Tetrahedron Letters,Vol.23,No.46,pp 4747-4750,1982 0040-4039/82/464747-04$03.00/0
Printed in Great Britain

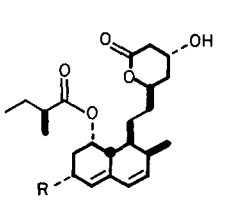
SYNTHESIS AND BIOLOGICAL ACTIVITY OF THE HEXALIN MOIETY OF COMPACTIN (ML-236B)

Clayton H. Heathcock, Michael J. Taschner, Terry Rosen, James A. Thomas, Cheri R. Hadley and George Popják**

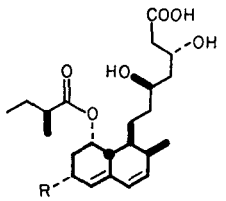
Department of Chemistry, University of California, Berkeley, CA 94720 and Department of Biological Chemistry, School of Medicine, University of California, Los Angeles, CA 90024

Summary: *Alcohols* **14** *and* **21** *and the derived half-glutarate esters* **15** *and* **22** *have been prepared and evaluated as inhibitors of HMG CoA reductase. Compound* **14**, *the hexalin moiety of the natural fungal metabolite compactin (ML-236B) and compound* **21**, *its isomeric diene, have essentially no inhibitory properties. Compounds* **15** *and* **22** *show definite activity, although several orders of magnitude lower than that shown by compactin.*

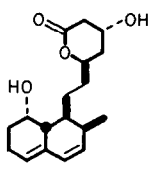
Compactin (**1**),[1] also known as ML-236B,[2] and the related compound mevinolin (**2**)[3] are potent inhibitors of the enzyme HMG CoA reductase. Because this enzyme mediates the rate-limiting step in cholesterol biosynthesis, compounds that affect its activity are of potential importance as hypocholesterolemic drugs. Indeed, it has recently been found in clinical trials that compactin effectively reduces serum cholesterol levels in patients with hypercholesterolemia.[4]



