DIHYDROCOMPACTIN, A NEW POTENT INHIBITOR OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME-A REDUCTASE FROM *PENICILLIUM CITRINUM*

Sir:

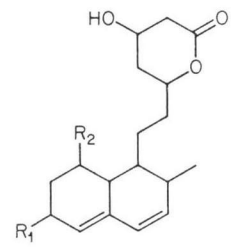
Compactin (Fig. 1) was first isolated from *Penicillium brevicompactum* by BROWN *et al.*[1] as an antifungal metabolite. Later, ENDO *et al.*[2] reported their isolation of compactin (designated as ML-236B) together with ML-236A and ML-236C as hypocholesteremic agents from *Penicillium citrinum*. ENDO also reported monacolin K from *Monascus ruber*[3]. Independently, the same compound (named mevinolin) was isolated by ALBERTS *et al.*[4] from *Aspergillus terreus*. 4a, 5-Dihydromevinolin (Fig. 2)* was also isolated from the same culture.[5] All of these five structurally-related entities are potent competitive inhibitors of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase (EC 1.1.1.34)[2~7], the rate-limiting enzyme in cholesterol synthesis, with mevinolin being the most potent. However, compactin is the most extensively studied. Its hypocholesteremic acitivity in several animal species including human subjects has been demonstrated[8~12], and a review of its pharmacology was also published[13].

In our search for new microbial metabolites having HMG-CoA reductase inhibition and ultimately hypocholesteremic activities, we have regrown *P. citrinum* in our laboratories and discovered a new component, designated as 4a,5-dihydrocompactin. This new compound apparently cannot be derived from compactin stereospecifically without lengthy chemical conversions. The present communication describes the isolation, physical and chemical properties of this unique compound. The inhibition of HMG-CoA reductase is also described.

P. citrinum (SANK 18767) was obtained as NRRL-8082[14] from the Northern Regional Research Laboratory, Northern Central Region, Agricultural Research Service, United States Department of Agriculture, Peoria, Illinois, U.S.A. It was grown aerobically in a medium containing 2% dextrose, 2% glycerol, 1% ardamine, 8 ppm $CoCl_2 \cdot 6H_2O$, and 0.25% polyglycol

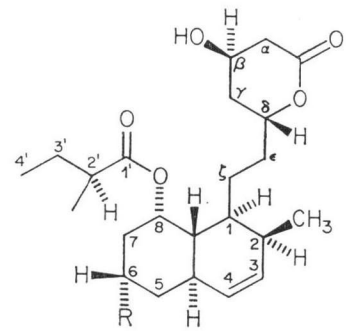
* The present numbering system conforms with the one used in ref. 4 for simplicity.

Fig. 1. Structures of the lactone forms of ML-236A, ML-236B (compactin), ML-236C and monacolin K (mevinolin).



ML-236A	$R_1 = -H$,	$R_2 = -OH$
ML-236B	$R_1 = -H$,	$R_2 = -OCOCH(CH_3)CH_2CH_3$
ML-236C	$R_1 = -H$,	$R_2 = -H$
Monacolin K	$R_1 = -CH_3$,	$R_2 = -OCOCH(CH_3)CH_2CH_3$

Fig. 2. Structures of the lactone forms of 4a,5-dihydrocompactin and 4a,5-dihydromevinolin.



4a,5-Dihydrocompactin R=-H
4a,5-Dihydromevinolin R=-CH_3

P2000 at pH 7.0 for 4 days. The culture filtrate (10 liters) was extracted with ethyl acetate (5 liters) at pH 4 (adjusted with 1 M HCl). The ethyl acetate layer was extracted with 0.5 M NaOH (2×2 liters). After being adjusted to pH 4 with 1 M HCl, the combined NaOH solutions were extracted with ethyl acetate (2 liters and 1 liter). The combined ethyl acetate layers were dried over Na_2SO_4, filtered, and then evaporated to dryness *in vacuo*. A toluene solution of the oily residue was refluxed for one hour to facilitate lactonization. After filtration, the toluene solution was evaporated to dryness *in vacuo* and the residue was dissolved in 18 ml hexane - toluene - methanol (4: 1: 1) and purified by partition chromatography in a Sephadex LH-20 column (3× 40 cm) equilibrated in the same solvent system. After eluting with 300 ml of solvent, a 10 ml

