B. Competitive Binding Assays. Aliquots (0.2 mL) of cytosol or 9000*g* supernatant were incubated, in turn, with 3.0 nM [^{3}H]estradiol or 1.4 nM [^{3}H]tamoxifen and increasing concentrations of test ligands. The extent of specific binding of ^{3}H ligands was determined as described previously,[10] and data were plotted as percentage of ^{3}H ligand bound as a function of the log of test ligand concentration.

C. Pretreatment of MCF 7 Subcellular Fractions with 5. To 6 mL of 9000*g* supernatant from 1×10^7 MCF 7 cells (see A) was added sufficient 0.2 M glycine buffer, pH 9.6, and 0.2 M Tris buffer, pH 7.4 (each buffer contained 10 mM EDTA), to give a final volume of ca. 12 mL with a pH of 8.7 (37 °C). To a 1.0-mL aliquot of this (found in preliminary experiments to contain ca. 770 fmol/mL of specific [^{3}H]tamoxifen binding sites) was added 0.4 nmol of **5** citrate in 20 μL of ethanol. After 3 h at 37 °C, the mixture was cooled to 0 °C. To 0.5 mL of the suspension was added 1.5 mL of dextran-coated charcoal. After 15 min, the mixture was centrifuged at 400*g* for 10 min. Aliquots (0.2 mL) of the supernatant were used for determination of total and nonspecific binding of [^{3}H]tamoxifen using established methods.[10] A control experiment was run exactly as described except that **5** citrate was added to the 1.0-mL aliquot just prior to addition of dextran-coated charcoal. Experiments with cytosol were carried out in exactly the same way at 25 °C, using [^{3}H]estradiol as the radioligand.

Acknowledgment. We gratefully acknowledge support of this research by Grant CA 28928 from the National Institutes of Health. Fast atom bombardment mass spectra were provided by the Massachusetts Institute of Technology Mass Spectrometry Laboratory (supported by NIH Division of Research Resources Grant 00317). We also thank W. Hall for preparing the manuscript.

Registry No. **3**, 117095-59-7; **3**·citrate, 117095-70-2; **4**, 117095-64-4; **4**·citrate, 117095-66-6; **5**, 117095-65-5; **5**·citrate, 117095-67-7; **6**, 35258-26-5; **7**, 117095-56-4; **8**, 117095-57-5; **9**, 117095-58-6; **10**, 796-77-0; **11a**, 4397-53-9; **11b**, 2426-87-1; **12a**, 117095-60-0; **12a**·citrate, 117120-05-5; **12b**, 117095-61-1; **12b**·citrate, 117095-69-9; **13**, 117095-68-8; $PhCH_2MgCl$, 6921-34-2; 2-methoxy-4-cyanophenol, 4421-08-3; 3-methoxy-4-hydroxybenzaldehyde, 121-33-5; *p*-hydroxybenzaldehyde, 123-08-0; 1-[4-[2-(diethylamino)ethoxy]phenyl]-1-phenyl-2-[(4-benzyloxy)phenyl]-2-hydroxyethene, 117095-62-2; 1-[4-[2-(diethylamino)ethoxy]-phenyl]-1-phenyl-2-hydroxy-2-[3-methoxy-4-(benzyloxy)-phenyl]ethene, 117095-63-3.

Synthesis and Biological Evaluation of a Monocyclic, Fully Functional Analogue of Compactin

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Department of Chemistry, University of California, Berkeley, California 94720. *Received January 19, 1988*

Compound **8**, a monocyclic analogue of compactin, has been prepared and its efficacy as an inhibitor of 3-hydroxy-3-methylglutarylcoenzyme A reductase (HMGR) evaluated. The synthesis (Schemes I and II) requires seven steps starting with di-(−)-menthyl fumarate (**9**) and employs the useful *RR*-phosphonate reagent **14** to attach the mevinic acid side chain to aldehyde **13**. A molecular mechanics study shows that the preferred conformations of **18** (a model for compactin) and **19** (a model for **8**) are nearly identical. Compound **8** inhibits HMGR with IC_{50} = 320 μM, compared to a corresponding value of 32 nM for the compactin ketone, **5**. The factor of 10 000 difference in the two inhibitors corresponds to a difference in binding energy of 5.45 kcal mol^{-1}, or 1.36 kcal mol^{-1} for each of the four carbons of **5** that are missing in analogue **8**. This quantitative difference is consistent with the idea that the decalin moiety of the mevinic acids plays a purely hydrophobic role in binding the inhibitors to the enzyme.

The mevinic acids compactin (**1**) and mevinolin (**2**) have been shown to be effective in lowering plasma cholesterol levels in clinical trials,[1-4] and it is clear that these and related compounds will have an important pharmacological use as hypocholesterolemic agents.[5] Compounds **1** and **2** function by inhibiting the enzyme 3-hydroxy-3-methylglutarylcoenzyme A reductase (HMGR) in its conversion of HMG CoA to mevalonate and coenzyme A. It has been shown that the effective inhibitors are the 3-(*R*),5(*R*)-dihydroxy acids **3** and **4**. Recent work has confirmed the importance of the 3*R* stereochemistry and has shown that the 5-keto analogues have inhibitory activity that is indistinguishable within experimental error from that of the parent dihydroxy acid.[6] Thus, the 5-keto compounds **5**,[6] **6**,[6] and **7**[7] have IC_{50} values of 32 nM, 1 nM, and 1.3 mM, respectively, compared with IC_{50} values of 13 nM, 1.6 nM, and 0.6 mM for the corresponding 3-(*R*),5(*R*)-dihydroxy acids.

Compounds **1**–**6** are exceedingly potent competitive inhibitors of HMGR; their IC_{50} values are on the order of 10^{-4} of K_m for the natural substrate of the enzyme.[6,8] Available evidence shows that the decalinic moiety is responsible for much of this tight binding. This bicyclic hydrocarbon unit might provide hydrophobic binding, or

(1) (a) Shigematsu, H.; Hata, Y.; Yamamoto, M.; Oikawa, T.; Yamauchi, Y.; Nakaya, N.; Goto, Y. *Geriatr. Med.* **1979**, *17*, 1564. (b) Tobert, J. A.; Bell, G. D.; Birtwell, J.; James, I.; Kokovetz, W. R.; Pryor, J. S.; Buntinx, A.; Holmes, I. B.; Chao, Y. S.; Bologese, J. A. *J. Clin. Invest.* **1982**, *69*, 913. (c) Yamamoto, A.; Sudo, H.; Endo, A. *Atherosclerosis* **1980**, *35*, 259. (d) Mabuchi, H.; Haba, T.; Tatami, R.; Miyamoto, S.; Sakai, Y.; Wakasugi, T.; Watanabe, A.; Koizumi, J.; Takeda, R. *N. Engl. J. Med.* **1981**, *305*, 478. (e) Mabuchi, H.; Sakai, T.; Sakai, Y.; Yoshimura, A.; Watanabe, A.; Wakasugi, T.; Koizumi, J.; Takeda, R. *N. Engl. J. Med.* **1983**, *308*, 609.

