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BIOTRANSFORMATION OF CHOLESTEROL BY FREE DISCAL FORM OF BROWN ALGA *Cladosiphon okamuranus* Tokida

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Abstract

The biotransformation of cholesterol by a free discal form of the Okinawan brown alga *Cladosiphon okamuranus* Tokida ("Okinawamozuku") was investigated. The metabolite **2** was obtained after incubation for one week. The structure of **2** (Cholesta-4, 6-dien-3-one) was determined based on spectral analysis using 1D-, 2D-NMR and GC-MS.

Key words : biotransformation, cholesterol, free discal from of marine alga, incubation, *Cladosiphon okamuranus*, cholesta-4, 6-dien-3-one

Introduction

The brown alga, *C. okamuranus* is commonly found in the Okinawan sea, and is traditionally known as an edible seaweed. More recently, it has been found that this seaweed contains useful compounds for human health, such as fucoidan,[1-2] and this has attracted considerable public attention. This organism is one of the brown algae that live in the subtidal zone of South-West islands of Japan.[3] Their sporophytes grow to about 30~40 cm in length and zoospores produced at the sporangia grow to the gametophyte stage through a discal form by settlement on rocks and reefs. The discal form has the ability to continue growing without settlement. Therefore, it is possible to incubate this organism producing a free discal form in sea water with aeration.[4]

The results of a biotransformation experiment using the discal form of *C. okamuranus* confirmed that they can metabolize the exogenous organic substrate, cholesterol (1) by oxidation. In this report, we describe the process of this biotransformation as well as the structural determination of metabolite **2** (**Fig. 1**). Besides, this is the first report about the biotransformation using the free discal form of marine algae.

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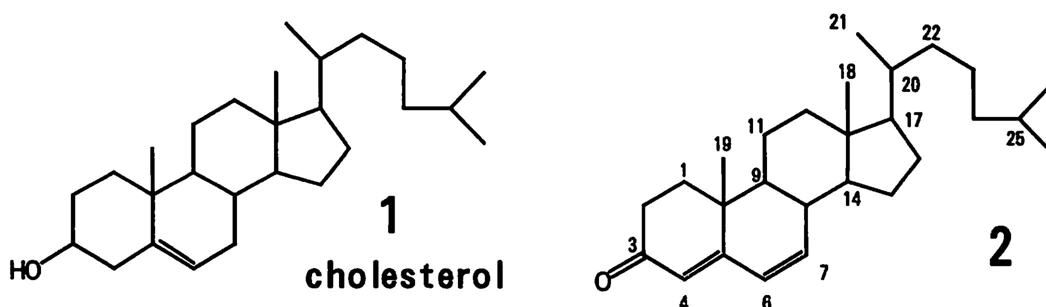


Figure 1. The structures of metabolite **2** and cholesterol **1** as the substrate

Table 1. The NMR spectral data of **2** in $CDCl_3$ [a]

No.	δH [b]	δC	HMBC (H→C)
1	2.21 (2H, dd, J=6.0, 11.0)	37.5 t	C-2, C-10
2	2.36 (2H, t, J=11.0)	42.6 t	C-1, C-3
3		209.1 s	
4	5.53 (1H, s)	137.3 d	C-3, C-5
5		160.2 s	
6	5.56 (1H, d, J=14.5)	136.6 d	C-5, C-7
7	5.64 (1H, dd, J=2.5, 14.5)	127.5 d	C-6, C-8
8	2.01 (1H,m)	35.4 d	C-7, C-9, C-14
9	2.01 (1H, t, J=2.5)	30.5 d	C-8, C-10, C-11
10		41.7 s	
11	2.53, 2.73 (2H, m)	21.2 t	C-9, C-12
12	2.53, 2.73 (2H, m)	28.3 t	C-11, C-13
13		42.4 s	
14	2.74 (1H, dd, J=7.0, 12.5)	51.8 d	C-8, C-13, C-15
15	1.81, 1.55 (2H, m)	24.3 t	C-14, C-16
16	1.42, 2.09 (2H, m)	28.7 t	C-15, C-17
17	1.56 (1H, m)	56.5 d	C-13, C-15, C-16
18	0.67 (3H, s)	12.0 q	C-13
19	1.00 (3H, s)	19.4 q	C-10
20	1.48 (1H, m)	35.8 d	C-17, C-21, C-22
21	0.91 (3H, d, J=6.5)	18.8 q	C-17, C-20
22	1.13, 1.65 (2H, m)	35.6 t	C-20, C-23
23	1.98, 2.19 (2H, m)	23.9 t	C-22, C-24
24	1.13, 1.65 (2H, m)	29.9 t	C-23, C-25
25	2.24 (1H, m)	28.0 d	C-24, C-26, C-27
26	0.87 (3H, d, J=7.0) [c]	22.8 q [c]	C-25, C-27
27	0.87 (3H, d, J=7.0) [c]	22.4 q [c]	C-25, C-26

