Notes

ISOLATION OF 3α-HYDROXY-3,5-DIHYDRO ML-236C (SODIUM SALT) FROM *Paecilomyces viridis* L-68

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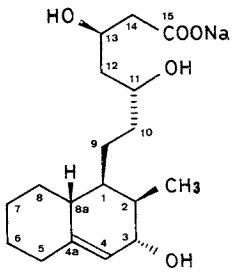
3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is the major rate-limiting enzyme in cholesterol biosynthesis. Potent inhibitors of HMG-CoA reductase have been isolated from *Penicillium citrinum* and several other fungal strains, which include ML-236B (compactin), ML-236A, ML-236C and 4a,5-dihydrocompactin[1~3]. This communication describes the isolation of 3α-hydroxy-3,5-dihydro ML-236C (sodium salt) (**1**), a new metabolite of the compactin family, from *Paecilomyces viridis* L-68 (Fig. 1).

P. viridis L-68 was grown aerobically at 25°C for 7 days in a medium containing 3% glucose, 2% glycerol, 3% soybean meal, 0.8% peptone, 0.2% $NaNO_3$ and 0.1% $MgSO_4 \cdot 7H_2O$. Under these growth conditions *P. viridis* L-68 produced ~20 μg/ml of **1**, which was detected by monitoring the UV absorption at 210 nm in HPLC (see below). The culture filtrate (2.5 liters) was adjusted to pH 10 with NaOH and passed through a column of Diaion HP-20 (3.6 × 26 cm) packed with water. The column was washed with water (1.2 liters) and then developed with a mixture of acetonitrile - water (2 : 3, 0.5 liter). The eluate containing **1** (62 ml) was concentrated to 35 ml under reduced pressure. The concentrated solution was adjusted to pH 3 with HCl and extracted three times with 30 ml of ethyl acetate. The solvent layers were mixed with first 10 ml and then 5 ml of water at pH 7.5 (adjusted with NaOH). The aqueous layers were pooled, adjusted to pH 10.0 with NaOH and then applied to a Diaion HP-20SS column (1.0 × 19.5 cm). After washing with 50 ml of water, the column was developed with a 400-ml linear gradient of 0 to 20% acetone. The active eluate obtained (66 ml) was concentrated under reduced pressure and then lyophilized to give 350 mg of residue. This material was dissolved in 2.5 ml of water and submitted to HPLC in a column of Inertsil PREP-ODS (30 × 250 mm, GL Sciences Co., Japan), using a solvent system of acetonitrile - 0.1% H_3PO_4 (30 : 70). Fractions containing **1** were pooled and applied to the same column, which was developed with a mixture of acetonitrile and 0.1% H_3PO_4 (25 : 75). The active eluate (150 ml) was extracted with 200 ml of ethyl acetate. The solvent layer was washed twice with 20 ml of water. The resultant solvent layer was mixed with 15 ml of water, followed by adjusting pH to 7.5 with NaOH. The aqueous layer was pooled and lyophilized, giving 27.1 mg of **1**.

Fig. 1. Sodium 3α-hydroxy-3,5-dihydro ML-236C (**1**).



1

The sodium salt of 3α-hydroxy-3,5-dihydro ML-236C is a white amorphous solid. It showed no UV spectrum characteristic of the conjugated double bond of the fused ring system. It was unstable under acidic conditions, giving ML-236C and its lactone[1].

Physico-chemical properties of **1** (sodium salt) are summarized in Table 1. The molecular formula of **1** (sodium salt) is determined to be $C_{18}H_{29}O_5Na$ by HRSI-MS spectrometry. The pseudo-molecular ion peak of **1** (sodium salt) is 18 mass units higher than that of ML-236C. ^{13}C NMR and DEPT experiments displayed one trisubstituted double bond as well as the presence of one carbonyl, three oxygenated methine, three aliphatic methine, eight methylene

Table 1. Physico-chemical properties of **1** (sodium salt).

