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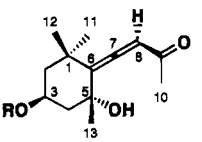
# ISOLATION OF APO-9′-FUCOXANTHINONE FROM THE CULTURED MARINE DINOFLAGELLATE *AMPHIDINIUM* SP.

YUKIKO DOI, MASAMI ISHIBASHI, NAOKO YAMAGUCHI, and JUN'ICHI KOBAYASHI*

*Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan*

ABSTRACT.—An allenic norterpenoid ketone [**1**] was isolated from the cultured marine dinoflagellate *Amphidinium* sp. and was identified by spectral data as (3*R*)-4-[(2*R*,4*S*)-4-acetoxy-2-hydroxy-2,6,6-trimethylcyclohexylidene]but-3-en-2-one, which was previously prepared via oxidative degradation from fucoxanthin. This is the first isolation of compound **1** from a natural source.

During our studies on bioactive substances from marine microalgae, we have investigated extracts of the laboratory-cultured dinoflagellate *Amphidinium* sp. (Gymnodinaceae), which is a symbiont of the marine flatworm *Amphiscolops* sp., and have reported previously a series of cytotoxic macrolides, named amphidinolides, as isolates from this alga (1,2). Further examination of the cytotoxic fraction of this organism has now resulted in the isolation of an allenic compound **1**, exhibiting cytotoxicity against murine lymphoma L-1210 and human epidermoid carcinoma KB cells in vitro with $IC_{50}$ values of 0.29 μg/ml and 0.24 μg/ml, respectively. Compound **1**, apo-9′-fucoxanthinone, was previously reported as a degradation product of fucoxanthin derived through permanganate oxidation (3), and its structure and absolute configuration were established through synthesis and X-ray crystallographic analysis as (3*R*)-4-[(2*R*,4*S*)-4-acetoxy-2-hydroxy-2,6,6-trimethylcyclohexylidene]but-3-en-2-one [**1**] (4). This report describes the isolation, identification, and full spectroscopic characterization of compound **1**.




**1** R=$COCH_3$
**2** R=H

The harvested algal cells (878 g, wet wt, from 3240 liters of culture) were extracted with MeOH-toluene (3:1), and the extracts were partitioned between toluene and $H_2O$. The toluene-soluble fraction was subjected to Si gel flash cc ($CHCl_3$-MeOH, 95:5), and the cytotoxic fractions were subsequently separated by gel filtration on Sephadex LH-20 ($CHCl_3$-MeOH, 1:1) followed by purification with mplc on ODS (59% MeOH) to afford compound **1** in 0.002% yield based on the wet wt of the alga.

Compound **1**, $C_{15}H_{22}O_4$ [hreims, *m/z* 266.1516 ($M^+$), Δ −0.2 mmu], had a characteristic ir absorption band at 1940 $cm^{-1}$, suggesting the presence of an allene group. The $^{13}C$-nmr chemical shifts for the allene moiety [$\delta_C$ 119.9 (C-6), 211.6 (C-7), and 101.5 (C-8)] corresponded approximately to the calculated values ($\delta_C$ 126.3, 201.7, and 101.2, respectively) (5). Spectral studies of compound **1** including 2D nmr experiments (Table 1) were carried out and indicated that compound **1** is apo-9′-fucoxanthinone, an oxidative degradation product of fucoxanthin (3,4). The relative stereochemistry of compound **1** was also confirmed to be identical with apo-9′-fucoxanthinone on the basis of *J* values ($J_{2eq,3}$=4.1 Hz, $J_{2ax,3}$=11.5 Hz, $J_{3,4eq}$=4.1 Hz, and $J_{3,4ax}$=11.5 Hz) and NOESY correlation data (H-3/$H_3$-11, H-2ax/$H_3$-10, and $H_3$-10/$H_3$-13). The cd spectrum of compound **1** ($\Delta\epsilon_{207}$ −3.27, $\Delta\epsilon_{230}$ +3.31, and $\Delta\epsilon_{257}$ −3.13) was almost superimposable upon that of a deacetyl

TABLE 1. $^1$H- and $^{13}$C-Nmr Data of **1** in $CD_3OD$.