(2) Havel, R. J.; Hunninghake, D. B.; Illingworth, D. R.; et al. *Circulation* **1985**, *72* (Suppl. 3), 198.

(3) Therapeutic Response to Lovastatin (Mevinolin) in Nonfamilial Hypercholesterolemia *JAMA, J. Am. Med. Assoc.* **1986**, *42*, 4909.

(4) (a) Nakaya, N.; Homma, Y.; Tamachi, H.; Goto, Y. *Atherosclerosis* **1986**, *61*, 125. (b) Alberts, A. W.; Chen, J.; Huff, J.; Hunt, V.; Kuron, G. *IXth Int. Symp. Drugs Affect. Lipid Metab. (Oct 22–25, Florence)* **1986**, 8. (c) Nakaya, N.; Goto, Y. *Ibid.* **1986**, 67.

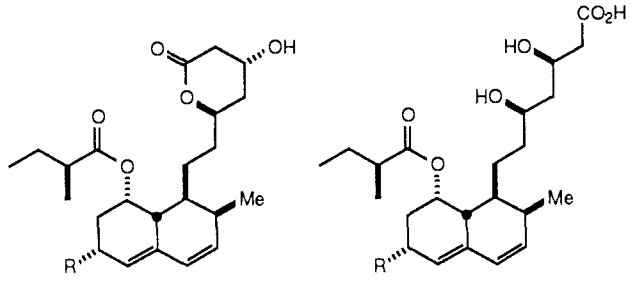
(5) Mevinolin (generic name lovastatin) was recently approved by the Food and Drug Administration for treatment of hypercholesterolemia; the drug is being marketed by Merck Sharp & Dohme under the name Mevacor.

(6) Heathcock, C. H.; Hadley, C. R.; Rosen, T.; Theisen, P. D.; Hecker, S. J. *J. Med. Chem.* **1987**, *30*, 1858.

(7) Heathcock, C. H.; Theisen, P. C.; Hadley, C. R. Unpublished results.

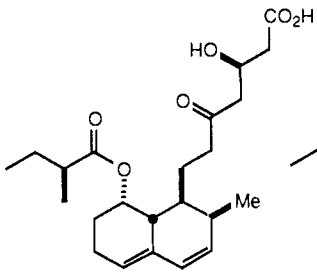
(8) (a) Tanazawa, K.; Endo, A. *Eur. J. Biochem.* **1979**, *98*, 195. (b) Alberts, A. W.; Chen, J.; Kuron, G.; Hunt, V.; Huff, J.; Hoffman, G.; Rothrock, J.; Lopez, M.; Joshua, H.; Harris, E.; Patchett, A.; Monaghan, R.; Currie, S.; Stapley, E.; Albers-Schonberg, G.; Hensens, O.; Hirschfield, J.; Hoogstein, K.; Liesch, J.; Springer , J. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3957.

0022-2623/89/1832-0197$01.50/0



1: R = H
2: R = Me

3: R = H
4: R = Me



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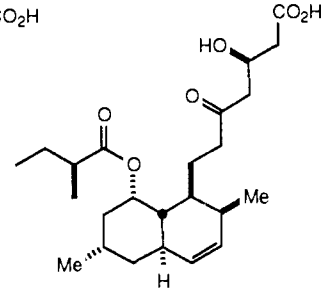

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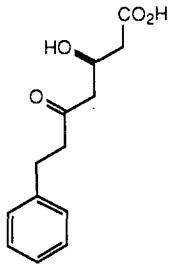
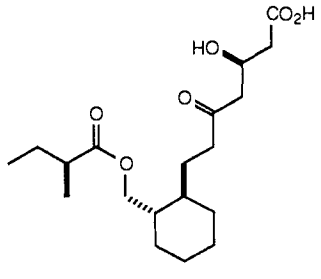


7

it might serve as a rigid template which positions the oxygen functions appropriately for interaction with H-bond donor sites on the enzyme. To provide more information on this point, we have prepared analogue 8, which retains all of the oxygen functionality of **5**, with the correct absolute and relative configuration, but which lacks four carbons of compactin (the ring-B methyl and three carbons of ring A).

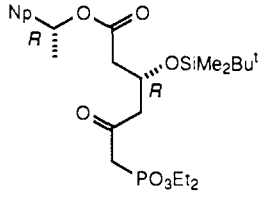


8

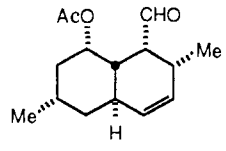
Results

As shown in Scheme I, the synthesis of 8 commenced with di-(−)-menthyl fumarate (**9**), which was obtained by morpholine-catalyzed isomerization of di-(−)-menthyl maleate according to the procedure of Yoshihara and his co-workers.[9] Lewis acid catalyzed reaction of the trans diester with 1,3-butadiene proceeded smoothly at −40 °C in hexane[10] and the resulting cyclic diester **10** was reduced to the crystalline diol **11**, which was readily obtained in enantiomerically pure form by recrystallization from ether/hexane. Reaction of the diol with (*S*)-(−)-2-methylbutanoic anhydride[11] gave the mono- and diesters each in about 33% yield, along with unchanged diol. A dramatic improvement was effected by the use of the procedure reported recently for the monosilylation of 1,*n*-diols.[12] Reaction of diol **11** in THF with 1 equiv of NaH gave a voluminous precipitate of the sodium salt of the monoanion. Reaction of this salt with the acid anhydride gave a 79% yield of the desired monoester **12**, accompanied by small, approximately equal amounts of the diester and diol. Oxidation of the primary alcohol function under Swern conditions[13] produced aldehyde **13** in 96% yield.

The stage was now set for the condensation of this aldehyde with phosphonate **14**[14] to construct the desired heptanoic acid side chain. However, it should be noted that since the aldehyde possesses an α-hydrogen atom, it may undergo base-catalyzed isomerization. In our previous work with reagents related to **14** we employed the Wadsworth–Emmons conditions developed by Masamune, Roush, and co-workers.[15] The base used to deprotonate the phosphonate, DBU, has pK_a = 11.6 and had proved to be basic enough to epimerize the axial aldehyde **15** to its equatorial epimer after 1 h at room temperature and 1 h at 0 °C.[16] When we used the same base in the horner–Wadsworth–Emmons reaction with the equatorial aldehyde, we did observe some aldehydic epimerization, but found, fortunately, that the axial aldehyde was unreactive toward the keto phosphonate.



