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SHORT COMMUNICATION

# STEROLS IN *ASCOPHYLLUM NODOSUM*

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**Abstract**—In stored, milled, dried *Ascophyllum nodosum*, saringosterol, 24-ketocholesterol, fucosterol, 24-methylenecholesterol, 24-methyl-$\Delta^{5,22}$-cholestadien-3$\beta$-ol, cholesterol and a trace of 24-ethylcholesterol were detected in the sterol fraction. Whereas, from freshly harvested and dried *A. nodosum* only fucosterol with traces of $C_{27}$ and $C_{28}$ sterol could be detected. When freshly harvested and dried seaweed was exposed to air for 4 weeks, 24-ketocholesterol and possibly saringosterol could be detected as additional components of the sterol fraction and it is suggested that these two compounds may arise as artefacts by aerial oxidation of fucosterol.

## INTRODUCTION

BECAUSE of a difference in their physiological effects upon the sporulation of the oomycete fungus *Phytophthora cactorum* (Leb. and Cohn) Schroet[1] it was desired to find satisfactory sources of both isomers at $C_{24}$ of 24-ethylidenecholesterol. $\Delta^5$-Avenasterol has been isolated from *Avena sativa* L.[2,3] whilst fucosterol is commonly found in brown algae.[4] Because *Ascophyllum nodosum* (L) Le Jol. was readily available in dried milled form by courtesy of Alginate Industries Ltd., this species was chosen first as a possible source of fucosterol and it is the purpose of this paper to describe the sterols of *A. nodosum*.

## RESULTS

Dried, milled *Ascophyllum nodosum* from Alginate Industries Ltd. was extracted with petroleum ether and sterols were isolated by chromatography on alumina of the non-saponifiable fraction. The crude sterol fraction contained three main zones by TLC and was purified by preparative TLC to give the three fractions.

The most mobile of these fractions had an $R_f$ identical to that of cholesterol and was found by gas–liquid chromatography (GLC) to consist of five components, a minor one of which was only partially resolved from the main component. Details of the GLC data are listed in the Experimental and these suggest that the first compound to be eluted was cholesterol, the second 24-methyl-$\Delta^{5,22}$-cholestadien-3$\beta$-ol (brassicasterol or its $C_{24}$ isomer), the third 24-methylcholesterol, the fourth 24-ethylcholesterol ($\beta$-sitosterol or its $C_{24}$ isomer clionasterol) and the main component to be fucosterol. Acetylated sterols were subjected to analysis by combined gas chromatography–mass spectrometry (GC–MS) and this confirmed the identities of these compounds, mass spectra being obtained similar to those described[5,6] previously for the various compounds indicated by GLC.

[1] C. G. ELLIOTT, M. R. HENDRIE and B. A. KNIGHTS, *J. Gen. Microbiol.* **42**, 425 (1966).
[2] D. R. IDLER, G. W. NICKSIC, D. R. JOHNSON, V. W. MELOCHE, H. A. SHUETTE and C. A. BAUMANN, *J. Am. Chem. Soc.* **75**, 1712 (1953).
[3] B. A. KNIGHTS, *Phytochem.* **4**, 857 (1965).
[4] K. TSUDA, R. HAYATSU, Y. KISHIDA and S. AKAGI, *J. Am. Chem. Soc.* **80**, 921 (1958).
[5] B. A. KNIGHTS, *J. Gas Chromatog.* **5**, 273 (1967).
[6] B. A. KNIGHTS and C. J. W. BROOKS, *Phytochem.* **8**, 463 (1969).

The second band obtained by TLC was shown to be largely a single compound by GLC and analysis by GC–MS suggested it to be 24-ketocholesterol (M-60 at *m/e* 382). This was confirmed by comparison by TLC, GLC and i.r. with an authentic sample kindly provided by Dr. N. Ikekawa.

