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Biosynthesis of Radioactive Fucosterol and 24-Methylene cholesterol by *Undaria pinnatifida* using Radioactive Methionine

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Summary

Radioactive fucosterol [(24*E*)-24-ethylidenecholest-5-en-3β-ol] and 24 methylenecholesterol [24-methylenecholest-5-en-3β-ol] were obtained from *Undaria pinnatifida* after injection of raidoactive methionine.

After injection *U. pinnatifida* was cultured in seawater. On the sterol metabolism of *U. pinnatifida*, sterol side chain elongation may occur by transmethylation with methionine.

Sterols are important structural components of cell and organella membranes of all higher organisms and several classes of microorganisms (1, 2). Cholesterol represents the primary sterol in animals. Analogs of cholesterol which are found in plants and lower organisms auch as yeast differ structually only in terms of additional substitution or unsaturation in the 8-numbered side chain and/or additional unsaturation in the B-ring. The sterol contents and composition of marine invertebrates are very complex and unique. For example, C_{26} sterols are proving to be ubiquitous in their distribution in small amounts in marine invertebrates (3-6). The origin of the C_{26} sterols is obscure but they do not appear to be synthesized *de novo* by animals, nor is there any evidence of modifiation of dietary sterols by invertebrates to give these compounds. Studies on sterol matabolism of marine invertebrates have progressed using radioactive precuesor (7), but species of radioactive sterols available are few. It is necessary that we should make suitable sterol precursors. It has been found that the additional carbon atoms found in the side chain of phytosterols are derived from *S*-adenosylmethionine by

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transmethylation. [methyl-^{14}C]-Methionine was injected in *U. pinnatifida* to make radioactive fucosterol and 24-methylenecholesterol.

Materials and Methods

L-[methy-^{14}C]-Methionine was injected into the rib of *U. pinnatifida* by micro sylinge. The alga was cultured in seawater that was filtrated (0.45 μm) under 3,000 lx white fluorescent illumination at 15°C for 10 days.

The lipids were extracted with chloroform-methanol (2 : 1) according to Folch's method (8). The lipids extracts were saponified using alcoholic potassium hydride for 60 min at 60–80°C. The non-saponifiable lipids were chromatographed on silica gel TLC developed with hexane-diethylether-acetic acid (70 : 30 : 1). The sterol fraction was acetylated with Ac_2O-pyridine (1 : 1) for 24 hr at room temperature. The steryl acetates were fractionated by preparative TLC on silver nitrated silica gel plates developed twice with hexane-benzene (5 : 2) (9). Bands were made visible under u.v. radiation aftoer spraying with a solution of dichlorofluorescein in isopropanol.

After development the plates were subjected to autoradiography for 14 days usinf FUJI X-ray film. The films were developed with Hi-RENDOL and fixed with Hi-RENFIX.

Radioactivity was measured with a Aloka liquid scintillation counter LSC-903 using a toluene solution of PPO (0.5%) and POPOP (0.15%).

The analytical gas chromatography (GC) was performed on a Hitachi 163 Gas Chromatograph, equipped with a 2 m $\times$ 3 mm i.d. stainless column packed with 1.5% OV-17 on Chromosorb W (80–100 mesh) and a FID detector. Column temperature 265°C, carrier gas flow rate 25 ml/min. Gas chromatograph-mass spectrometry (GC-MS) was performed on a Shimadzu LKB 9000 equipped with a 2 m $\times$ 3 mm i.d. glass column packed with 0.75% OV-17 on Chromosorb W (80–100 mesh) and TIC. Column temperature 265°C, carrier gas flow rate 30 ml/min.

Results

Gas-liquid chromatography of *U. pinnatifida* sterols showed three components. *U. pinnatifida* contained cholesterol (cholest-5-en-3β-ol, 3.6% of total sterols), 24-methylenecholesterol (24-methylenecholest-5-en-3β-ol, 17.0%) and fucosterol (24-ethylidene-cholest-5-en-3β-ol, 79.4%). They could be separated by $AgNO_3$-impregnated TLC from each other as shown in Fig. 1.

Thin layer radioscan and autoradiogram after $AgNO_3$-impregnated TLC indicated the presence of radioactivity corresponding to fraction II (Fraction II ; 24-ethylidenecholest-5-en-3β-ol) and fraction III (Fraction III ; 24-methylenecholest-5-en-3β-ol). Specific radioactivity of purified fucosterol (Fr. II) and 24-methylenecholesterol (Fr. III) was 25.9×10^3 dpm/mg and 5.8×10^3 dpm/mg, respectively.