14

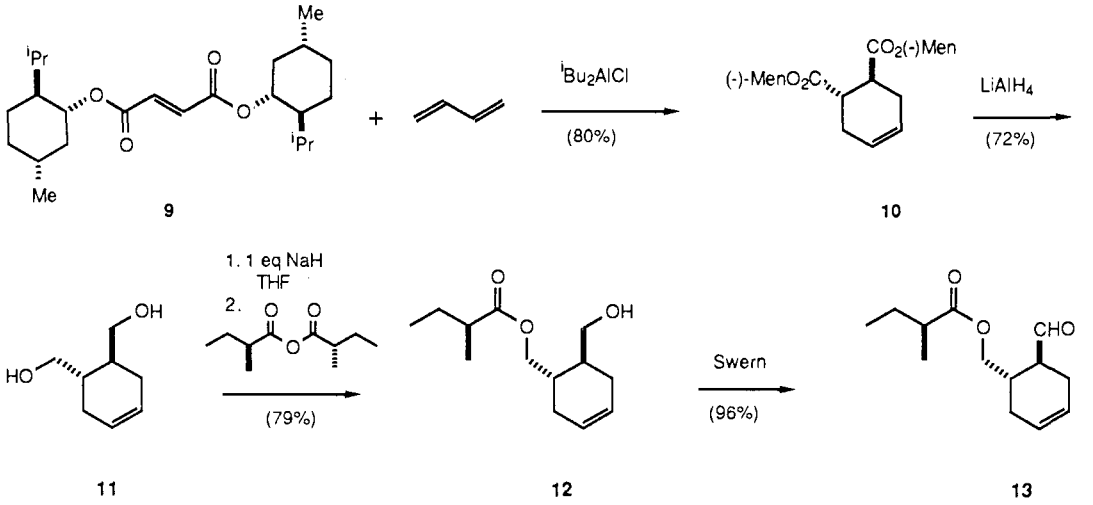


15

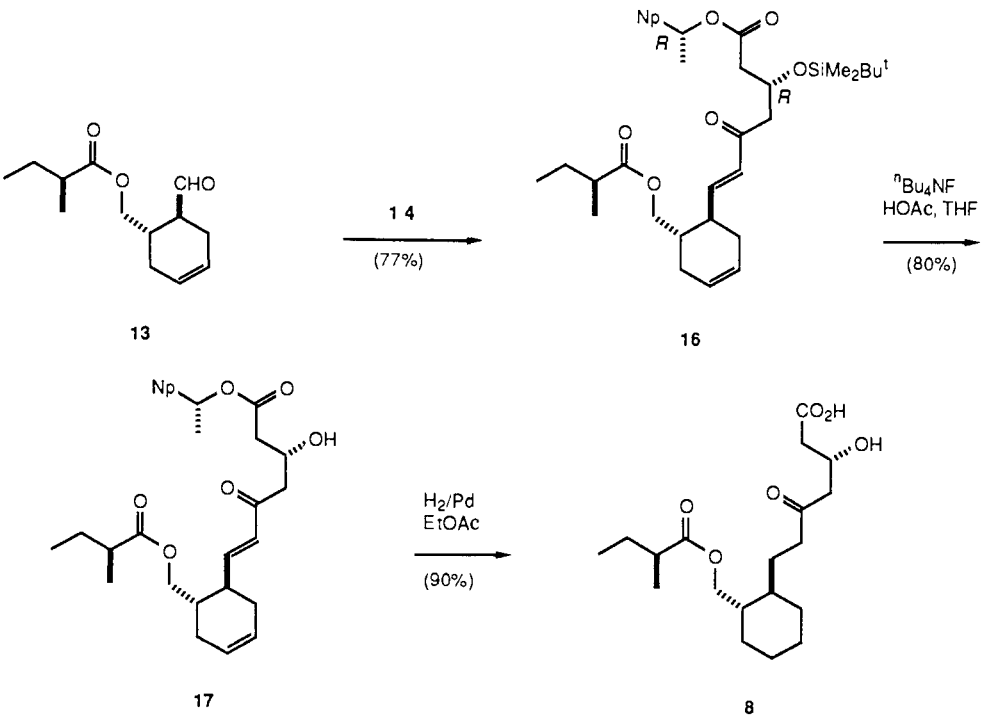
We, therefore, studied the behavior of the aldehyde **13** toward DBU and toward triethylamine (pK_a = 10), the base used by Rathke and Nowak[17] in their studies of this condensation reaction. DBU in CD_3CN rapidly isomerized aldehyde **13**, giving a 3:1 mixture of trans and cis isomers after 45 min and a 2:1 mixture after some hours. By contrast, the aldehyde was less reactive toward triethylamine under similar conditions, producing only a few percent of the cis isomer after 20 h and reaching equilibrium only after several weeks. In the ^1H NMR spectrum of the trans isomer, the aldehyde proton appears as a doublet (J = 2.3 Hz) at δ 9.74, whereas the corresponding signal in the cis isomer (axial CHO group) appears as a

(9) Nozaki, K.; Yoshihara, M.; Matsubara, Y.; Maeshima, T. *Phosphorus Sulfur* **1985**, *22*, 1.
(10) Furata, K.; Iwanaga, K.; Yamamoto, H. *Tetrahedron Lett.* **1986**, *27*, 4507.
(11) Hsu, C.-T.; Wang, N. Y.; Latimer, L. H.; Sih, C. J. *J. Am. Chem. Soc.* **1983**, *105*, 593.
(12) McDougal, P. G.; Rico, J. G.; Oh, Y.-I.; Condon, B. D. *J. Org. Chem.* **1986**, *51*, 3388.
(13) Mancuso, A. J.; Huang, S.-L.; Swern, D. *J. Org. Chem.* **1978**, *73*, 2480.
(14) Theisen, P. D.; Heathcock, C. H. *J. Org. Chem.* Submitted for publication.
(15) Blanchette, M. A.; Choy, W.; Davis, J. T.; Essenfeld, A. P.; Masamune, S.; Roush, W. R.; Sakai, T. *Tetrahedron Lett.* **1984**, 2183.
(16) Hecker, S. J.; Heathcock, C. H. *J. Am. Chem. Soc.* **1986**, *108*, 4586.
(17) Rathke, M. W.; Nowak, M. *J. Org. Chem.* **1985**, *50*, 2624.

Scheme I



Scheme II



broad singlet at lower field (δ 9.82). Similar spectroscopic behavior has been observed in related systems.[18] The relative proportion of axial aldehyde in the equilibrium mixture (ca. 30%) indicates an energy difference of some 500 cal mol^{-1}.