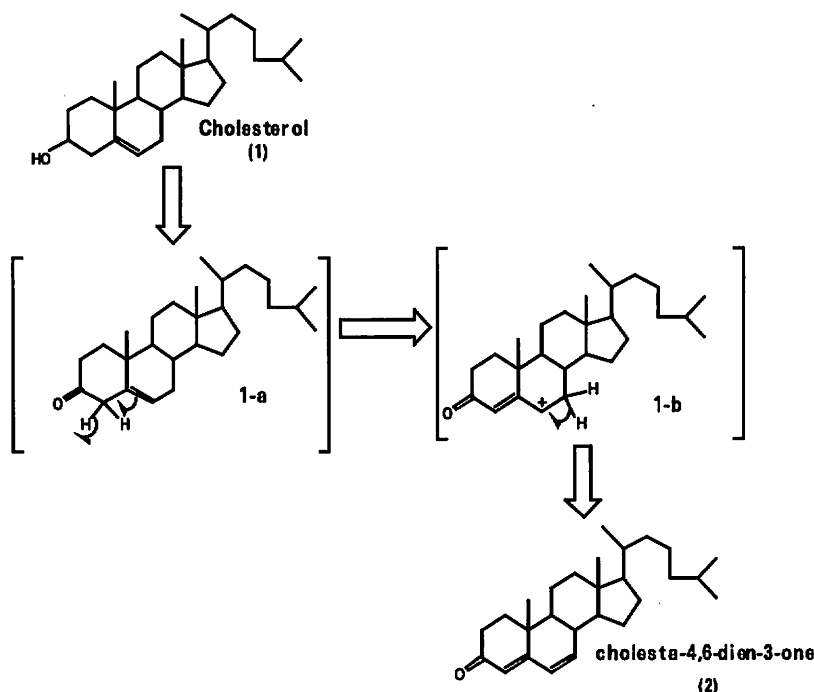
[a] The ^{1}H-NMR and ^{13}C-NMR spectra were measured at 500 MHz.
[b] δ and J values are given in ppm and Hz, respectively.
[c] Assignments are interchangeable.

Results and discussion

The molecular ion peak ($[M]^+$) at m/z 381.8625 detected in HREIMS of **2** was assigned the molecular formula $C_{27}H_{42}O$. The ^{1}H-NMR spectrum **(Table 1)** showed the signals of two olefins at δ 5.70, 5.42 and 5.34, and no signal indicating the presence of hydroxyl group. The ^{13}C-NMR spectrum **(Table 1)** indicated the presences of a carbonyl carbon at δ 209.1 and conjugated olefinic carbons at δ 137.3, 160.2, 136.6 and 127.5. The DEPT and HMQC spectra exhibited the correct carbon multiplicities and heteronuclear couplings between carbon atoms and the protons directly attached on them, respectively. Further information regarding the partial structure of **2** was obtained by analysis of the principal HMBC spectral data. The conjugated olefinic proton at C-4 showed correlations with carbonyl carbon at C-3 and olefinic carbon at C-5. Two conjugated olefinic carbons at C-5 and C-7 showed correlations with the olefinic proton of C-6. Other H-C correlations were observed as shown in the **Table 1**.

Based on these 1D- and 2D-NMR data, the structure of **2** was determined to be cholesta-4, 6-dien-3-one **(Fig.1)**. This compound has a carbonyl group instead of a hydroxyl group at C-3, and two double bonds at C-4 and C-6, when compared with the substrate, cholesterol.

Based on these structural findings, the following metabolism pathway can be proposed **(Scheme 1)**: the hydroxyl group at C-3 of cholesterol (1) was initially oxidized, and was



Scheme 1. The plausible pathway of oxidation reaction

converted into a ketone (**1-a**). The double bond at C-5 was then transferred to C-4 due to elimination of the allyl hydrogen at C-4, and **1-a** was converted to the structure of **2** via the intermediate **1-b**.

We also performed incubation experiments for 2 and 3 weeks under the same conditions. These results suggest the discal form further metabolize the product **2**. The results of these extensional experiments will be reported in a future paper in detail.

Experimental Section

General Experimental Procedures. Cholesterol (99% pure by GC, Yoneyama Chemical Industries Ltd.) was obtained commercially and used without further purification. All NMR spectral analyses were performed on a JEOL α 500 MHz NMR instrument using $CDCl_3$ as solvent with tetramethylsilane (TMS) as internal reference. One-bond heteronuclear ^1H-^{13}C connectivities were determined by HMQC; two- and three-bond connectivities were determined by HMBC. GC-MS (EI) analyses were performed on a Shimadzu GCMS-QP5050 with an ionizing energy of 70 eV. HPLC was performed using a JASCO 880 PU (pump) and 830 RI (refractometer). Merck Si gel 60 was used for column chromatography. All solvents were distilled prior to use. All mediums were autoclaved before using.

Incubation and Isolation. Free discal forms of C. *okamuranus* were incubated in 2 L of sea water with nutrients (Kw 21)[4] and antibiotics (200 ppm of chloramphenicol). One gram (wet wt.) of the discal form was separated from the culture, and moved into a 100 ml round bottom flask containing 80 ml of the above culture medium. Ten milligrams of cholesterol as the substrate was then added, and the flask was incubated for one week with aeration at 23 ℃, under 5000 lx-18 h/day light conditions.[5-8] After one week, the culture was transfused into a 200 ml Erlenmeyer flask, and 100 ml of EtOH was added to stop the reaction. After homogenization, the culture was filtered, concentrated and extracted with ethyl acetate (EtOAc). This process yielded 15 mg of crude extract after concentration of the EtOAc layer.

The GC-MS data for the crude extract showed the presence of a metabolite other than the substrate. Subsequently, 4 mg of **2** was acquired through purification by column chromatography (Si-60) and normal phase HPLC (Hexane-EtOAc solvent system).

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