The most polar substance upon TLC was shown by i.r. to have a hindered hydroxyl group, not acetylated by acetic anhydride–pyridine and a vinyl group. Analysis by GLC and GC–MS of the acetate showed it to be a pure compound and suggested the presence of a hydroxyl group and possibly a vinyl group in a $C_{29}$ sterol. Analysis by NMR confirmed the presence of a vinyl group and a double bond at $C_5$. These data suggested that the compound could be saringosterol.[7] The melting point of the diol was in agreement with this but the acetate had a higher melting point than that quoted by Ikekawa. However, comparison with authentic material confirmed that the sterol from *A. nodosum* was in fact saringosterol, i.r., GLC and mass spectral data closely corresponding in each case and Ikekawa[8] kindly confirmed the identity of the melting points of the two acetate samples after further recrystallization of his sample.

*A. nodosum* was harvested fresh from below the tide line in the Gareloch and, after oven-drying overnight, was extracted for sterols. Only one spot was noted upon TLC and by GLC this material was shown to be nearly pure (>95 per cent) fucosterol with only small amounts of cholesterol and 24-methylenecholesterol as contaminants.

A sample of this dried algal material was retained and left exposed to air in a greenhouse for 4 weeks. Extraction afforded a sterol fraction much less pure than that obtained from the freshly dried and extracted material. Three zones could be detected upon TLC each corresponding to one of the three zones obtained from the commercial sample of *A. nodosum*. By GLC it was confirmed that the mobile zone consisted of fucosterol with small amounts of cholesterol, 24-methyl-$\Delta^{5,22}$-cholestadien-3$\beta$-ol and the 24-methylenecholesterol. The middle zone had an i.r. spectrum similar to that for 24-ketocholesterol and this identification was confirmed by GLC. The third TLC zone had mobility identical to that of saringosterol but was not further analysed successfully.

## DISCUSSION

The presence of only a relatively small amount of fucosterol together with significant amounts of 24-ketocholesterol and saringosterol in the sterol fraction from the commercial sample of *Ascophyllum nodosum* suggested that the two latter compounds had been derived from the former by successive aerial oxidation steps. This was thought likely since the algal sample was extracted in batches over a period of several months and was not protected from aerial degradation prior to extraction. As a confirmation, fresh material was harvested from below the low tide line and extracted immediately following overnight air drying. No trace of either saringosterol or 24-ketocholesterol could be found and, by contrast with the old sample of seaweed, fucosterol was present as a nearly pure compound in the sterol fraction. Further confirmation that saringosterol and 24-ketocholesterol might be derived by oxidation of fucosterol was obtained when additional, more polar, sterols were obtained from a sample of the fresh material, which had been exposed to air for 4 weeks prior to extraction. One of these compounds was shown to be 24-ketocholesterol and from TLC data it seems likely that the other was saringosterol. It must be stressed that, whilst it seems most likely that these

[7] N. IKEKAWA, K. TSUDA and N. MORISAKI, *Chem. Ind.* 1179 (1966).
[8] N. IKEKAWA, personal communication.

compounds were formed by air oxidation of fucosterol, it is possible that degradation of the structure of the dry powder by air to release 24-ketocholesterol and saringosterol from a non-extractable complex form may be taking place. The author is not aware of any reported finding of 24-ketocholesterol in plant sources although saringosterol has been reported to occur in a number of marine brown algae.[9]

## EXPERIMENTAL

Ascophyllum nodosum. Commercial material was obtained as a dried milled powder from Alginate Industries Ltd., Girvan, Ayrshire. Fresh material was harvested from below the low tide mark in the upper reaches of the Gareloch, Dunbartonshire and was air dried at 100° overnight.

*Sterols.* Dried alga was extracted with petroleum ether and, after removal of the solvent, the extract was saponified with NaOH. The non-saponifiable fraction was chromatographed on neutral alumina, sterols being eluted by increasing concentrations of ethyl acetate in petroleum ether.

*TLC.* Sterols were acetylated ($Ac_2O$/pyridine[10]) and then applied as strips to prewashed silica gel G layers (20 × 20 × 0·1 cm). Plates were developed using 10% ethyl acetate in petroleum ether and zones were located using dichlorofluorescein. Recovery of sterols was achieved by mechanical transfer of indicated zones into small chromatography columns followed by elution with ether.