Triethylamine was thus the base of choice for the condensation reaction, which is summarized in Scheme II. The best yields (70–80%) of the product (**16**) were obtained when LiBr and the relatively nonpolar acetonitrile were added to the aldehyde, followed by phosphonate **14**. The mixture was stirred for 30 min at room temperature before the base was added and the reaction was complete after stirring overnight. Some epimerization at the aldehydic carbon was evident in most runs (as evidenced by a downfield doublet of doublets due to the proton at C-7 in the product) but this could be minimized by careful adherence to the conditions described. Desilylation of the protected hydroxyl group in **16** was cleanly, if slowly, effected by the use of tetra-*n*-butylammonium fluoride in THF buffered with a slight excess of acetic acid,[19] giving alcohol **17** in 80% yield. The two double bonds and the α-naphthyl ethyl ester group were then removed by catalytic hydrogenation to give acid **8** as a colorless oil in 89% yield.

In order to examine the likely conformation of this molecule, and to see how closely it models the bicyclic mevinic acids, a molecular mechanics study was performed, using Allinger's MM2 force field and Still's Macromodel program. The model compounds studied were simplified analogues **18** and **19**. Compound **18** was assumed to exist in a chair–chair conformation and **19** was assumed to exist in the chair conformation with the two side chains both equatorial. In both calculations, the two C–O single bonds were allowed to rotate through a full 360°, in 60° increments, so as to find the lowest energy conformation with respect to this side chain. The calculations revealed that

(18) (a) Overman, L. E.; Jessup, P. J. *J. Am. Chem. Soc.* **1978**, *100*, 5179. (b) Overman, L. E.; Lesuisse, D.; Hashimoto, M. *J. Am. Chem. Soc.* **1983**, *105*, 5373. (c) Buchanan, G. W.; McCarvill, A. R. *Can. J. Chem.* **1972**, *50*, 1965.

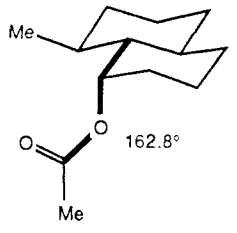
(19) Hoffman, W. F.; Alberts, A. W.; Anderson, P. S.; Chen, J. S.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* **1986**, *29*, 849.

Table I. Inhibition of HMGR by 8[a]

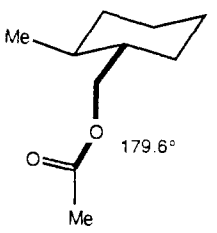
[8], μM	dpm of [^{14}C]mevalonate produced (av)	% inhibn
2500	883 ± 71	97
250	17400 ± 348	47
25	26050 ± 782	21
2.5	28800 ± 576	12
0	32800 ± 1312	0

[a] Assays at each inhibitor concentration were performed in duplicate. Initial velocities were measured in all cases and radioassay background value (670 dpm) amounted to 2% of control. Above data are corrected for mevalonate recovery and assay background.

the two models have similar lowest energy conformations (below). There is a quantitative difference between the dihedral angles highlighted in the two molecules, which is 162.8° in **18** and 179.6° in **19**. However, the distortion of **19** to a conformation in which this dihedral angle is 162.8° requires only a trivial amount of energy.



18



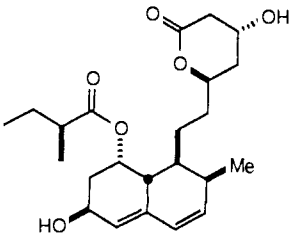
19

The ability of acid **8** to inhibit HMGR was measured by the standard radioassay procedure which has been published elsewhere.[6] Assay results and percent inhibition values obtained at four concentrations of **8** are shown in Table I. Interpolation of a plot of percent inhibition versus log [8] provides an IC_{50} value of 320 μM for keto acid **8**. This inhibitory activity is to be compared with a corresponding value of 32 nM for **5**, the analogous compactin keto acid.[6]

Discussion

Compound **8** is 10 000 times less potent than **5**, corresponding to a difference of 5.45 kcal mol^{-1} in the free energy of binding. Of course, we have no knowledge about the preferred conformation of binding of either inhibitor. However, it is reasonable to assume that the 3(*R*)-hydroxy-5-oxoheptanoic acid side chains are bound identically and that the (2(*S*)-methylbutanoyl)oxy groups are bound at least similarly. Since our molecular mechanics investigation revealed that **18** and **19** have nearly identical ground-state conformations, it is unlikely that the $\Delta\Delta G$ of binding has a significant conformational component. This finding is consistent with the idea that the decalinic moiety plays the role of a hydrophobic anchor in binding the mevinic acids to the enzyme active site. The $\Delta\Delta G$ of 5.45 kcal mol^{-1} corresponds to 1.36 kcal mol^{-1} for each of the four carbons in **5** that is missing in **8** (i.e., the three missing carbons of ring A and the missing methyl group in ring B), a value that is not unreasonable for purely hydrophobic binding.[20] In our previous work, we found that the 5-keto analogue of dihydromevinolin (**6**) has an IC_{50} value of 1 nM. The difference of a factor of 32 between **6** and **5** corresponds to a $\Delta\Delta G$ of 2.1 kcal mol^{-1}, suggesting that the enhanced activity of mevinolin relative to compactin[8b] is due to additional hydrophobic binding of the C-3 methyl group in the former compound.

The importance of a hydrophobic anchoring group for HMGR inhibition has also been demonstrated by the Merck group in a series of structure–activity relationship (SAR) papers.[19,21] In this extensive study, dozens of analogues were prepared in which the mevinic acid decalinic moiety was replaced by various aromatic and hydroaromatic surrogates.[21a-c] The resulting SAR studies led to the development of a series of potent HMGR inhibitors in which the decalinic moiety is replaced by substituted biphenyls. In addition, it was shown that extensive modification of the (*S*)-2-methylbutanoyl side chain is possible.[19] It should be noted, however, that compound **20**, which has an equatorial hydroxy group at C-3, has



20

about the same inhibitory activity as mevinolin.[22] A possible inference that may be drawn from this observation is that the HMGR binding site normally occupied by the mevinic acid decalin moiety has some group which may serve as a hydrogen-bond acceptor on one of its faces, and that there is a substantial hydrophobic pocket extending away from this surface. It is into this hydrophobic pocket that the axial methyl group of mevinolin and the axial acyloxy group extends.

Nakamura and Abeles have reported a study of HMGR's reverse reaction, in which mevaldate and CoA are dehydrogenated to HMG CoA.[23] Kinetic analysis showed that compactin and CoA are competitive in this process, suggesting that the decalinic portion of the mevinic acids binds in a region normally occupied by CoA. The interesting question arises of why HMGR would have evolved to have a binding region for CoA that appears to be hydrophobic to a major degree. A possible answer is that this region was evolved so that the reaction product, CoA, would itself not be bound too strongly. Thus, an interesting scenario is that the enzyme has a groove (represented in Figure 1)[24] in which HMG CoA is bound, principally by the Coulombic and H-bond attractive forces in "the binding region" and by the hydrophilic attactive forces in the "hydrophilic region". The forces binding the two ends of the substrate to the enzyme are then sufficient to overcome the lack of complementarity in the "hydrophobic region". After the

(20) Fersht, A. *Enzyme Structure and Mechanism*, 2nd ed.; W. H. Freeman: New York, 1985; pp 299–301.