| Position | $\delta_H$ | $J$ (Hz) | $\delta_C$ | HMBC correlations |
|---|---|---|---|---|
| 1 ..... | | | 37.2 s | H-2ax, H-4ax, $H_3$-11, $H_3$-12 |
| 2 ax..... | 1.60 dd | 12.7, 11.5 | 46.4 t | H-4ax, H-4eq, $H_3$-11, $H_3$-12 |
| eq..... | 2.04 ddd | 12.7, 4.1, 2.2 | | |
| 3 ..... | 5.42 tt | 11.5, 4.1 | 69.2 d | H-2ax, H-2eq, H-4ax |
| 4 ax..... | 1.49 dd | 12.7, 11.5 | 46.3 t | H-2ax, H-2eq, $H_3$-13 |
| eq..... | 2.27 ddd | 12.7, 4.1, 2.2 | | |
| 5 ..... | | | 72.4 s | H-4eq, $H_3$-13 |
| 6 ..... | | | 119.9 s | H-2eq, H-4eq, H-8, $H_3$-11, $H_3$-12, $H_3$-13 |
| 7 ..... | | | 211.6 s | H-8 |
| 8 ..... | 5.90 s | | 101.5 d | $H_3$-10 |
| 9 ..... | | | 200.9 s | H-8, $H_3$-10 |
| 10 ..... | 2.24 s | | 26.9 q | H-8 |
| 11 ..... | 1.46 s | | 29.5 q | H-2ax, $H_3$-12 |
| 12 ..... | 1.20 s | | 32.3 q | $H_3$-11 |
| 13 ..... | 1.43 s | | 30.8 q | H-4ax |
| 14 ..... | | | 172.5 s | $H_3$-15 |
| 15 ..... | 2.07 s | | 21.4 q | |

derivative (**2**: $\Delta\epsilon_{211}$ −1.83, $\Delta\epsilon_{229}$ +2.98, and $\Delta\epsilon_{255}$ −3.43) described in the literature (4), suggesting that **1** has the 3*S*,5*R*,8*R*-configuration.

This is the first report of the isolation of **1** from a natural source and of its cytotoxicity, although the deacetyl derivative [**2**] has been isolated from antrepellent secretions of the large flightless grasshopper *Romalea microptera* (6) and flowers of *Edgeworthia chrysantha* (7).

## EXPERIMENTAL

ANIMAL MATERIAL.—The taxonomy and cultivation procedures of the alga have been described previously (8).

EXTRACTION AND ISOLATION.—Harvested cells of the cultured dinoflagellate *Amphidinium* sp. (strain #Y-5; 878 g, wet wt, from 3240 liters of culture) were extracted with MeOH-toluene (3:1; 1 liter×3). After addition of 1 M NaCl (1.5 liter), the mixture was extracted with toluene (500 ml×5). The toluene-soluble fraction was evaporated under reduced pressure to give a residue (40 g), of which a portion (26 g) was subjected to flash cc on Si gel (4.5×40 cm) eluted with $CHCl_3$-MeOH (95:5). The fraction eluting from 540 to 940 ml (1.6 g) was partially (950 mg) dissolved in 80% MeOH and passed through a Sep-Pak cartridge $C_{18}$ (9×12 mm, Waters). The eluate (708 mg) was then separated by gel filtration on a Sephadex LH-20 column (Pharmacia, 3.5×108 cm) with $CHCl_3$-MeOH (1:1) to give a fraction (270–330 ml, 86 mg), which was subsequently separated by reversed-phase mplc (CPO-HS-221-20, Kusano Kagakukikai, 22×100 mm, 20 μm; flow rate: 2.5 ml/min; detection: uv at 222 nm and refractive index; eluent: 59% $CH_3CN$) to give compound **1** (*R*, 6.4 min, 10.7 mg, 0.002% yield, wet wt).

*(3R)-4-[(2R,4S)-4-Acetoxy-2-hydroxy-2,6,6-trimethylcyclohexylidene]but-3-en-2-one* [**1**].—Colorless amorphous solid; $[\alpha]^{19}_D$ −284° ($c$=0.1, MeOH); uv (MeOH) λ max 280 (ε 700), 231 nm (2800); ir (KBr) ν max 3400, 2960, 2920, 1940, 1740, 1680, 1360, 1240, 1160, 1030, 750 $cm^{-1}$; cd (EtOH) $\Delta\epsilon_{207}$ −3.27, $\Delta\epsilon_{230}$ +3.31, $\Delta\epsilon_{257}$ −3.13; $^1$H- and $^{13}$C-nmr data, see Table 1; fabms (negative, diethanolamine matrix) *m/z* 265 $(M-H)^-$, 253, 205 $(M-CH_3COOH)$, 162, 148, 132, 86, 59; eims *m/z* 266 $(M^+)$, 251 $(M-CH_3)$, 206 $(M-CH_3COOH)$, 191, 173, 163, 149, 123, 85, 55; found *m/z* 266.1516, calcd for $C_{15}H_{22}O_4$ $(M^+)$, 266.1518.

BIOLOGICAL TESTING.—The cytotoxicity of compound **1** was determined by the procedures described previously (9).

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