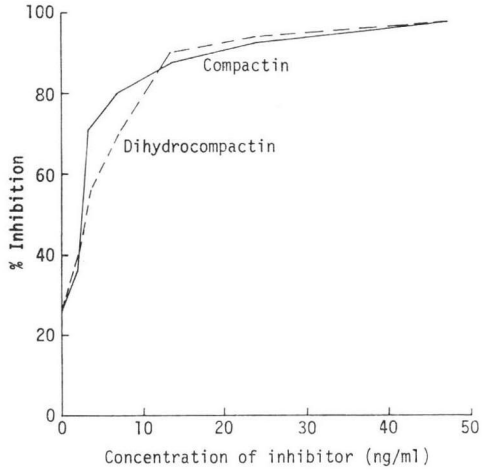
fraction was obtained which yielded an oil on concentration to dryness *in vacuo*. Reverse phase high pressure liquid chromatography of this material on an ES Industries Chromegabond C-18 column (0.9×50 cm) using acetonitrile - water (60: 40) as the mobile phase yielded 45 mg of homogeneous (by TLC and HPLC) 4a,5-dihydrocompactin.

Exact mass measurements of 4a,5-dihydrocompactin established its molecular formula to be $C_{23}H_{36}O_5$ (Calcd: 392.2560; Found: 392.2558). Except for the obvious difference in mass units, the mass spectrum of our compound compared well with that of monacolin K published in reference 3 and the data for compactin in reference 1. In addition to peaks at *m/z* 392 (M^+), 307 (M−85), 290 (M−102), and 272 (M−120), prominent peaks at 186 (M−206), 159 (M−233) and 145 (M−247) were observed. The FT-IR spectrum (KBr) displayed absorptions at 3424, 2965, 1724 (lactone C=O), 1704 (ester C=O), 1258 and 1239 cm^{-1}. The ^{1}H-NMR spectrum ($CDCl_3$) showed resonances in complete agreement with the assigned structure. In comparison with those of compactin and dihydromevinolin, the following proton assignments were observed: δ 5.60 (1H, m, H-3), δ 5.43 (1H, bd, J=10 Hz, H-4), δ 5.20 (1H, dt, J=2.5 Hz, H-8), δ 4.73 (1H, m, H-δ), δ 4.39 (1H, m, H-β), δ 2.75 (1H, dd, J=5, 18 Hz, H-α_a), δ 2.61 (1H, ddd, J=1.5, 4, 18 Hz, H-α_e), δ 1.12 (3H, d, J=7 Hz, C-2′-CH_3), δ 0.90 (3H, t, J=7 Hz, H-4′), δ 0.85 (3H, d, J=7 Hz, C-2-CH_3). In particular, the transoid stereochemistry at the ring juncture of the decalin system was strongly favored by the appearance of H-8a as a doublet (J=2.5 Hz) of a triplet (J=11 Hz) at δ 1.22. A large coupling of 11 Hz between the diaxial protons H-4a and H-8a was also observed for dihydromevinolin[5]. The Rf values on TLC (E. Merck, Kieselgel 60) were 0.40 in ethyl acetate - dichloromethane (4: 6) and 0.18 in hexane - acetone (1: 1).

Rat liver HMG-CoA reductase preparation and assay were performed as described by ALBERTS *et al.*[4] Fig. 3 clearly shows the similarity in potency between the inhibition of HMG-CoA reductase by compactin and dihydrocompactin. The I_{50} for both Na^+ salts (open hydroxy-carboxylate) are approximately 2.5 ng/ml. In a separate experiment, the *Ki*'s of the ammonium salts were determined to be 3.7 nM for dihydrocompactin and 3.8 nM for compactin.

Fig. 3. % Inhibition of rat liver HMG-CoA reductase by Na^+ salts of compactin and dihydrocompactin.

The preparation and assay of HMG-CoA reductase were as described by ALBERTS *et al.*[4]



The compactin used as reference in this work was isolated from the same culture as dihydrocompactin in our laboratories and fully characterized to be identical to that described in references 1 and 14 by IR, NMR, UV and mass spectrometries, optical rotation, elemental analysis, and m.p. (149~150°C).