(21) (a) Stokker, G. E.; Hoffman, W. F.; Alberts, A. W.; Cragoe, E. J., Jr.; Deana, A. A.; Gilfillan, J. L.; Huff, J. W.; Novello, F. C.; Prugh, J. D.; Smith, R. L.; Willard, A. K. *J. Med. Chem.* **1985**, *28*, 347. (b) Hoffman, W. F.; Alberts, A. W.; Cragoe, E. J., Jr.; Deana, A. A.; Evans, B. E.; Gilfillan, J. L.; Gould, N. P.; Huff, J. W.; Novello, F. C.; Prugh, J. D.; Rittle, K. E.; Smith, R. L.; Stokker, G. E.; Willard, A. K. *Ibid.* **1986**, *29*, 159. (c) Stokker, G. E., Alberts, A. W.; Anderson, P. S.; Cragoe, E. J., Jr.; Deanna, A. A.; Gilfillan, J. L.; Hirshfield, J.; Holtz, W. J.; Hoffman, W. F.; Huff, J. W.; Lee, T. J.; Novello, F. C.; Prugh, J. D.; Rooney, C. S.; Smith, R. L.; Willard, A. K. *Ibid.* **1986**, *29*, 170.

(22) (a) Serizawa, N.; Nakagawa, K.; Hamano, K.; Tsuijita, Y.; Terahara, A.; Kuwano, M. *J. Antibiot.* **1983**, *36*, 604. (b) Serizawa, N.; Serizawa, S.; Nakagawa, K.; Furuya, K.; Okazaki, T.; Terahara, A. *Ibid.* **1983**, *36*, 887.

(23) Nakamura, C. E.; Abeles, R. H. *Biochemistry* **1985**, *24*, 1364.

(24) The orientation of the HMG CoA molecule in Figure 1 is purely fanciful, and is intended only to convey a schematic sense of one manner in which the molecule might be bound in a site having three rather different domains.

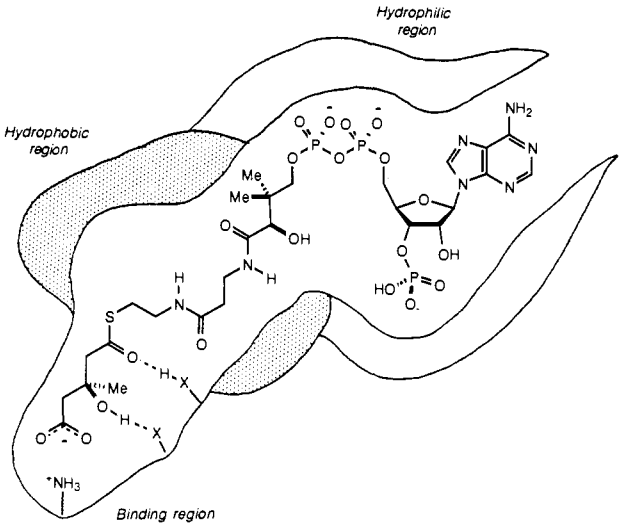


Figure 1. Hypothetical HMGR binding site, with HMG CoA in place.

two-stage reduction, the product CoA is bound only in the "hydrophilic region", and is actually repelled in the "hydrophobic region". The lack of complementarity in this region would assist in ushering the product from the binding site, thus facilitating turnover. It is hypothesized that Nature (in the form of the fungi that biosynthesize the mevinic acids) capitalized on this situation and evolved the mevinic acids as agents of chemical warfare against yeasts, organisms with which they compete for the same ecological niche.

Experimental Section

General Procedures. Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AM 500 spectrometer using chloroform as an internal standard ($CHCl_3$ = 7.26). All NMR spectra were recorded in $CDCl_3$, unless otherwise specified, and chemical shifts are expressed in ppm downfield from tetramethylsilane. Proton NMR data are presented in the order: number of hydrogens, multiplicity, coupling constant in hertz, assignment. Carbon NMR data are followed by assignment, confirmed by DEPT spectra, and where noted, by the use of 2D NMR spectra. Infrared (IR) spectra were recorded with a Perkin-Elmer 1420 spectrometer with the polystyrene absorption at 1601 cm^{-1} as reference. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at the sodium D line. Low-resolution mass spectra were recorded with an AEI MS9 spectrometer. Analytical thin-layer chromatography (TLC) was performed on Analtech silica gel GF plates. Visualization was accomplished by staining with a solution of phosphomolybdic acid and/or UV illumination. Flash column chromatography was performed according to the procedure of Still, Kahn, and Mitra,[25] using Kieselgel 60 (230–400 mesh). Melting points were determined in open capillary tubes and are uncorrected. Elemental analyses were performed by the Microanalytical Laboratory operated by the College of Chemistry, University of California, Berkeley.

Di-(−)-menthyl Maleate. Maleic anhydride (18.8 g, 0.192 mol), (−)-menthol (60 g, 0.385 mol), and *p*-toluenesulfonic acid (2.7 g, 0.0192 mol) were dissolved in benzene (200 mL) and heated under reflux, with a Dean–Stark water separator. After 13.25 h, a total of 3.5 mL of water was collected (theory = 3.46 mL). Ether was added and the solution was washed with saturated aqueous $NaHCO_3$, water, and brine and dried ($MgSO_4$). Acidification of the bicarbonate washing gave a white solid (1.39 g). Evaporation of the solvent and recrystallization of the residue from methanol gave 63.5 g (85%) of di-(−)-menthyl maleate: mp 97–98 °C; $[\alpha]_D$ −95.3° (*c* 1.76, C_6H_6) [lit.[9] mp 97–98 °C; $[\alpha]_D$ −95.67° (*c* 1.2, C_6H_6)]; IR ($CHCl_3$) 1720 cm^{-1}; ^1H NMR δ 6.19 (s, vinyl H). Further material was obtained from the mother liquors.

Di-(−)-menthyl Fumarate (9).[9] Morpholine (0.87 g, 0.01 mol) in benzene (10 mL) was added to a solution of di-(−)-menthyl maleate (39.2 g, 0.1 mol) in benzene (90 mL) and the solution heated under reflux. TLC analysis (1:10 ether/hexane) showed the reaction to be substantially complete after 3 h. After 8 h, the cooled solution was poured into ether, and the organic extracts were washed with aqueous HCl (2 M, 2 × 20 mL), saturated aqueous $NaHCO_3$, and brine and dried ($MgSO_4$). Removal of solvent gave di-(−)-menthyl fumarate as an oil: bp 185–188 °C (0.13 kPa); $[\alpha]_D$ −96° (*c* 1.20, C_6H_6) [lit.[9] $[\alpha]_D$ −104.3° (*c* 0.90, C_6H_6)]; IR (film) 1730 cm^{-1}; ^1H NMR δ 6.82 (2 s, vinyl). Later experiments were carried out with heating for 4 h and showed no maleate by ^1H NMR or TLC.

