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Ergosteryl Myristate, a New Ergosterol Derivative from Unidentified Marine Algicolous Fungus

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Abstract – A new ergosteryl myristate (**1**) and ergosterol (**2**) have been isolated from the organic extract of the mycelium of unidentified marine algicolous fungus, isolate MF001. The structure of a new compound was assigned on the basis of comprehensive spectroscopic analyses and chemical synthesis.
Key words – Ergosteryl myristate, ergosterol, ergosterol derivative, marine algicolous fungus.

Introduction

Marine natural product research is now focusing more on marine microorganisms, mainly bacteria and fungi that can be cultured (Fenical, 1993; Pietra, 1997; Son *et al.*, 1999).

This led us search for new bioactive compounds from microorganisms inhabiting the marine environment. The chemical search in the marine microorganism is essential to our basic understanding of their biology and biological activity.

As part of a search for the bioactive metabolites from the marine fungi found in association with seaweeds, we have investigated and isolated new ergosteryl myristate (**1**) together with ergosterol (**2**) from the mycelium extract.

This paper deals with details of the structural elucidation of new metabolite.

Experimental

General – Optical rotations were measured on JASCO DIP-1000 digital polarimeter. IR spectra were obtained by using the Bruker FT-IR, IFS48 spectrometer. NMR Spectra were measured with JEOL EX 90A (90 MHz) and Varian Unity Plus-300 (300 MHz) spectrometers. MS was taken on Autospec-Ultima E spectrometer. The following experimental conditions were used for chromatography : HPLC, Spectra system P2000 : column chromatography, silica gel 60 (Merck, 70~230 mesh) : TLC, silica gel 60 F_{254} (Merck, pre-coated TLC plate) (detection by spraying with 1% $Ce(SO_4)_2$/10% aq. H_2SO_4 followed by heating.

Fungal Isolation, Fermentation, and Extraction – The unidentified fungal strain, MF001 was isolated from the surface of the green alga *Sargassum thunbergii* collected at Chungsapo of Pusan in 1997.

The fungus was incubated in static liquid culture medium of 20 L scale comprised of 0.5% yeast extract, 0.5% peptone, 1.0% glucose, 0.2% fish meal and 100% sea water. Following a 20 day fermentation period at 27°C, the mycelium and broth were separated by filtration. The mycelial mat was freeze-dried and extracted twice with CH_2Cl_2 - MeOH (1:1).

Isolation of ergosteryl myristate (1) and ergosterol (2) – The combined extract was fractioned by flash silica gel column with a stepwise gradient of AcOEt (0~100%) in hexane, and a total of 4 fractions were collected. Further purification of fraction 1 (30.9 mg) over silica gel using *n*-hexane / AcOEt (20:1 → 10:1), followed by HPLC [*n*-hexane / AcOEt (30:1)] yielded ergosteryl myristate (**1**) (11.0 mg) and ergosterol (8.0 mg).

Ergosteryl myristate (**1**) : colorless solid; mp 100.4~101.4; $[\alpha]_D$ −59° (c = 0.16, $CHCl_3$); UV (dioxane) 262 nm (ε8,000) (*sh*), 273(12,000), 282 (15,000), 295 (7,000); IR(KBr) 2954, 2916, 2851, 1741, 1462, 1382, 1200, 1179 cm^{-1}; LREIMS *m/z* = 606[(M)$^+$; rel. int. 7], 378(94), 253(73), 228(31), 199(22), 183(34), 169(56), 149(64), 127(72), 113(79), 97(89), 71(90), 57(100); HREIMS (M^+) *m/z* obsd. 606.5380,

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Table 1. ^{1}H-and ^{13}C-NMR Data for Ergosteryl myristate (**1**)[1]

carbon#	δ_H(mult, *J*(Hz))[2]	δ_C		carbon#	δ_H (mult, *J*(Hz))[2]	δ_C	
1		38.0	t[3]	19	0.95 s	16.2	q[3]
2		28.2[4]	t	20		40.4	d
3	4.72, tt, 11.5, 4.0	72.5	d	21	1.03, d, 6.6	21.1	q
4		36.8	t	22	5.18, dd, 15.1, 12.2	135.6	d
5		138.7	s	23	5.22, dd, 15.1, 12.2	132.1	d
6	5.57, dd, 5.5, 2.3	120.2	d	24		42.9	d
7	5.38, ddd, 5.5, 2.7, 2.5	116.4	d	25		33.1[4]	d
8		141.5	s	26	0.82[4], d, 4.6	20.0[4]	q
9		46.1	d	27	0.84[4], d, 4.6	19.7	q
10		37.2	s	28	0.92, d, 6.8	17.6	q
11		22.7	t	1'		173.3	s
12		39.1	t	2'		34.8	t
13		42.9	s	3'		29.7	
14		54.6	d	⋮	1.25, br.s	⋮	all t
15		23.0[4]	t	12'		29.2	
16		32.0	t	13'		25.1	t
17		55.9	d	14'	0.88, t, 7.1	14.1	q
18	0.63, s	12.1	q				

[1]Recorded in $CDCl_3$ at 300 MHz (^{1}H) and 22.5 MHz (^{13}C). Chemical shifts are relative to internal TMS (δ=0).
[2]Shown with clearly assignable signals.
[3]Multiplicities determined by DEPT spectrum.
[4]Interchangeable in each column.