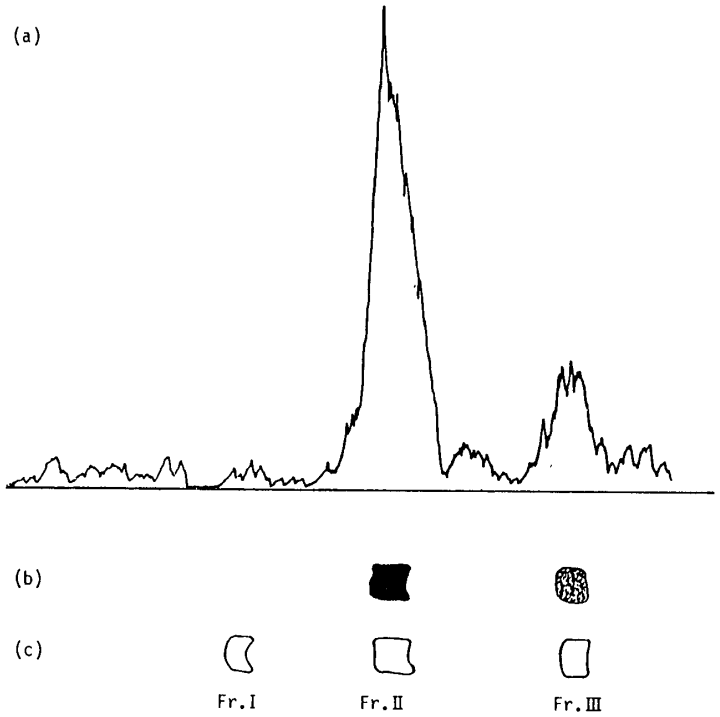

FIG. 1. (a) Thin layer radioscannogram of the acetylated sterols isolated from *U. pinnatifida* after jnjection of radioactive methionine
(b) Autoradiogram of the acetylated sterols.
(c) Thin layer chromatogram of the acetylated sterols.

TABLE 1. Sterol Composition of *Undaria pinnatifida*

Sterol No.	Sterol Systematic Nomenclature	RRT*	%(GC)
1	Cholest-5-en-3β-ol	1.00	3.6
2	24-Methylenecholest-5-en-3β-ol	1.37	17.0
3	24-ethylidenecholest-5-en-3β-ol	1.64	79.4

*RRT = reletive retention time to cholesterol acetate

Discussion

Sterols represent one of the triterpenoids. Algal sterols differ from animal sterols in their diversity, nevertheless the formation of plant sterols followed the same pathway as that in animals up to the synthesis of the first C_{30} intermediate, squalene (10-12). Cyclization of squalene occurs in animals with the formation of lanosterol which is then converted into sterols. In the case of higher plants and algae, cycloartenol is formed as the first cyclic product which is converted into sterols (13, 14). The reactions unique to the pathway from cycloartenol to plant sterols are opening of the cyclopropane ring and alkylation of the side chain at C-24. So plants produce sterols in which the cholesterol molecule is modified by

TABLE 2. Salient Mass Spectrometric Data of the Steryl Acetates

Fragment*	Sterol No.		
	1	2	3
M-AcOH(60)	368(100)	380(100)	394(29)
M-AcOH-CH_3	353(22)	365(22)	379(7)
M-AcOH-(C_{22}-C_{23})**-H	—	296(50)	296(100)
M-AcOH-CH_3-(C_{22}-C_{23})-H	—	281(22)	281(23)
M-AcOH-C_8H_{12}	260(27)	—	—
M-AcOH-CgH_{13}	247(22)	—	—
M-AcOH-SC***	255(22)	255(17)	255(8)
M-AcOH-SC-2H	253(8)	253(35)	253(18)
M-AcOH-SC-42	213(18)	213(22)	213(18)

* m/z(%)

** (C_{22}-C_{23})=cleavage at C_{22},C_{23}

*** SC=side chain

the addition of one or two supernumerary carbon atoms at C-24. The addition of C_1 and C_2 side chain at C-24 requires either a single or double methylation which involves one and two molecules of S-adenosylmethionine, respectively. These were first demonstrated in higher plants and then in algae (15, 16).

The sterol composition of algae is different from that of terrestrial higher plants which almost always contain sitosterol 24α-ethylcholest-5-en-3β-ol) or stigmasterol (24α-ethylcholesta-5, 22-dien-3β-ol) as the major sterol. The major sterol of red algae so far examined contain predominantly Δ5C27 sterol. The dominant sterol found in all the brown algae so far examined is fucosterol which has a 24-ethylidene group with the *E*-configuration. The major sterol of green algae is usually isofucosterol which has a 24-ethylidene group with the *Z*-configuration. Sterol composition of marine algae may have some value in biochemical taxonomy, and phylogenetic significance.

Marine invertebrates also contain a complex sterol mixture and their sterol metabolism and sterol origin is not so clear. In studying sterol metabolism, though radioactive acetate of mevalonate have been often used as a precurser (7), derived sterols have so low a level of radioactivity that they are unsuitable to use for elucidating the sterol bioconversion.

In studying the sterol metabolism of algae or phytoplanktons, there is a problem of how to inject the precurser into the organisms. It is necessary to incorporate a radioactive precurser into sterols effectively. So we injected it directly into the rib of U. pinnatifida, and obtained the radioactive sterols, fucosterol (25.9×10^3 dpm/mg) and 24-methylenecholesterol (5.8×10^3 dpm/mg).

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