(1*S*,2*S*)-Di-(−)-menthyl Cyclohex-4-ene-1,2-dicarboxylate (10). Di-(−)-menthyl fumarate (19.5 g, 0.05 mol) in hexane (300 mL) was dried by azeotropic distillation and cooled to −40 °C ($MeCN/CO_2$ bath). Diisobutylaluminum chloride in hexane (100 mL, 25% solution) was added by syringe through a septum, producing an intense orange color. The solution stood for 30 min and buta-1,3-diene (11.8 g, 0.22 mol) was passed into the cooled reaction mixture. The solution was stirred at −40 °C; TLC analysis (1:10 ether/hexane) showed that the reaction was substantially complete after 4 h. The mixture was kept at −15 °C overnight, poured into dilute aqueous HCl, and extracted with ether. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine and dried ($MgSO_4$). Flash chromatography on silica gel (100 g) and elution with hexane and 1:10 ether/hexane gave an oil (17.90 g, 80%) which crystallized on standing. Crystallization from ethanol gave plates of the diester: mp 59–60.5 °C (lit.[26] mp 56–58 °C); $[\alpha]_D$ −29.3° (*c* 2.15, $CHCl_3$); IR ($CHCl_3$) 1730 cm^{-1}; ^1H NMR δ 2.40–2.44 (4, m, 3, 6), 2.86 (2, m, 1, 2), 5.68 (2, m, 4, 5), and signals due to two menthol residues; ^{13}C NMR δ 15.96, 20.88, 22.04, 23.18, 25.99, 27.89, 31.42, 34.33, 40.74, 41.26, 47.04, 74.34, 125.02, 174.49.

(1*S*,2*S*)-Cyclohex-4-ene-1,2-dimethanol (11). (1*S*,2*S*)-Di-(−)-menthyl cyclohex-4-ene-1,2-dicarboxylate (4.10 g, 9.2 mmol) in ether was added to a suspension of $LiAlH_4$ (0.58 g, 15 mmol) in ether with cooling and stirring. Reaction was complete after 89 h (TLC); water (0.58 mL), 3 M aqueous NaOH (0.58 mL), and water (1.74 mL) were added, and the mixture was stirred for 1 h. Removal of salts and solvent gave an oil (3.82 g) which was subjected to flash chromatography on silica gel (50 g, 1:1 ether/hexane) to give menthol and diol 11 (1.11 g, 85%) as needles from ether/hexane: mp 61.5–62 °C; $[\alpha]_D$ +75.1° (*c* 1.40, $CHCl_3$) [lit.[27] mp 62–63 °C $[\alpha]_D$ +73°]; IR 3360 cm^{-1}; ^1H NMR δ 1.68–1.70, 1.87–1.89 (4, M), 2.01–2.04 (2, m), 3.04 (1, t, *J* = 5.5), 3.58–3.61, 3.71–3.75 (4, m), 5.66 (2, m); ^{13}C NMR δ 28.04 (C-3, C-6), 39.22 (C-1, C-2), 65.72 (CH_2OH), 125.79 (C-4, C-5).

In another experiment, a major portion of the menthol was removed by sublimation (30–40 °C, 0.1 kPa) before flash chromatography.

(1*S*,2*S*,2′*S*)-1-(Hydroxymethyl)-2-[[(2′-methyl-1-oxobutyl)oxy]methyl]cyclohex-4-ene (12). Diol 11 (284 mg, 2 mmol) in THF (5 mL) was added to NaH (80 mg, 60%, 2 mmol) in THF (5 mL) with stirring, to give a dense white gel. After stirring of the mixture for 2 h to ensure complete formation of the monoanion, (*S*)-2-methylbutanoic anhydride (372 mg, 0.41 mL, 2 mmol) was added dropwise. The reaction mixture was stirred for 30 min, diluted with ether, and poured into 1 M aqueous H_3PO_4. The aqueous layer was reextracted with ether and the combined organic layer washed with saturated aqueous $NaHCO_3$ and brine. After drying ($MgSO_4$), the solvent was removed and the product chromatographed on silica gel (10 g). Elution with 1:5 ether/hexane yielded the diester (71 mg, 11%); further elution with 1:2 ether/hexane gave the monoester (**12**, 357 mg, 79%): $[\alpha]_D$ +67.4° (*c* 1.79, $CHCl_3$); IR 3460, 1740 cm^{-1}; ^1H NMR δ 0.90 (3, t, *J* = 7.4), 1.14 (3, d, *J* = 7.0), 1.47, 1.69 (2, m), 1.76–2.20 (6, m), 2.38 (1, sextet, *J* = 6.9), 3.65 (2, m), 4.08 (1, dd, *J* = 11.1, 5.68), 4.13 (1, dd, *J* = 11.1, 6.24), 5.63, 5.65 (2, d, *J* = 2.2); ^{13}C NMR δ 11.51 (C-4′), 16.47 (C-2′ Me), 26.63, 26.70, 27.03 (C-3, C-6, C-3′),

(25) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, 43, 2923.

(26) Walborsky, H. M.; Barash, L.; Davis, T. C. *Tetrahedron* **1963**, *19*, 2333.

(27) Lythgoe, B.; Manwaring, R.; Milner, J. R.; Moran, T. A.; Nambudiry, M. E. N.; Tideswell, J. *J. Chem. Soc., Perkin Trans. 1* **1978**, 387.

33.71 (C-1), 37.18 (C-2), 41.06 (C-2'), 64.46, 66.47 (CH_2O), 124.98, 125.72 (C-4, C-5), 176.82 (C-1'); mass spectrum, m/z (relative intensity) 226 (0.3), 208 (2.4), 124 (53.6), 106 (67.2), 103 (39.6), 91 (73.9), 85 (55.1), 78 (82.3), 68 (47.5), 57 (100). Anal. ($C_{13}H_{22}O_3$) C, H.

Acylation of Cyclohex-4-ene-1,2-dimethanol (11) with (*S*)-2-Methylbutanoic Anhydride in the Presence of Pyridine/DMAP. Diol 11 (710 mg, 5 mmol) in pyridine (5 mL) containing 4-(*N*,*N*-dimethylamino)pyridine (61 mg, 0.5 mmol) was treated with (*S*)-2-methylbutanoic anhydride (960 mg, 5.16 mmol). After 91 h at room temperature the reaction mixture was poured into 1.2 M aqueous HCl (60 mL), and the products were extracted with ether (2 × 40 mL). The organic extract was washed with saturated aqueous $NaHCO_3$, brine, and dried ($MgSO_4$). Evaporation of solvent and flash chromatography (silica gel, 16 g; 1:3 ether/hexane) yielded the following.