Appearance	White amorphous powder
Molecular formula	$C_{18}H_{29}O_5Na$
SI-MS (*m/z*)	347 $(M-H)^-$, 325 $(M-Na)^-$
HRSI-MS (*m/z*)	
Found:	325.2014 $(M-Na)^-$
Calcd:	325.2014 for $C_{18}H_{29}O_5$
UV (H_2O)	End absorption
IR (KBr) (cm^{-1})	3410, 2910, 1560, 1410

Table 2. ^{13}C and ^{1}H NMR data of **1** (sodium salt)[a].

No.	^{13}C (δ)	^{1}H (δ)
1	40.8 (d)	1.73 (1H, m[b])[c]
2	37.0 (d)	1.87 (1H, m)
2-CH_3	12.6 (q)	0.84 (3H, d, 6.7)
3	71.4 (d)	3.81 (1H, br dd, 2.9, 4.3)
4	120.8 (d)	5.43 (1H, br d, 4.3)
4a	145.6 (s)	—
5	36.7 (t)	2.03 (1H, br t, 12.3), 2.27 (1H, br d, 12.3)
6	29.0 (t)	1.32 (1H, m[b]), 1.83 (1H, m[b])
7	27.6 (t)	1.44 (1H, m[b]), 1.83 (1H, m[b])
8	34.5 (t)	1.11 (1H, br q, 12.0), 2.07 (1H, br d, 12.0)
8a	42.1 (d)	1.72 (1H, m[b])
9	25.9 (t)	1.48 (2H, m[b])
10	36.6 (t)	1.52 (2H, m[b])
11	71.4 (d)	3.81 (1H, m[b])
12	44.9 (t)	1.65 (2H, t, 6.7)
13	69.3 (d)	4.14 (1H, ddt, 5.5, 6.7, 7.3)
14	45.5 (t)	2.32 (1H, dd, 7.3, 15.3), 2.41 (1H, dd, 5.5, 15.3)
15	180.5 (s)	—

[a] ^{13}C NMR (67.5 MHz) and ^{1}H NMR (270 MHz) were measured in CD_3OD at 25°C.
[b] Overlapping multiplets.
[c] Proton number, multiplicity and coupling constants in Hz are indicated in parentheses.

and one methyl groups. Based on COSY spectral data, one of the oxygenated methine signal at 3.81 ppm (3-H) showed coupling to a methyl substituted methine signal at 1.87 ppm (2-H) and to an olefinic methine signal at 5.43 ppm (4-H). Since this olefinic methine proton signal showed long-range coupling to methylene carbon at 36.7 ppm (C-5) in correlation spectroscopy *via* long-range couplings (COLOC), the trisubstituted double bond was determined to be at the ring junction of 3-hydroxy 4-ene decalin system. Assignments for the remaining part of the molecule were comparable to those for ML-236C. The ^{1}H and ^{13}C assignments are listed in Table 2.

Irradiation of the 2-methyl showed enhancement of 3.5% to 8a-H, 7.0% to 9-H_2, 7.5% to 2-H and 9.6% to 3-H in NOE difference spectroscopy, respectively. Thus the configuration of the 3-hydroxy was assigned as α. The ^{1}H spin-spin coupling constants between this oxygen bearing methine proton (3-H) and 2-H and 4-H were $J=2.9$ and 4.3 Hz, respectively. These results were comparable to those for 3α-hydroxy-3,5-dihydromonacolin L[4], phenacyloxy derivative of 3α-hydroxy-3,5-dihydromonacolin L[5] and 3α,5β-dihydroxy ML-236B[6].

When **1** was incubated at pH 2.0 and 25°C for 10 hours, it was converted quantitatively to a lactone derivative. The converted lactone was purified and analyzed spectrophotometrically. All the spectral data including $[\alpha]_D$ were identical to those for authentic ML-236C (data not shown). Thus the relative configurations of 11-H and 13-OH of **1** could be assigned as β.

The *in vitro* activity of HMG-CoA reductase, assayed as described previously[7], was inhibited approximately 50% by **1** at 10 μM.

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