Notes

MONACOLINS J AND L, NEW INHIBITORS OF CHOLESTEROL BIOSYNTHESIS PRODUCED BY *MONASCUS RUBER*

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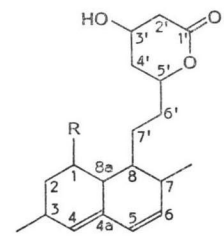
There is considerable interest in the discovery of hypocholesterolemic drugs whose mode of action is the inhibition of sterol biosynthesis. Culture broths of microorganisms have yielded active compounds that specifically inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis. The first of these to be described was ML-236B (compactin)[1]. Its isolation from *Penicillium citrinum* and hypocholesterolemic properties were extensively studied by ENDO and his associates[2,3]. The clinical efficacy of ML-236B in lowering plasma cholesterol has also been reported[2~5].

Later, a more potent relative of ML-236B, designated monacolin K (mevinolin), was isolated by ENDO and ALBERTS *et al.* from *Monascus ruber*[6,7] and *Aspergillus terreus*[8]. Along with these two compounds, several metabolites related to either ML-236B or monacolin K were isolated[1,9,10]. The present communication describes the isolation, structure and some of the biological properties of two new compounds designated monacolins J and L (Fig. 1).

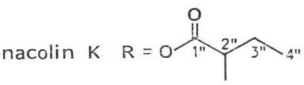
Monascus ruber No. 1005 was grown aerobically in a medium containing glucose 6%, peptone 2.5%, corn steep liquor (Corn Products Co., U.S.A.) 0.5%, ammonium chloride 0.5% at 25°C for 10 days. The culture filtrate (5 liters) was adjusted to pH 3 with HCl and extracted with ethyl acetate (5 liters). The solvent layer was dried over Na_2SO_4, filtered, and then evaporated to dryness *in vacuo*. A benzene solution of the oily residue was, after filtration, washed twice with 5% $NaHCO_3$ (100 ml) and then mixed with 100 ml of 0.2 M NaOH. The mixture was stirred at room temperature for 2 hours to hydrolyze the δ-lactone ring. The aqueous layer was adjusted to pH 3 with HCl and then extracted twice with ethyl acetate (100 ml). The solvent layers were dried over Na_2SO_4 and then evaporated to dryness. The resulting oily residue (260 mg) was dissolved in a small volume of benzene; crystals formed were removed by filtration, and purified by chromatography in a silica gel (Wako gel C-200) column (10 g). The column was developed first with 50 ml of dichloromethane, then with 600 ml of dichloromethane - ethyl acetate (9:1) followed by 900 ml of dichloromethane - ethyl acetate (8:2) to elute monacolins L, K and J, respectively. The dichloromethane fraction was evaporated to dryness and applied to a silica gel column (Wako gel C-200, 5 g). The column was washed with 30 ml of *n*-hexane, then developed with 500 ml of *n*-hexane - acetone (9:1) to elute monacolin L. The active fraction was evaporated to dryness and dissolved in 10 ml of benzene. The solution was washed twice with 5 ml of 5% $NaHCO_3$, dried over Na_2SO_4 and evaporated to dryness, yielding 24 mg (white powder) of monacolin L.

The dichloromethane - ethyl acete (8:2) fraction obtained above was evaporated to dryness, dissolved in 10 ml of benzene and washed twice with 5 ml of 5% $NaHCO_3$. The benzene solu-

Fig. 1.



Monacolin J R = OH



Monacolin L R = H

Table 1. Assignment of ^1H NMR spectra of monacolins J, K and L by ^1H shift correlated 2D-NMR (360 MHz, $CDCl_3$, TMS).

