonstrated that Δ^5-avenasterol is 28-isofucosterol† which had previously been synthesized.[7] 28-Isofucosterol (I) is the isomer of fucosterol (II), the typical sterol of the marine brown algae (Phaeophyceae).[2,3,8] Therefore we considered it would be of both biogenetic and taxonomic interest to reinvestigate the major sterols found



I 28-Isofucosterol



II Fucosterol

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† Professor E. Lederer has pointed out to us that this compound is more correctly named 28-isofucosterol rather than the usual 29-isofucosterol.

[1] I. M. HEILBRON, E. G. PARRY and R. F. PHIPERS, *Biochem. J.* **29**, 1376 (1935).
[2] P. W. CARTER, I. M. HEILBRON and B. LYTHGOE, *Proc. Roy. Soc.* B **128**, 82 (1939).
[3] I. M. HEILBRON, *J. Chem. Soc.* 79 (1942).
[4] K. TSUDA and K. SAKAI *Chem. Pharm. Bull. Tokyo* **8**, 554 (1960).
[5] D. R. IDLER, S. W. NICKSIC, D. R. JOHNSON, V. W. MELOCHE, H. A. SCHUETTE and C. A. BAUMANN, *J. Am. Chem. Soc.* **75**, 1712 (1953).
[6] B. A. KNIGHTS, *Phytochem.* **4**, 857 (1965).
[7] J. P. DUSZA, *J. Org. Chem.* **25**, 93 (1960).
[8] K. TSUDA, S. AKAGI, Y. KISHIDA, R. HAYATSU and K. SAKUI, *Chem. Pharm. Bull. Tokyo* **6**, 724 (1958).

in marine green algae belonging to the Chlorophyceae. The present communication reports the identification of 28-isofucosterol in *E. intestinalis* and *U. lactuca*.

RESULTS

The non-saponifiable lipids were extracted from batches of carefully selected *Enteromorpha intestinalis* and *Ulva lactuca* and the sterols isolated by digitonin precipitation followed by chromatography on alumina. The Liebermann–Burchard reaction gave the typical slow reacting green colour of a Δ^5 sterol.[9] Gas–liquid chromatography (GLC) of the sterols using QF-1 or SE-30 as stationary phase showed a single compound with a retention time identical to that of β-sitosterol or fucosterol, which do not separate on these columns. GLC on hexadimethanol succinate (Hi-EFF 8B) showed that the sterol from both algae was the same but that it had a slightly longer retention time than either β-sitosterol or fucosterol which are separated by this stationary phase. This observation indicated that the sterol might be 28-isofucosterol which has been reported to have a longer retention time than

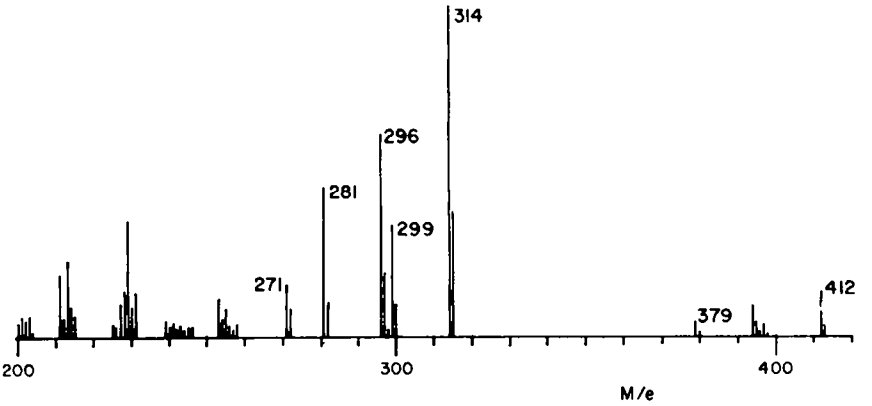


FIG. 1. MASS SPECTRUM OF THE STEROL ISOLATED FROM *Enteromorpha intestinalis*.