1: R = H
2: R = Me



3: R = H
4: R = Me

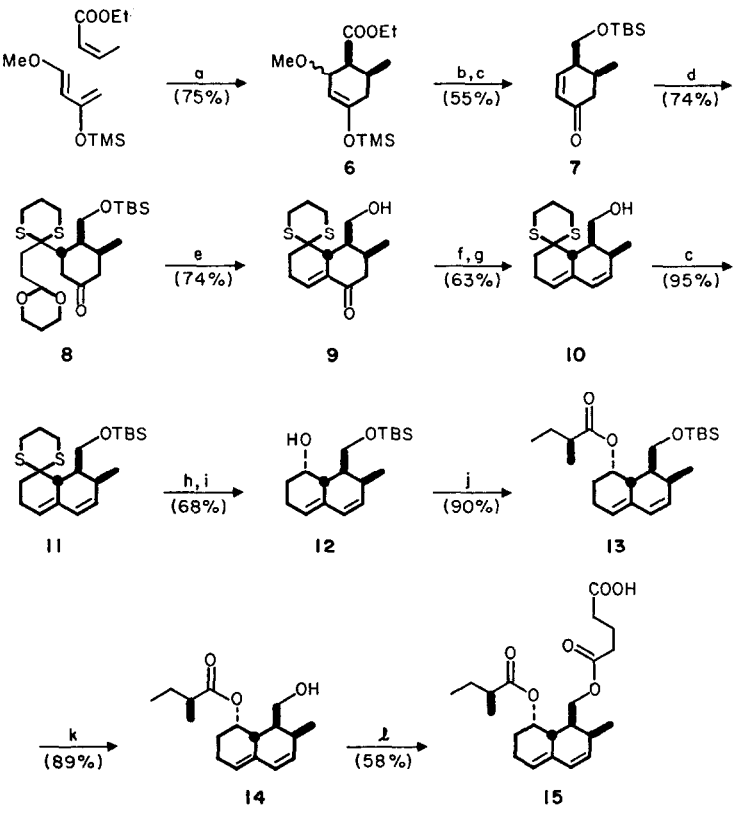


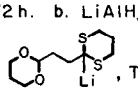
5

One of the interesting questions in connection with the inhibitory activity of compactin and mevinolin is the function of the hexalin portion of the molecule. It is known that the active forms of the inhibitors are the dihydroxy acids **3**[5] and **4**[3], and that they bind at the active site for at least the first step of the two-stage reduction of HMG CoA to mevalonic acid.[2b] The 3,5-dihydroxypentanoic acid side-chain in **3** and **4** bears a close structural resemblance to the natural substrate and product of the reduction, and it is probable that this portion contributes an important part of the binding affinity. In fact, compounds **3** and **4** may function as transition state analogs for this reduction.[6] However, the structure of the hexalin unit is also important, since the additional methyl group in mevinolin confers additional activity[3] and removal of the (*S*)-2-methylbutanoyl group to give ML-236A (**5**) results in greatly diminished activity.[5] One interpretation of these observations is that the hexalin portion of the molecule plays an important role in binding compounds **3** and **4** to the active site of HMG CoA reductase. Thus, it might be expected that this part of the molecule, excised from the 3,5-dihydroxypentanoic acid residue, would function as a competitive inhibitor of the enzyme. In this letter, we report the total synthesis and biological evaluation of alcohol **14**, its half glutarate ester **15** and the isomeric dienes **21** and **22**.[7]

The synthesis of compounds **14** and **15** is summarized in Scheme I. Thermal cycloaddition of ethyl (*Z*)-crotonate[9] and Danishefsky's diene[10] provides an adduct (**6**) which is reduced with lithium aluminum hydride to produce, after hydrolytic workup and protection of the primary alcohol with *t*-butyldimethylsilyl chloride, the enone ether **7**. This material is subjected to a cyclohexene annelation process previously developed for this purpose[11] to obtain enone **9**. Treatment of the derived 1,3,5-triisopropylbenzenesulfonyl hydrazone[12] with four equivalents of *n*-butyllithium provides diene **10**, which is reprotected to obtain **11**. Hydrolysis of the dithiane group,[13] followed by reduction of the resulting ketone with L-selectride[14] affords axial alcohol **12**, which is esterified with (*S*)-2-methylbutanoic anhydride[8a] to obtain ester **13**.[15] Desilylation of **13** affords alcohol **14**, which is treated with glutaric anhydride to obtain diester acid **15**.

