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C-24 Configuration of 24-Methylcholesta-5, 7, 22-trienol from a Marine Occurring Yeast

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Abstract

A marine-occurring yeast isolated from the seaurchin contained 24-methylcholesta-5, 7, 22-trienol as the major sterol. This sterol was isolated from the sterol mixture as a pure compound by column chromatography on a $AgNO_3$-silicic acid with benzene-hexane and the chemical structure, especially the C-24 configuration, was elucidated by 400 MHz nuclear magnetic resonance spectrometry. The 24-methylcholesta-5, 7, 22-trienol was identified as (24R)-24-methylcholesta-5, 7, 22-trien-3β-ol (ergosterol) mainly on the basis of the chemical shift of a C-21 methyl group.

Recent advances in the investigation of marine invertebrate sterols resulted in the finding of many sterols with unprecedented structures[1,2]. But, the information on the origin of sterols in marine invertebrates is still only a little[3]. As pointed out by GOAD[3], marine yeast may play some role in relation to the origin of invertebrate sterols because of their important ecological role in marine environments. Previously, we have shown that marine yeast contained 24 - methylcholesta-5, 7, 22-trienol as the major sterol[4]. However, none of the informations elucidated reliably the C-24 configuration of 24-methylcholesta-5, 7, 22-trienol occurring in marine yeast. The three-dimentional characteristics of sterols are interesting in two points. First, sterols play the major role in biological systems as the architectural components of membranes, and the fit of molecules in the lipid leaflet may be concerned with the stereochemistry of sterols. Second, there appears to be a marked relation between the evolution of organisms and the C-24 configuration of sterols[3,5]. In the present study, therefore, we examined the C-24 configuration of 24-methylcholesta-5, 7, 22-trienol isolated from a marine yeast mainly on the basis of 400 MHz nuclear magnetic resonance (NMR) spectral data.

Materials and Methods

A marine yeast was isolated from the ovaries of the seaurchin collected near the

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Fisheries Research Laboratory of Kagoshima University and unidentified taxonomically*[1]. The marine yeast was cultured at room temperature (25-29 °C) for 2-3 days in the medium (pH 5.4-5.5); the artificial sea water (33 ‰) containing glucose (50 g), sodium glutamate (300 mg), $(NH_4)_2SO_4$ (2 g), KH_2PO_4 (1 g), biotin (10 μg), calcium pantothenate (25 μg), nicotinamide (50 μg), and choline chloride (50 μg). Lipids (970 mg) were extracted with chloroform-methanol-water (2 : 2 : 1) from the wet cells of yeast (100 g) and saponified with 10 % ethanolic KOH to give the unsaponifiable matters (489 mg). Crude sterols (338 mg) were isolated from the unsaponifiable matters by alumina column chromatography with hexane-ether and then acetylated with dry pyridine-acetic anhydride(1 : 1)[6].

The sterol components were characterized by gas-liquid chromatography (GLC) on 1.5 % OV-17 (3 m x 3 mm i.d., column temp. 250 °C)[6], combined GLC/mass spectrometry (ionizing energy, 70 eV)[7], and 400 MHz NMR spectrometry. NMR spectra were obtained in $CDCl_3$ on a Japan Electron Optics JMS Gx400 spectrometer. The isolation of sterol samples for NMR determination was conducted by column chromatography on 10 % $AgNO_3$-silicic acid with increasing proportions of benzene in hexane[8] and by crystallizations from methanol.

Results and Discussion

The steryl acetates of yeast gave 3 peaks (RRT = 1.00, 1.33, and 1.57; cholesteryl acetate = 1.00) in analytical GLC on 1.5 % OV-17. The major component was identified as 24-methylcholesta-5, 7, 22-trienyl acetate by GLC/Mass spectrometry : m/e (28 %, M^+), 378 (100 %, M^+ −AcOH), 363 (36 %, M^+ -AcOH - CH_3), 337 [2 %, M^+ -C(1) to C(3)-AcOH], 253 (43 %, M^+ -R-AcOH, R = side chain), 226 (4%, M^+ -R-27-AcOH), 211 (7 %, M^+ -R - 42 -AcOH), 157 (16 %), and 143 (14 %). The 400 MHz NMR spectrum of this steryl acetate gave the signals at δ 0.625 (s, 3H, C-18 H), 0.959 (s, 3H, C-19 H), 0.829 (d, 3H, J = 6.8 Hz, C-27 H), 0.845 (d, 3H, J = 6.2 Hz, C-26 H), 0.891 (d, 3H, J = 6.8 Hz, C-28 H), 1.039 (d, 3H, J = 6.5 Hz, C-21 H), 2.055 (s, 3H, acetoxymethyl group), 4.708 (m, 1H, C-H), 5.200 (m, 2H, C-22, 23 H), 5.383 (broad s, 1H, C-7 H), and 5.567 (broad s, 1H, C-6 H).