(a) The diester, (1*S*,2*S*,2'*S*)-1,2-bis[(2'-methyl-1-oxobutoxy)-methyl]cyclohex-4-ene (576 mg, 37%): $[\alpha]_D$ +74.2° (*c* 1.82, $CHCl_3$); IR (film) 1740 cm^{-1} (C=O); ^1H NMR δ 0.90 (3, t, *J* = 7.4), 1.14 (3, d, *J* = 7.0), 1.47 and 1.68 (2, 2 m), 1.91–2.18 (6, m), 4.04 (1, dd, *J* = 11.1, 5.85), 4.13 (1, dd, *J* = 11.1, 4.87), 5.63 (2, s); ^{13}C NMR δ 11.48 (4'), 16.44 (2'-Me), 26.61 (3'), 26.74 (3, 6), 33.93 (1, 2), 40.99 (2'), 65.80 (CH_2O), 125.10 (4, 5); assignments were confirmed by $^1H/^{13}C$ NMR correlation spectroscopy; mass spectrum, m/z (relative intensity) 310 (0.6), 208 (45.8), 106 (88.1), 91 (65.7), 85 (59.6), 78 (100), 57 (76.8). Anal. ($C_{18}H_{30}O_4$) C, H.

(b) The monoester **12**, 360 mg, 32%, identical (^1H NMR) with material prepared previously.

(c) Unchanged diol **11**, 103 mg, 15%.

(1*S*,2*S*,2'*S*)-2-[(2'-Methyl-1'-oxobutoxy)methyl]cyclohex-4-enecarboxaldehyde (13). According to the Swern protocol,[13] to a stirring solution of oxalyl chloride (250 mg, 1.97 mmol) in CH_2Cl_2 (5 mL) at −78 °C was added a solution of dimethyl sulfoxide (300 mg, 3.84 mmol) in CH_2Cl_2 (1 mL). The solution was stirred for 2 min at −60 °C and a solution of alcohol **12** (345 mg, 1.54 mmol) in CH_2Cl_2 (3 mL) was added. After stirring of the mixture for 25 min at −78 °C, triethylamine (800 mg, 7.9 mmol) was added; the mixture was stirred for 5 min at the same temperature and allowed to warm to room temperature. Ether (30 mL) was added and the mixture was washed with 1 M aqueous H_3PO_4, saturated aqueous $NaHCO_3$, and brine. The ether solution was dried ($MgSO_4$) and filtered and the solvent removed. The crude product was purified by flash chromatography on silica gel (7.5 g, 1:8 ether/hexane) to afford aldehyde **13** (329 mg, 96%) as a colorless oil: $[\alpha]_D$ +53.8° (*c* 1.11, $CHCl_3$); IR (film) 2715, 1734 cm^{-1}; ^1H NMR δ 0.90 (3, t, *J* = 7.4), 1.13 (3, d, *J* = 7.0), 1.47, 1.67 (2, m), 1.86–1.91, 2.14–2.53 (7, m), 4.03 (1, dd, *J* = 11.1, 7.35), 4.12 (1, dd, *J* = 1.1, 5.94), 5.65–5.71 (2, m), 9.67 (1, d, *J* = 2.3); ^{13}C NMR δ 11.36 (C-4'), 16.27 (C-3' Me), 22.50, 25.39, 26.45 (C-3, C-6, C-3'), 40.77 (C-2'), 47.67 (C-1), 65.59 (CH_2O), 123.92, 125.16 (C-4, C-5), 176.12 (C-1'), 203.04 (CHO); mass spectrum, m/z (relative intensity) 224 (0.6), 139 (47.6), 122 (75.1), 107 (54.1), 104 (63.5), 94 (83.8), 85 (70.4), 79 (78.4), 68 (40.9), 57 (100). Anal. ($C_{13}H_{20}O_3$) C, H.

(3*R*,1'*R*,2'*S*,2''*S*,1'''*R*)-1'''-(1-Naphthyl)ethyl 7-[2'-[(2''-Methyl-1''-oxobutoxy)methyl]-1'-cyclohex-4'-enyl]-3-[(*tert*-butyldimethylsilyl)oxy]-5-oxohept-6-enoate (16). Lithium bromide (144 mg, 1.56 mmol) and acetonitrile (6 mL) were added to aldehyde **13** (279 mg, 1.25 mmol). Phosphonate **14**[14] (736 mg, 1.41 mmol) was added dropwise and the mixture stirred at room temperature for 30 min. Triethylamine (140 mg, 1.38 mmol) was added and the mixture stirred at room temperature for 16 h. The light yellow-orange reaction mixture was cooled in ice, ether was added, and the solution was poured into cold aqueous 1 M H_3PO_4. The ether layer was washed with saturated aqueous $NaHCO_3$ and brine and dried ($MgSO_4$). After filtration, the solvent was removed and the product (857 mg) subjected to flash chromatography on silica gel (30 g, 1:8 ether/hexane) to yield diester **16** as a colorless oil (597 mg, 77%): $[\alpha]_D$ +49.5° (*c* 0.95, $CHCl_3$); IR ($CHCl_3$) 1732, 1667, 1624 cm^{-1}; ^1H NMR δ 0.02 (3, s), 0.03 (3, s), 0.80 (9, s), 0.89 (3, t, *J* = 7.4), 1.12 (3, d, *J* = 7.0), 1.46 and 1.65 (2, 2 m), 1.70 (3, d, *J* = 6.7), 1.89–2.20 (5, m), 2.37 (1, m), 2.60 (2, m), 2.79 (2, m), 3.91, (1, dd, *J* = 11.1, 6.2), 4.03 (1, dd, *J* = 11.1, 4.6), 5.67 (2, m), 6.08 (1, d, *J* = 15.7), 6.65 (1, q, *J* = 6.7), 6.69 (1, dd, *J* = 15.8, 9.0), 7.44–8.09 (7, m); ^{13}C NMR δ −5.09, −4.88, 11.61 (C-4''), 16.56 (C-2'' Me), 17.80, 25.66 (*t*-Bu), 21.76 (C-2'''), 26.71 (C-3''), 27.25 (C-3'), 29.94 (C-6'), 36.58 (C-2'), 39.26 (C-1'), 41.04 (C-2''), 42.59 (C-2), 47.25(C-4), 65.97 (C-3), 66.33 (OCH_2), 69.56 (C-1'''), 124.69, 125.67 (C-4', C-5'), 130.67 (C-6), 123.10, 123.26, 125.32, 125.58, 126.23, 128.34, 128.82, 130.11, 133.73, 137.32, 149.56 (C-7), 170.20 (C-1), 176.49 (C-1''), 198.02 (C-5). Anal. ($C_{37}H_{52}O_6Si$) C, H.