fucosterol on this stationary phase.[6] Moreover the melting points of the algal sterols (133·5°) and their acetates (132°) were in good agreement with the reported values for synthetic 28-isofucosterol[7] and Δ^5-avenasterol.[5]

Mass spectrometry of both algal sterols gave spectra (Fig. 1) similar to that of fucosterol[10] with a parent ion at *m/e* 412 and fragmentation ions at *m/e* 397 [M^+-CH_3]; 394 [M^+-H_2O]; 379 [M^+-(CH_3+H_2O]; 314 [M^+-part of side chain (C_7H_{14})]; 299 [M^+-(C_7H_{14}+CH_3)]; 296 [M^+-(C_7H_{14}+H_2O)]; 281 [M^+-(C_7H_{14}+CH_3+H_2O)] and 271 [M^+-(side chain+2H)]. The loss of part of the side chain (C_7H_{14}) is characteristic of sterols with a $\Delta^{24(28)}$ bond.[10,10a]

The i.r. spectrum of the algal sterol resembled that of fucosterol with peaks at 840 cm^{-1} and 800 cm^{-1} for the Δ^5 bond (Fig. 2). The i.r. spectrum of fucosterol had a further peak at 825 cm^{-1} attributed[7] to the out-of-plane bending frequency of the hydrogen at C 28. In the algal sterol this peak was displaced at 812 cm^{-1} and was also of relatively greater intensity. This is in complete accord with the reported i.r. spectrum of synthetic 28-isofucosterol;[7]

[9] P. R. MOORE and C. A. BAUMANN, *J. Biol. Chem.* **195**, 615 (1952).
[10] J. BERGMAN, B. O. LINDGREN and C. M. SVAHN, *Acta Chem. Scand.* **19**, 1661 (1965).
[10a] P. BENVENISTE, L. HIRTH and G. OURISSON, *Phytochem.* **5**, 31 (1966).

the 812 cm^{-1} peak has been ascribed to a *trans* arrangement of the ethylidene methyl group and the terminal isopropyl group[7] (I).

Further confirmatory evidence for the identity of the algal sterols with 28-isofucosterol was provided by a comparison of the NMR spectrum of the sterol from *E. intestinalis* with that of fucosterol isolated from *Fucus spiralis* (Fig. 3). Fucosterol gave a doublet at 8·48τ ($J \simeq 6{\cdot}9$ c/s) for the protons at C 29, a quartet at 4·88τ ($J \simeq 6{\cdot}9$ c/s) for the proton at C 28, a multiplet at 4·71τ for the proton at C 6 and a multiplet at 6·6τ for the C 3 proton. This is in agreement with the results recently published in confirmation of the structure of fucosterol.[11] The NMR spectrum of the *E. intestinalis* sterol was similar to that of fucosterol except that the doublet for the C 29 protons was displaced slightly downfield to 8·46τ ($J \simeq 6{\cdot}9$ c/s) whilst the quartet for the C 28 proton was displaced upfield to 4·95τ ($J \simeq 6{\cdot}9$ c/s).* These data confirm the presence of an ethylidene group in the algal sterol and are in complete accord with its identification as 28-isofucosterol.