Assignment	Monacolin J (ppm)	Monacolin K (ppm)	Monacolin L (ppm)
H-1	4.23	5.38	1.18
			1.76
H-2	1.88 (2H)	1.95 (2H)	1.58
			1.70
H-3	2.46	2.45	2.33
H-3-CH_3	1.19 (3H)d	1.08 (3H)d	0.98 (3H)d
H-4	5.54	5.53	5.43
H-5	5.98 d	5.99 d	5.91 d
H-6	5.80 dd	5.79 dd	5.72 dd
H-7	2.38	2.38	2.31
H-7-CH_3	0.90 (3H)d	0.90 (3H)d	0.89 (3H)d
H-8	1.82	1.72	1.38
H-8a	2.16 dd	2.27 dd	2.05
H-2′ eq	2.63 ddd	2.63 ddd	2.64 ddd
H-2′ ax	2.72 dd	2.72 dd	2.72 dd
H-3′	4.38	4.36	4.38
H-4′ ax	1.75	1.65	1.80
H-4′ eq	1.98	1.98	1.97
H-5′	4.72	4.63	4.72
H-6′	1.53	1.29	1.47
	1.78	1.89	1.82
H-7′	1.48	1.38	1.42
	1.83	1.48	1.77
H-2″	—	2.36	—
H-2″-CH_3	—	1.11 (3H)d	—
H-3″	—	1.42	—
		1.63	
H-4″	—	0.88 (3H)t	—

tion was dried over Na_2SO_4 and evaporated to dryness, giving 43 mg (powder) of monacolin J.

Exact mass measurements of monacolin J established its molecular formula to be $C_{19}H_{28}O_4$. Except for the difference in molecular ion peak at m/z 320 for monacolin J and 404 for monacolin K, the mass spectrum of monacolin J was very similar to that of monacolin K[6,8]. In addition to peaks at m/z 320 (M^+), 302 (M−18) and 284 (M−36), prominent peaks at 269 (M−51), 224 (M−96), 198 (M−122), 172 (M−148), 159 (M−161, 100%) and 157 (M−163) were observed. The ^1H NMR spectrum ($CDCl_3$) showed resonances in complete agreement with the assigned structure (Fig. 1). In comparison with those of ML-236B (compactin)[12], the ^1H NMR spectrum of monacolin J as well as those for monacolins K and L were completely interpreted (Table 1). Strong IR absorbance of monacolin J near 1720 cm^{-1} is in part assigned for the six-membered lactone. Consistent with the high-resolution mass spectroscopy measurements, the ^{13}C NMR spectrum on gated decoupling indicated a total of 19 carbons consisting of two methyl, five methylene, and four methine carbons in the high-field region, three methine carbons carrying oxygen substituents, four olefinic signals consistent with a trisubstituted diene, and one ester carbonyl. UV absorbance at 230 (ε 22,100), 237 (25,600) and 247 nm (16,100) was identical to those of ML-236B and monacolin K.

Mass spectrometric analysis of monacolin L gave a molecular formula of $C_{19}H_{28}O_3$. In addition to a molecular ion peak at m/z 304 (M^+), prominent peaks were observed at m/z 286 (M−18), 200 (M−104), 174 (M−130), 159 (M−145, 100%) and 145 (M−159). This fragmentation pattern closely parallels that of ML-236C[1]. As shown in Table 1, the H-1 observed at 4.23 ppm (1H) and 5.38 (1H) in monacolins J and K respectively, shifted to the methylene or methine region (1.18 ppm, 1H; 1.76 ppm, 1H), suggesting that the hydroxyl group at C-10 of monacolin J is substituted by hydrogen in monacolin L. The ^{13}C NMR spectrum showed a total of 19 carbons including two methyl, six methylene, and four methine carbons in the high-field region and two oxygen-bearing carbons. IR absorbance of monacolin L was observed at 1700, 2950 and 3400 cm^{-1}. UV absorbance at 230 (ε 20,300), 237 (25,100) and 247 nm (15,900) was identical to that of monacolins J and K. From the data described above, structure of monacolin L was determined as shown in Fig. 1.

Rat liver microsomal HMG-CoA reductase was isolated and assayed as described by ENDO *et al.*[11]. Concentrations required for 50% inhibition of the reductase (I_{50}) were 1.2 and 0.94 μg/ml for Na salts (open hydroxycarboxylate) of monacolins J and L, respectively. Under the same conditions, I_{50} values were 0.38 and 0.69 μg/ml for Na salts of monacolin K and ML-236B, respectively.

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