$C_{42}H_{70}O_2$, dev (–0.7 ppm); ^{1}H and ^{13}C NMR data, see Table 1.

Ergosterol (**2**) : colorless solid, $[\alpha]_D$, IR, UV, MS, ^{1}HNMR and ^{13}C NMR data for ergosterol (**2**) were identical to those reported in the literature (Goad and Akihisa, 1997).

Synthesis of ergosteryl myristate – Myristoyl chloride (0.3 ml) (0.1 mM) was added to a solution of ergosterol (**2**) (200 mg) (0.5 mmol) and *N,N*-dimethylaniline (0.1 ml) (0.8 mM) in pyridine at 0°C under N_2 atmosphere, and then the mixture was stirred for 12 hours at 60°C. The reaction mixture was then poured into water and extracted with AcOEt. The AcOEt extract was washed with aq. HCl (5%) and brine, then dried over $MgSO_4$.

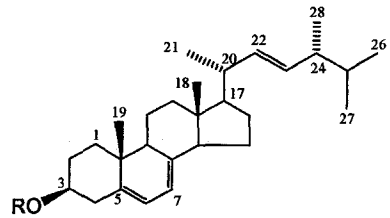
Removal of the solvent under reduced pressure from the AcOEt extract gave a product, which was purified by column chromatography (*n*-hexane-AcOEt = 20:1) to furnish ergosteryl myristate (124 mg).

Synthetic compound was shown to be identical with ergosteryl myristate (**1**) by TLC, $[\alpha]_D$, ^{1}H NMR and ^{13}C NMR.

Results and Discussion

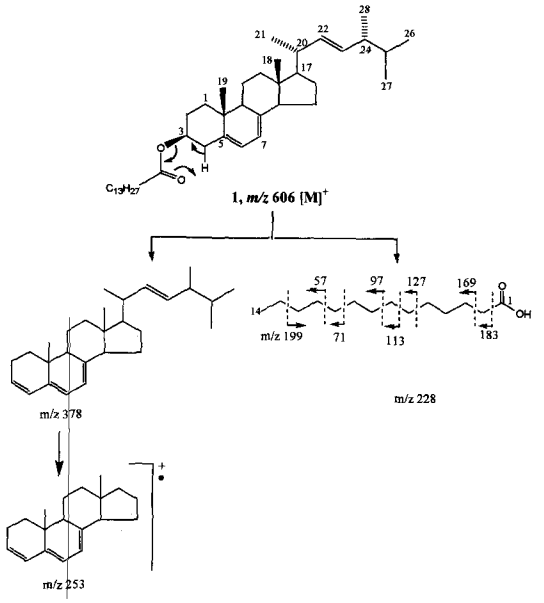
Ergosteryl myristate (**1**) was isolated as a colorless solid which analyzed for $C_{42}H_{70}O_2$ by HREIMS and ^{13}C NMR methods. The IR spectrum of **1** showed band characteristic of an ester functionality (1741, 1200 cm^{-1}). The ^{1}H NMR and ^{13}C NMR spectra of **1** contained one 1,1,4,4-tetrasubstituted conjugated diene, one 1,2-disubstituted double bond, one oxygenated methine carbon, two *tertiary* methyls, four *secondary* methyls, one ester, one terminal methyl and a number of methylenes functionalities (Table 1).

The physicochemical features outlined above suggested that **1** was a fatty acid ester of sterol. Detailed comparison of the data for compound **1** with those of ergosterol (Goad and Akihisa, 1997) and ergosterol acetate (Goad and Akihisa, 1997) illustrated that **1** was ergosteryl myristate. This conclusion was sup-



1, R = - $OCCH_2(CH_2)_{10}CH_2CH_3$
2, R = H

Scheme (1)



Scheme (2)

ported by homoannular conjugated diene in UV spectrum [262 nm (ε8,000) (*sh*), 273(12,000), 282(15,000), 295(7,000)] and by mass fragments of ergosterol moiety (*m/z* 378, 253) and of myristate moiety (*m/z* 228, 199, 183, 169, 127, 113, 97, 71, 57) in mass spectrum (Scheme 1).

On the basis of these data, the structure of **1** was proposed as the ergosteryl myristate. In order to clarify the structure of **1**, we have synthesized ergosteryl myristate from ergosterol and myristoyl chloride using amine base. The spectral data of synthetic compound were identical to those of compound **1** in all aspects. Accordingly, the structure of compound **1** was determined as ergosteryl myristate. Ergosterol was frequently found in yeast and fungi as the main steroid (Parks and Casey, 1995) and transformed to vitamin D_2 by UV irradiation (Kawazoe and Yuasa, 1995).

Ergosterol exerted a regulatory effect on gene transcription in the yeast (Smith *et al.*, 1996), and also inhibited 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation in mice (Yasukawa *et al.*, 1996). Several derivatives of ergosterol, ergosteryl palmitate (Buckingham *et al.*, 1994), ergosteryl galactoside (Takahashi *et al.*, 1991) and ergosterol peroxide (Kim *et al.*, 1997; Mizushima *et al.*, 1998), have been reported. Among them, ergosterol peroxide inhibited tumor-promoting effect of TPA (Mizushima *et al.*, 1998) and showed anticomplementary activity (Kim *et al.*, 1997) as well. Since ergosterol showed diverse functions in mammalian system, the physiological functions of ergosteryl myristate (**1**) is an interesting subject for investigation.

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