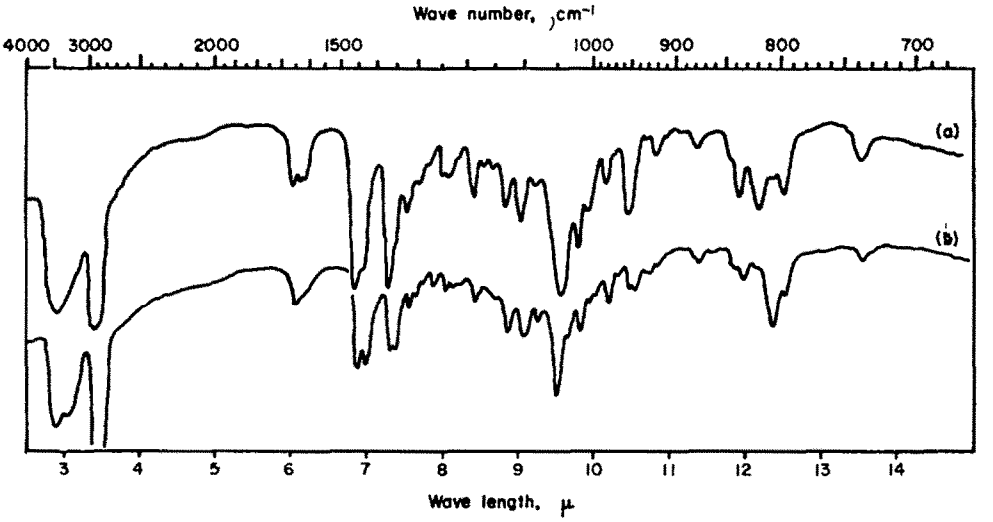


FIG. 2. I.R. SPECTRA OF (*a*) FUCOSTEROL ISOLATED FROM *Fucus spiralis* (*b*) THE STEROL ISOLATED FROM *Enteromorpha intestinalis*.

The 4,4-dimethyl and 4-methyl sterols of the two algae were purified by thin-layer chromatography on silica gel and examined by GLC on a QF-1 column. The 4,4-dimethyl sterol fraction of *E. intestinalis* was found to be a complex mixture containing several components and the possibility that some of these may have been non-sterol components cannot be eliminated. However, the major peak had a retention time identical to 24-methylene cycloartanol whilst two other more minor peaks had retention times close to those of cycloartenol and lanosterol or butyrospermol respectively. The 4,4-dimethyl sterol fraction of *U. lactuca* contained only two components with retention times close to those of cyloartenol and 24-methylenecycloartanol respectively, the latter sterol predominating. The 4-methyl sterol fraction of *E. intestinalis* showed two main components which had retention data the

* A multiplet at 7·2τ in the NMR spectrum of 24-ethylidene lophenol has previously been assigned to the proton on carbon 25 of this sterol.[10] A multiplet at 7·2τ was also present in the NMR spectra of the sterols isolated from *Enteromorpha intestinalis* and *Ulva lactuca*. However, such a peak was not apparent in the spectrum of fucosterol (Fig. 3).

[11] W. R. NES, M. CASTLE, J. L. MCCLANAHAN and J. M. SETTINE, *Steroids* **8**, 655 (1966).

same as those of 24-methylene- and 24-ethylidene-lophenol respectively. Unfortunately the very small amounts obtained of the various 4-methyl sterols did not permit a more detailed examination and the above identifications should be regarded as provisional.