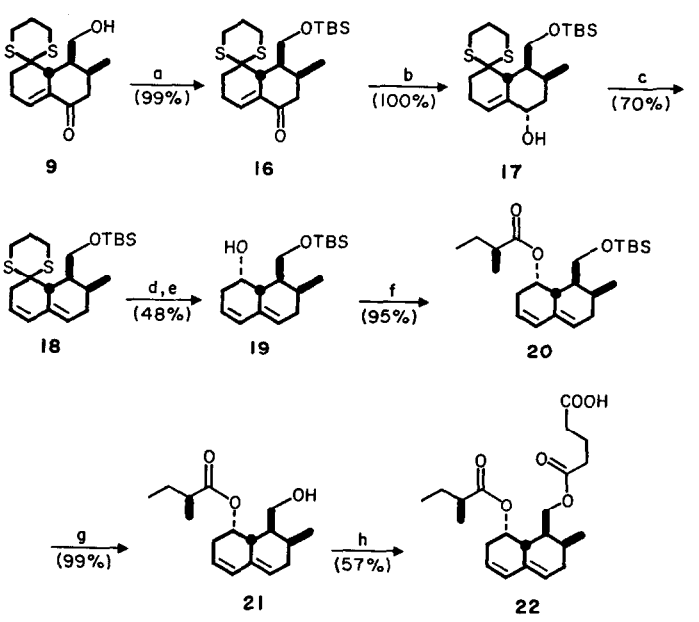
Scheme I



a. 145°C, 72 h. b. $LiAlH_4$, ether, -78°C. c. *t*-$BuMe_2SiCl$, Et_3N, DMAP, CH_2Cl_2. d.  , THF, HMPT, -78°C. e. 10% HCl, MeOH, reflux. f. 2,4,6-$(i\text{-}Pr)_3C_6H_2SO_2NHNH_2$, MeOH, HCl. g. *n*-BuLi, hexane, TMEDA. h. NCS, $AgNO_3$, collidine, 0°C. i. $LiHB(s\text{-}Bu)_3$. j. (*S*)-2-methylbutanoic anhydride, Et_3N, DMAP, CH_2Cl_2. k. *n*-$Bu_4N^+F^-$, THF. l. glutaric anhydride, Et_3N, DMAP, CH_2Cl_2.

The isomeric diene **21** and its glutarate ester **22** are obtained by the modified synthesis summarized in Scheme II. Reduction of enone **16** with sodium borohydride-ceric chloride[16] provides the equatorial allylic alcohol **17**, which undergoes smooth dehydration upon treatment with pyridinium *p*-toluenesulfonate[17] in refluxing 1,2-dichloroethane to give dienes **18** and **11** in a ratio of 92:8. The major isomer from this dehydration is converted into alcohol **21** and the derived glutarate **22**[15] in the same manner as is used for the preparation of **14** and **15**.

Scheme II



a. *t*-$BuMe_2SiCl$, Et_3N, DMAP, CH_2Cl_2. b. $NaBH_4$, $CeCl_3$, MeOH. c. PPTS, $ClCH_2CH_2Cl$, reflux. d. NCS, $AgNO_3$, collidine, 0°C. e. $LiHB(s\text{-}Bu)_3$. f. (*S*)-2-methylbutanoic anhydride, Et_3N, DMAP, CH_2Cl_2. g. $n\text{-}Bu_4N^+F^-$, THF. h. glutaric anhydride, Et_3N, DMAP, CH_2Cl_2.

Compounds **14**, **15**, **21**, and **22** were evaluated as inhibitors of HMG CoA reductase by measuring their effect on mevalonate production from radiolabelled HMG CoA by rat liver microsomes.[18] Data are summarized in the Table. It is noteworthy that neither alcohol **14** nor alcohol **21** shows significant inhibitory activity at any concentration examined. Glutarates **15** and **22** do show definite inhibitory activity, but of a much lower magnitude than that exhibited by compactin. The relatively low activity of **15**, given its rather close structural resemblance to acid **3** suggests that compound **3** indeed may be functioning as a transition state analog, and that an effective inhibitor of HMG CoA reductase may require the secondary carbinol at C-5. Further experiments to test this idea are in progress.

Acknowledgements: The work at Berkeley was supported by USPH postdoctoral fellowships to J.A. Thomas (GM07487) and M.J. Taschner (CA06815). The work at UCLA was supported by a grant from the USPH (HL12745). We also thank Dr. A.G. Brown, of Beecham Pharmaceuticals, for a sample of compactin.

Table. Effect of Compounds **14**, **15**, **21** and **22** on HMG CoA Reductase Activity[a]

Compound	% Inhibition of Enzymatic Reaction[b]			
	200 n*M*	40 μM	200 μM	Compactin, 200 n*M*
14	2.3	11.0	9.0	70.8
15	0	41.0	72.0	63.0
21	3.3	0	11.0	72.3
22	0	27.0	63.0	72.0

(a) The HMG CoA reductase was assayed in 0.5 mL incubations containing 150 m*M* potassium phosphate buffer, pH 6.8, 10 m*M* dithiothreitol, 4 m*M* EDTA, 200 m*M* KCl, 2 m*M* NADPH (regenerated with glucose-6-phosphate and glucose-6-phosphate dehydrogenase), 18 μM [3-^{14}C]-HMG CoA and 60 μg of microsomal protein. Compactin or a test compound was added in 10 μL of DMSO; control incubations also contained 10 μL of DMSO. Compactin was added in lactone form. (b) Values are the % inhibition of the rate of mevalonate production in the presence of the appropriate test compound relative to an uninhibited control. All values are the average of three determinations. Assays are thought to be accurate to ±5%.