*GLC.* Carried out using 3% OV-17 and 5% SE-30 stationary phases as previously described.[11] Retention indices were determined using $nC_{30}H_{62}$, $nC_{32}H_{66}$, $nC_{34}H_{70}$ and $nC_{36}H_{74}$ as standards.[12]

*GC-MS.* An LKB 9000 gas chromatograph–mass spectrometer was used as previously described.[6] The column (1% OV-17) was operated at 240° for the mobile sterol zone (fucosterol) and at 265° for the other two zones. Acetylated sterols were used and the ion of highest mass obtained for each sample always corresponded to *m/e* M-60.[5,6]

*Saringosterol acetate.* The acetate (GLC: $I_{250}^{SE-30}$ 3530, $I_{255}^{OV-17}$ 3880) was recrystallized from methanol to constant m.p. at 176–178° (Ikekawa[8] reports the same melting point rather than the literature figure (163–164°[7]). The mass spectrum exhibited characteristic ions as follows: M-60, 410 (15%); M-(60 + 18), 392 (6%); M-(60 + 28), 382 (3%); M-(60 + 18 + 15), 377 (2%); M-(60 + 28 + 15), 367 (3%); M-(60 + 18 + 43), 349 (7%); M-157, 313 (4%); M-(60 + 114), 296 (9%); M-(60 + 127), 283 (9%); M-(60 + 157), 253 (24%); base peak *m/e* 43 (100%).

*Infrared spectrum.* (S.P. 200; nujol mull) 3530, 1715, 1270, 1250, 1035, 995, 980, 960, 917, 800 $cm^{-1}$.

*NMR.* (Varian H.A. 100; $CDCl_3$ solution, TMS standard.) Methyl singlets: 9·34, 9·0, 8·0 $\tau$; multiplet 5·38 $\tau$ ($C_3$ proton); doublet 4·66 $\tau$ ($J$ 2 cps) ($C_6$ proton); and a vinyl group septet centred at 4·2 $\tau$ (quartet $J$ 11 and 17 cps) ($C_{28}$ proton) and at 4·81 $\tau$ (triplet) (2 × $C_{29}$ protons).

*Saringosterol.* The acetate was hydrolysed to afford the free diol, m.p. (*aq.* methanol) 160–163°, lit.[7] 160–161°. The mass spectrum (GEC-AEI MS-12) corresponded to the literature spectrum[7,8] as did the i.r. spectrum.

*24-Ketocholesterol acetate* failed to crystallize, the mass spectrum of the TLC purified material exhibited ions for M-60, 382 (66%); M-(60 + 86), 296 (14%); M-(60 + 127), 255 (16%); M-(60 + 129), 253 (12%); base peak: *m/e* 43 (100%).

*Infra-red spectrum.* (liquid film). 1730, 1710, 1475, 1450, 1380, 1250, 1040, 960, 905, 850, 805 $cm^{-1}$, comparable with peaks from an authentic sample.

*Fucosterol fraction.* GLC indicated five components, peak 1 (15%) $I^{SE-30}$ 3185, $I^{OV-17}$ 3450; peak 2 (7%) $I^{SE-30}$ 3225, $I^{OV-17}$ 3500; peak 3 (17%) $I^{SE-30}$ 3275, $I^{OV-17}$ 3560; peak 4 (trace) $I^{OV-17}$ 3645; peak 5 (61%) $I^{SE-30}$ 3370, $I^{OV-17}$ 3650.

*GC-MS* showed ions of highest mass (M-60) at *m/e* 368 (peak 1), 380 (peak 2), 380 (peak 3), 396 (peak 4), 394 (peak 5) and fragment ions as described previously.[5,6]

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[9] N. Ikekawa, N. Morisaki and K. Tsuda, *Steroids* **12**, 41 (1968).
[10] B. A. Knights, *Memoirs of the Society for Endocrinology*, No. 16 (edited by J. K. Grant), p. 211 (1967).
[11] D. S. Ingram, B. A. Knights, I. J. McEvoy and P. McKay, *Phytochem.* **7**, 1241 (1968).
[12] B. A. Knights, *J. Gas Chromatog.* **4**, 329 (1966).