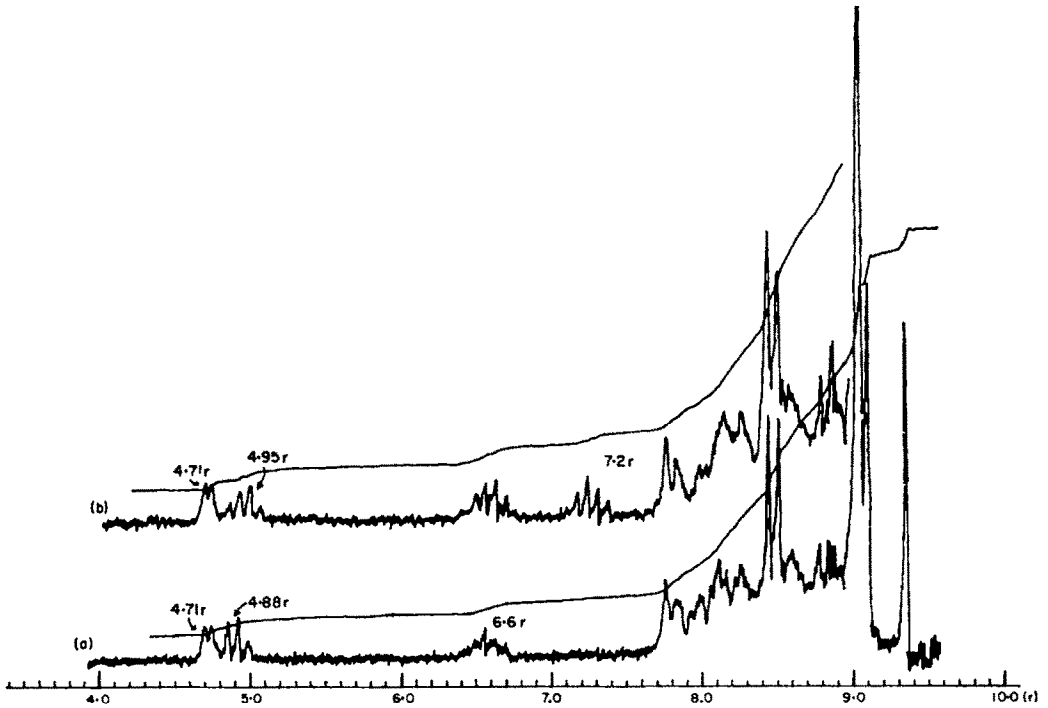


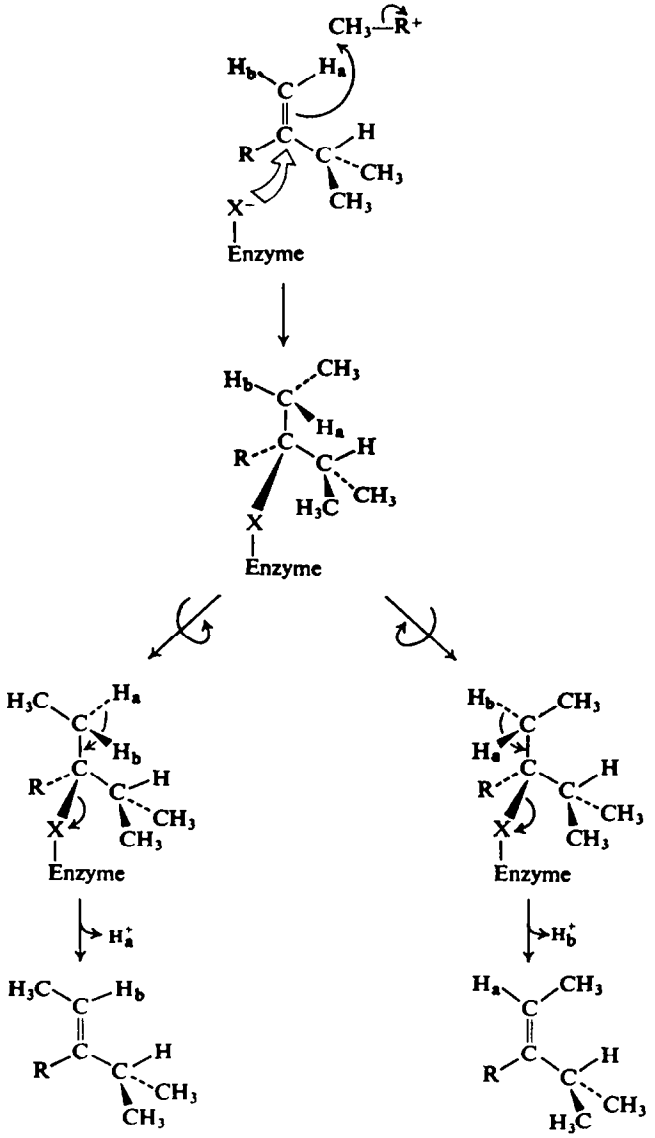
FIG. 3. NUCLEAR MAGNETIC RESONANCE SPECTRA AT 100 Mc OF (*a*) FUCOSTEROL AND (*b*) THE STEROL ISOLATED FROM *Enteromorpha intestinalis*.