(3*R*,1'*R*,2'*S*,2''*S*,1'''*R*)-1'''-(1-Naphthyl)ethyl 7-[2'-[(2''-Methyl-1''-oxobutoxy)methyl]-1'-cyclohex-4'-enyl]-3-hydroxy-5-oxohept-6-enoate (17). To silyl ether **16** (331 mg, 0.53 mmol) in dry THF (2 mL) was added acetic acid (130 mg, 2.2 mmol) and tetra-*n*-butylammonium fluoride trihydrate (518 mg, 1.64 mmol) in dry THF (2 mL). TLC analysis showed the reaction to be substantially complete after 48 h. Ether was added and the organic layer washed with saturated aqueous $NaHCO_3$ and brine and dried ($MgSO_4$). The solvent was removed and the product (290 mg) subjected to flash chromatography on silica gel (7.5 g, 1:1 ether/hexane). Combination of fractions on the basis of TLC analysis gave 21 mg of a 1:1 mixture of two diastereomers and 214 mg (80%) of pure alcohol **17** as a colorless oil: $[\alpha]_D$ +59.0° (*c* 1.37, $CHCl_3$); IR (film) 1728, 1665, 1625, 804, 785, 735 cm^{-1}; ^1H NMR δ 0.89 (3, t, *J* = 7.4), 1.12 (3, d, *J* = 7.0), 1.46 and 1.67 (2, 2 m), 1.72 (3, d, *J* = 6.6), 1.90–2.19 (5, m), 2.37 (1, m), 2.63 (2, m), 2.76 (2, m), 3.51 (1, d, *J* = 3.8, OH), 3.91 (1, dd, *J* = 11.1, 6.1), 4.02 (1, dd, *J* = 11.1, 4.6), 4.53 (1, m), 5.64–5.68 (2, m), 6.08 (2, d, *J* = 15.8), 6.68 (1, q, *J* = 6.6), 6.73 (1, dd, *J* = 15.8, 9.1), 7.44–8.08 (7, m); ^{13}C NMR δ 11.57 (C-4''), 16.49 (C-2'' Me), 21.64 (C-2'''), 26.65 (C-3''), 27.25 (C-3'), 2.92 (C-6'), 36.56 (C-2'), 39.38 (C-1'), 40.8 (C-2''), 41.01 (C-2), 45.59 (C-4), 64.50 (C-3), 66.29 (CH_2O), 69.87 (C-1'''), 124.57, 125.67 (C-4', C-5'), 122.91, 123.11, 125.26, 125.61, 128.42, 128.84, 130.04, 133.69, 137.05, 139.01 (C-6), 150.39 (C-7), 171.00 (C-1), 176.48 (C-1''), 199.13 (C-5). Anal. ($C_{31}H_{38}O_6$) C, H.

(3*R*,1'*R*,2'*S*,2''*S*)-7-[2'-[(2''-Methyl-1''-oxobutoxy)-methyl]-1'-cyclohexyl]-3-hydroxy-5-oxoheptanoic Acid (8). Diester **17** (62 mg, 0.122 mmol) in EtOAc (10 mL) containing 10% palladium on carbon was stirred under an atmosphere of hydrogen for 9 h. Removal of solvent and catalyst gave an oil (60 mg) which was dissolved in ether and extracted with dilute aqueous NaOH (0.16 mmol). The ether layer was washed with water, and the combined aqueous layers were acidified with aqueous HCl (0.1 M, 2 mL). The aqueous acidic fraction was extracted with ether (3 × 10 mL), and the ether extract was washed with water and brine and dried ($MgSO_4$). Removal of solvent gave 39 mg (89%) of acid **8**: $[\alpha]_D$ +49.9° (*c* 1.68, $CHCl_3$); IR 3500, 1725 cm^{-1}; ^1H NMR δ 0.87 (3, t, *J* = 7.4), 0.92 (1, m), 1.11 (3, d, *J* = 7.0), 1.10 (1, m), 1.16 (1, m), 1.18 (2, m), 1.38 (1, m), 1.40 (1, m), 1.45, 1.63 (2, m), 1.68 (3, m), 1.76 (1, m), 1.84 (1, m), 2.37, 2.57 (2, m), 2.55 (2, m), 2.65 (2, m), 3.95, (1, dd, *J* = 11.1, 6.5), 4.11 (1, dd, *J* = 11.1, 3.8), 4.47 (1, m); ^{13}C NMR δ 11.61 (C-4''), 16.53 (C-2'' Me), 25.56 (C-5'), 25.77 (C-4), 26.58 (C-7), 26.67 (C-2''), 29.72 (C-3'), 31.14 (C-6'), 38.03 (C-1'), 40.36, 40.40 (C-2, C-6), 40.94 (C-2'), 41.13 (C-2''), 47.95 (C-4), 64.22 (C-3), 66.93 (OCH_2), 175.93 (C-1), 177.08 (C-1''), 210.75 (C-5). Assignments were made on the basis of DEPT, COSY, and $^1H/^{13}C$ correlation spectra and some in the cyclohexane ring may be interchanged. Anal. ($C_{19}H_{32}O_6$) C, H.

Enzymatic Assay. Compound **8** was evaluated for its ability to inhibit the activity of HMGR by the previously published procedure.[6] The method measures the rate of production of [^{14}C]mevalonate from [^{14}C]-(*R*,*S*)-HMG CoA in the presence of NADPH and rat liver microsomes.

Acknowledgment. This research was supported by funds from the University of California Committee on Research. B.R.D. thanks the University of Auckland for a leave during 1987 and acknowledges the New Zealand–United States Educational Foundation for a Fulbright Travel Grant. We thank Dr. Kenneth Feingold for the gift of rat liver microsomes.

Registry No. **8**, 117678-63-4; **11**, 15679-27-3; **12**, 117678-57-6; **13**, 117678-59-8; **14**, 117678-60-1; **16**, 117678-61-2; **17**, 117678-62-3; **18**, 117678-64-5; **19**, 117709-11-2; HMGR, 9028-35-7; maleic anhydride, 1121-34-2; (−)-menthol, 2216-51-5; di-(−)-menthyl maleate, 34212-60-7; di-(−)-menthyl fumarate, 34675-24-6; buta-1,3-diene, 106-99-0; (1*S*,2*S*)-di-(−)-menthyl cyclohex-4-ene-1,2-dicarboxylate, 109062-95-5; (*S*)-2-methylbutanoic anhydride, 84131-91-9; (1*S*,2*S*,2'*S*)-1,2-bis[(2'-methyl-1'-oxobutoxy)methyl]cyclohex-4-ene, 117678-58-7.