Acknowledgments

The authors wish to thank Dr. D. D. SAPERSTEIN and Mr. J. L. SMITH for recording infrared and mass spectra respectively, and Ms. J. CHEN and Mr. R. JETER for their assistance in preparing HMG-CoA reductase.

Y. K. TONY LAM
V. P. GULLO
R. T. GOEGELMAN
D. JORN
L. HUANG
C. DERISO
R. L. MONAGHAN
I. PUTTER
Merck Sharp & Dohme Research Laboratories
Rahway, N. J. 07065, U.S.A.

(Received January 19, 1981)

References

1) BROWN, A. G.; T. C. SMALE, T. J. KING, R. HASENKAMP & R. H. THOMPSON: Crystal and

molecular structure of compactin, a new antifungal metabolite from *Penicillium brevicompactum*. J. Chem. Soc. Perkin I 1976: 1165~1170, 1976

2) ENDO, A.; M. KURODA & Y. TSUJITA: ML-236A, ML-236B, and ML-236C, new inhibitors of cholesterogenesis produced by *Penicillium citrinum*. J. Antibiotics 29: 1346~1348, 1976
3) ENDO, A.: Monacolin K, a new hypocholesterolemic agent produced by a *Monascus* species. J. Antibiotics 32: 852~854, 1979
4) ALBERTS, A. W.; J. CHEN, G. KURON, V. HUNT, J. HUFF, C. HOFFMAN, J. ROTHROCK, M. LOPEZ, H. JOSHUA, E. HARRIS, A. PATCHETT, R. MONAGHAN, S. CURRIE, E. STAPLEY, G. ALBERS-SCHÖNBERG, O. HENSENS, J. HIRSHFIELD, K. HOOGSTEEN, J. LIESCH & J. SPRINGER: Mevinolin, a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and cholesterol-lowering agent. Proc. Natl. Acad. Sci. U.S.A. 77: 3957~3961, 1980
5) ALBERS-SCHÖNBERG, G.; H. JOSHUA, M. LOPEZ, O. HENSENS, J. SPRINGER, J. CHEN, S. OSTROVE, C. HOFFMAN, A. ALBERTS & A. PATCHETT: Dihydromevinolin, a potent hypocholesterolemic metabolite produced by *Aspergillus terreus*. J. Antibiotics 34: 507~512, 1981
6) ENDO, A.; M. KURODA & K. TANZAWA: Competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase by ML-236A and ML-236B, fungal metabolites having hypocholesterolemic activity. FEBS Lett. 72: 323~326, 1976
7) ENDO, A.: Monacolin K, a new hypocholesterolemic agent that specifically inhibits 3-hydroxy-3-methylglutaryl coenzyme A reductase. J. Antibiotics 33: 334~336, 1980
8) KURODA, M.; K. TANZAWA, Y. TSUJITA & A. ENDO: Mechanism for elevation of hepatic cholesterol synthesis and serum cholesterol levels in Triton WR-1339-induced hypercholesterolemia. Biochim. Biophys. Acta 489: 119~125, 1977
9) TSUJITA, Y.; M. KURODA, K. TANZAWA, N. KITANO & A. ENDO: Hypolipidemic effects in dogs of ML-236B, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Atherosclerosis 32: 307~313, 1979
10) KURODA, M.; Y. TSUJITA, K. TANZAWA & A. ENDO: Hypolipidemic effects in monkeys of ML-236B, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Lipids 14: 585~589, 1979
11) ENDO, A.; Y. TSUJITA, M. KURODA & K. TANZAWA: Effects of ML-236B on cholesterol metabolism in mice and rats: Lack of hypocholesterolemic activity in normal animals. Biochim. Biophys. Acta 575: 266~276, 1979
12) YAMAMOTO, A.; H. SUDO & A. ENDO: Therapeutic effects of ML-236B in primary hypercholesterolemia. Atherosclerosis 35: 259~266, 1980
13) ENDO, A.: Pharmacology of ML-236B, a cholesterol synthesis inhibitor. Kagaku to Seibutsu 17: 672~676, 1979 (in Japanese)
14) ENDO, A.; M. KURODA, A. TERAHARA, Y. TSUJITA & C. TAMURA: Physiologically active substances and fermentative process for producing the same. U.S. Pat. 4,049,495, Sept. 20, 1977