DISCUSSION

The present identification of 28-isofucosterol in *Enteromorpha intestinalis* and *Ulva lactuca* in addition to the evidence for its presence in *E. linza*[4] leads to speculation that this sterol may be characteristic of the Ulvaceae. Clearly an examination of many other members of this family would seem warranted and may prove to be of taxonomic value. The accumulation of 28-isofucosterol in *E. intestinalis* and *U. lactuca* coupled with the preponderance of fucosterol in the members of the Phaeophyceae[2,8] is also of interest biogenetically. A mechanism for the introduction of the C-24 ethyl and ethylidene side-chains of phytosterols has been proposed[12] and has been examined in some detail in a number of laboratories.[13–20]

[12] M. CASTLE, G. BLONDIN and W. R. NES, *J. Am. Chem. Soc.* **85**, 3306 (1963).
[13] G. JAURÉGUIBERRY, J. H. LAW, J. MCCLOSKEY and E. LEDERER, *Biochemistry* **4**, 347 (1965).
[14] S. BADER, L. GUGLIELMETTI and D. ARIGONI, *Proc. Chem. Soc.* 16 (1964).
[15] V. VILLANUEVA, M. BARBIER and E. LEDERER *Bull. Soc. Chim. Fr.* 1423 (1964).
[16] L. J. GOAD, A. S. A. HAMMAN, A. DENNIS and T. W. GOODWIN, *Nature* **210**, 1322 (1966).
[17] M. LENFANT, E. ZISSMANN and E. LEDERER, *Tetrahedron Letters* 1049 (1967).
[18] M. AKHTAR, P. F. HUNT and M. A. PARVEZ, *Biochem. J.* **103**, 616 (1967).
[19] A. R. H. SMITH, L. J. GOAD, T. W. GOODWIN and E. LEDERER, *Biochem. J.* **104**, 56c (1967).
[20] L. J. GOAD In *Terpenoids in Plants* (edited by R. B. PRIDHAM), p. 139. Academic Press, London (1967).

The isolation of fucosterol and 28-isofucosterol from natural sources now demonstrates that the transmethylation mechanism operative in a particular organism must involve an en-



SCHEME. HYPOTHETICAL MECHANISM FOR THE BIOSYNTHETIC FORMATION OF THE FUCOSTEROL AND 28-ISOFUCOSTEROL SIDE-CHAINS.

zymatically controlled stereospecific hydrogen elimination to produce one or other of the C 24 ethylidene isomers. This is shown in its simplest form in the scheme; however, with the evidence at present available, further elaboration of the mechanism would not seem appropriate.

EXPERIMENTAL

Methods were generally as described previously.[21,22]

Enteromorpha intestinalis and *Ulva lactuca* were collected from the shore at Aberystwyth, Cardiganshire, Wales, and carefully sorted and washed before extraction. The algae (1 kg wet weight) were homogenized with ethanol and refluxed with potassium hydroxide (10 per cent) for 2 hr. The non-saponifiable lipids were extracted with diethyl ether in the usual manner and the sterols obtained by digitonin precipitation. The sterols were then separated by chromatography on alumina (Brockmann grade III) to obtain the major sterols (*E. intestinalis*: 271 mg; *U. lactuca*: 202 mg). These sterols were crystallized from $CHCl_3$—MeOH before further analysis. Very small amounts of 4,4-dimethyl (1 mg) and 4-methyl (1 mg) sterols were obtained from both algae. These were purified by preparative silica gel thin-layer chromatography before analysis by gas–liquid chromatography.

Gas–liquid chromatography was carried out on a Varian-Aerograph 1522 instrument fitted with hydrogen flame ionization detectors. All columns (1 per cent SE-30, 1 per cent QF-1, and 0·7 per cent Hi EFF 8B) and operating parameters were as described previously.[21]

I.r. spectra were determined in 13-mm KBr discs using a Perkin-Elmer 247 instrument.

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[21] G. F. GIBBONS, L. J. GOAD and T. W. GOODWIN, *Phytochem.* **6**, 677 (1967).
[22] L. J. GOAD and T. W. GOODWIN, *Biochem. J.* **99**, 735 (1966).