References and Notes

1. A.G. Brown, T.C. Smale, T.J. King, R. Hasenkamp and R.H. Thompson, *J. Chem. Soc., Perkin I,* 1165 (1976).
2. (a) A. Endo, M. Kuroda and Y. Tsujita, *J. Antibiot.*, **29**, 1346 (1976); (b) A. Endo, Y. Tsujita, M. Kuroda and K Tanzawa, *Eur. J. Biochem*, **77**, 31 (1977).
3. A.W. Alberts, J. Chen, G. Kuron, V. Hunt, J. Huff, C. Hoffman, J. Rothrock, M. Lopez, E. Harris, A. Patchett, R. Monaghan, S. Currie, E. Stapley, G. Albers-Schonberg, O. Hensens, J. Hirshfield, K. Hoogsteen, J. Liesch and J. Springer, *Proc. Natl. Acad. Sci. USA,* 77, 3957 (1980).
4. (a) A. Yamamoto, H. Sudo and A. Endo, *Atherosclerosis,* **35**, 259 (1980); (b) H. Mabuchi, T. Haba, R. Tatami, S. Miyamoto, Y. Sakai, T. Wakasugi, A. Watanabe, J. Koizumi and R. Takeda, *N. Engl. J. Med.* **305**, 478 (1981).
5. A. Endo, M. Kuroda and K. Tanzawa, *FEBS Lett.*, **72**, 323 (1976).
6. This hypothesis has clear implications with regard to the stereochemistry of the initial reduction process.
7. Total syntheses of compactin itself,[8a] and of alcohol **12**,[8b] and of an alcohol related to **12**[8c] have recently been described.
8. (a) N.-Y. Wang, C.-T. Hsu and C.J. Sih, *J. Am. Chem. Soc.*, **103**, 6538 (1981); (b) R.L. Funk and W.E. Zeller, *J. Org. Chem.*, **47**, 180 (1982); (c) E.A. Deutsch and B.B. Snider, *ibid.*, **47**, 2682 (1982).
9. Ethyl (*Z*)-crotonate is prepared in 95% overall yield on a 0.25 mol scale by acylation of 1-lithiopropyne with ethyl chloroformate, followed by catalytic hydrogen of the derived methyl propiolate over Lindlar's catalyst.
10. S. Danishefsky, T. Kitahara, C.F. Van and J. Morris, *J. Am. Chem. Soc.*, **101**, 6996 (1979).
11. J.A. Thomas and C.H. Heathcock, *Tetrahedron Lett.*, 3255 (1980).
12. A.R. Chamberlin, J.E. Stemke and F.T. Bond, *J. Org. Chem.*, **43**, 147 (1978).
13. E.J. Corey and B.W. Ericson, *J. Org. Chem.*, **36**, 4144 (1971).
14. H.C. Brown and S. Krishnamurthy, *J. Am. Chem. Soc.*, **94**, 7159 (1972).
15. Compounds **6-12** and **16-19** have thus far been prepared only in racemic form, although only one enantiomorph is represented in Schemes I and II. Compounds **13-15** and **20-22** are actually diastereomeric mixtures of the indicated compounds and the diastereomers also having the *S*-configuration in the appendage, but with the enantiomeric configuration at the four chiral centers on the hexalin unit.
16. A.L. Gemal and J.L. Luche, *J. Am. Chem. Soc.*, **103**, 5454 (1981).
17. M. Miyashita, A. Yoshikoshi and P.A. Grieco, *J. Org. Chem.*, **42**, 3772 (1977).
18. P. A. Edwards, D. Lemongello and A. N. Fogelman, *J. Lipid Res.*, **20**, 40 (1979).

(Received in USA 15 June 1982)