NES *et al.*[9] have shown that the primitive tracheophyta, *Lycopodium complanatum*, contained both ergosterol (24R-24-methylcholesta-5, 7, 22E-trienol) and epiergosterol (24S-24-methylcholesta-5, 7, 22E-trienol), indicating that the 24S- and 24R-isomers gave the doublets of C-21 methyl group at δ 1.029 and 1.039, respectively. The 24-methylcholesta-5, 7, 22-trienyl acetate isolated from the marine yeast in the present

*[1] The yeast is likely to come from the intestinal content contaminated during the removal of ovaries from the seaurchin bodies.

Table 1. Sterols of the marine yeast isolated from the seaurchin

Remark	Sterol *1			
	Cholesterol	Ergosterol	Episterol	7-Ergostenol
RRT in GLC	1.00	1.33	1.57	1.57
Mass spectrometry				
M^+	386 (100) *2	396 (40)	398 (16)	400 (100)
M^+ - HOH	368 (50)	378 (60)	380 (16)	382 (38)
M^+ - 33	353 (43)	363 (75)	365 (10)	367 (18)
M^+ - (R+2H)		269 (25)	271 (100)	271 (58)
M^+ - (R+HOH)	255 (40)	253 (100)	255 (25)	255 (90)
M^+ - (R+HOH+2H)		251 (53)	253 (40)	253 (36)
M^+ - (R+27)	246 (10)		246 (8)	246 (5)
M^+ - (R+27+HOH)	229 (20)		228 (10)	228 (16)
M^+ - (R+42)	231 (41)	229 (4)	231 (20)	231 (31)
M^+ - (R+42+HOH)	213 (45)	211 (35)	213 (15)	213 (60)
Other peaks	275 (60)	337 (17)	314 (42)	314 (6)
	301 (64)			
	247 (30)			

*1 The steryl acetates obtained by argentic column chromatography were saponified with 5% ethanolic KOH to give free sterols for GLC/Mass spectrometry

*2 Relative intensity (%).

study gave the C-21 methyl doublet at δ 1.039, indicating the 24R-configuration. Thus, the marine yeast examined was found to contain ergosterol as the major sterol (97% of total sterols). In some marine invertebrates such as molluscs[3] and sponges[3,10], ergosterol have been found as the minor or major components, however the C-24 configuration was not characterized rigorously in most case. We have demonstrated that the oyster, *Crassostrea virginica*, contained both ergosterol and epiergosterol in the sterol mixture, suspecting the diversity of food habits in this mollusc[8].

The minor sterol (RRT 1.00, 0.5% of total sterols) was identified as cholesterol on the basis of the mass spectral data. Another minor peak (RRT 1.57, 2.5%) was not homogenous and composed of 24-methylcholest-7-enyl and 24-methylenecholest-7-enyl acetates. The 2 steryl acetates were identified mainly on the basis of mass spectral data : 24-methylcholest-7-enyl acetate, m/e 442 (100%, M^+), 427 (18%), 382 (20%), 367 (15%), 288 (12%), 273 (13%), 255 (80%, M^+-R-AcOH), 229 (28%), and 213 (40%); 24-methylenecholest-7-enyl acetate, 440 (35%, M^+), 425 (13%), 380 (2%), 365 [50%, M^+-C (23) to C (28)], 313 (100%, M^+-R-2H), 255 (35%), 229 (8%), 227 (18%), and 213 (21%).

As mentioned above, the marine yeast contained ergosterol (24 β/24 R configuration), typical sterol of terrestrial yeast, as the major sterol, and had the similar sterol composi-

tion (%) to those of baker's yeast[11] and the several marine yeast examined previously[4] except for the minor components. It warrants further studies to clarify the contribution of marine yeast as the source of dietary sterols to invertebrate sterols and the ecological role of marine yeast in the movement and transformation of sterols in marine environments.

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