tively constant deviation occurred for similar conformational differences (e.g., the values calculated by the Rekker method for all the *exo*-amines differed by -0.26 ± 0.15 from the log P value observed experimentally for each *exo*-amine). These deviations are summarized in Table II. We have utilized these average deviations for each conformational type (e.g., gauche) to derive the appropriate correction factor to be used to include conformation in the calculated log P value. These correction factors are shown in Table III. Inclusion of the correction factors for conformation then allowed a calculation of the Rekker [log $P_{\text{calcd(Rekker,corr)}}$] and Leo [log $P_{\text{calcd(Leo,corr)}}$] values listed in Table I. In the modification of both the Rekker or Leo procedure, the appropriate additional factor from Table III was added after the normal fragment calculation was completed to compensate for the effect of conformation on hydrophobicity. An example set of calculated log P values is shown in Chart I. The corrected log P values calculated from both the Rekker and Leo methods are shown graphically in Figures 2 and 3, respectively. As can be seen from the plots in Figures 2 and 3, the calculated (corrected) log P for this diverse set of amines is in excellent agreement with the observed values. The regression equations for both plots are also shown in Figures 2 and 3; the correlation coefficients, r, are 0.989 for the Rekker method and 0.983 for the Leo method. Thus, when our new conformational correction factors are applied to the compounds of this data set, excellent agreement between calculated and measured values arises. It is significant to note that the correlation applies over a wide log P range (0.4–3.3).

Since there is some controversy[14] as to the choice of the Rekker[3] or Leo[4,5] fragment approach, we have determined new correction factors for both methods. Although the theoretical foundation of both methods is different, the two methods appear to predict the log P values of these conformationally defined systems well. The Leo method predicts 14 compounds better than the Rekker method, while the latter predicts eight compounds better than the former. The remaining two compounds are predicted equally well by both methods.

In summary, a valuable extension of the popular fragment method for calculating log P values has been presented for conformationally defined compounds. With our new correction factor, both the Rekker and Leo fragment procedures give excellent agreement with measured log P values for a wide range of pharmacologically important, conformationally defined amines. This approach is easy to use and only requires one additional correction factor per isomer. With these new correction factors, a beginning has been made toward the inclusion of conformation into the calculation of log P values.

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Registry No. **1**, 86943-77-3; **2**, 83118-50-7; **3**, 83118-51-8; **4**, 86992-67-8; **5**, 83118-48-3; **6**, 86022-72-2; **7**, 86943-78-4; **8**, 18883-05-1; **9**, 86992-68-9; **10**, 18883-06-2; **11**, 86943-79-5; **12**, 62624-27-5; **13**, 86992-69-0; **14**, 15537-20-9; **15**, 58742-05-5; **16**, 14342-36-0; **17**, 14098-20-5; **18**, 62624-26-4; **19**, 72597-35-4; **20**, 58742-04-4; **21**, 86943-80-8; **22**, 73159-84-9; **23**, 86992-70-3; **24**, 73208-84-1; NMT, 9037-68-7.

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Synthesis of 3-Hydroxy-3-cyclohexylbutyric Acid Derivatives. 1. Cyclic Homologues of 3-Hydroxy-3-methylglutaric Acid

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Z and *E* isomers of 3-methyl-3-(carboxymethyl)hexahydro-1(3*H*)-isobenzofuranones (I), lactones of 3-hydroxy-3-(2-carboxycyclohexyl)butyric acids (II), were prepared and tested on cholesterol biosynthesis in vitro. Compound I of the *Z* series was prepared through its ethyl ester by hydrogenation, over Rh/Al_2O_3 catalyst, of the phenyl ring of 3-methyl-3-[(ethoxycarbonyl)methyl]-1(3*H*)-isobenzofuranone. Compound I of the *E* series was prepared, through its ethyl ester, by Reformatsky reaction from ethyl (*E*)-2-acetylcyclohexanecarboxylate. 3-Methyl-3-(carboxymethyl)-5,6,7,8-tetrahydro-1(3*H*)-isobenzofuranone, 3-methyl-3-ethyl-5,6,7,8-tetrahydro-1(3*H*)-isobenzofuranone, and 3-methyl-3-(carboxymethyl)-1(3*H*)-isobenzofuranone were also prepared and tested. The above compounds inhibited acetate incorporation in cholesterol and fatty acids in rat liver slices at 5×10^{-3} M but lack specific inhibitory activity on HMG-CoA reductase.

3-Hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) is the metabolic precursor of mevalonic acid in cholesterol biosynthesis, and its reduction by HMG-CoA reductase is considered to be the rate-limiting step in the biosynthetic pathway from acetate to cholesterol.[1] Moreover, 3-hydroxy-3-methylglutaric acid (HMG) reportedly has regulatory effects on cholesterol biosynthesis both in vitro and in vivo[2] and blood cholesterol lowering activity in rats and humans.[3]

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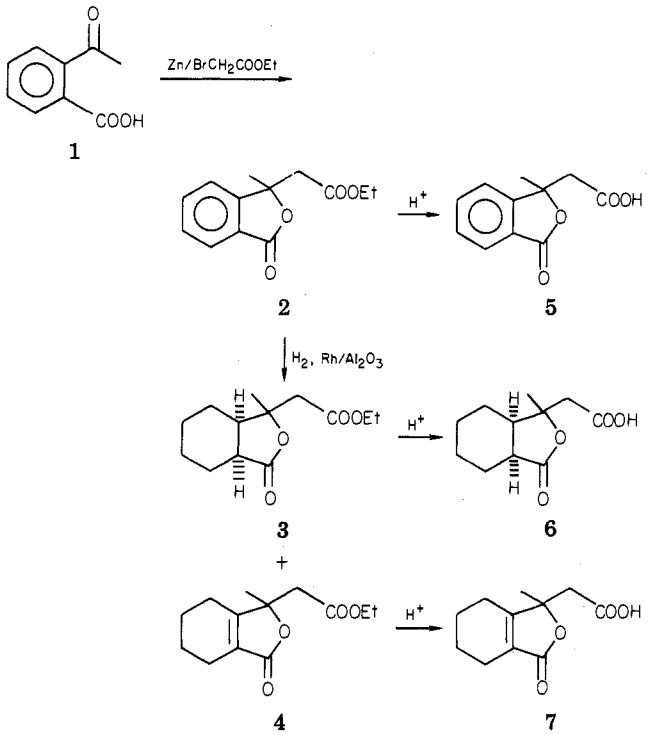
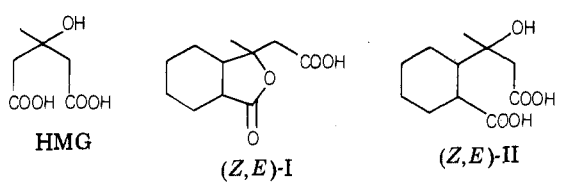
Scheme I



Some derivatives of HMG, mainly monoesters[4] and some analogues,[5] have been described as inhibitors of cholesterol biosynthesis. We now report the synthesis and preliminary biological evaluation of some alicyclic homologues of HMG: (*Z*)- and (*E*)-3-methyl-3-(carboxymethyl)hexahydro-1-(3*H*)-isobenzofuranones (I), the lactones of the corresponding 3-hydroxy-3-(2-carboxycyclohexyl)butyric acids (II) and some related compounds.

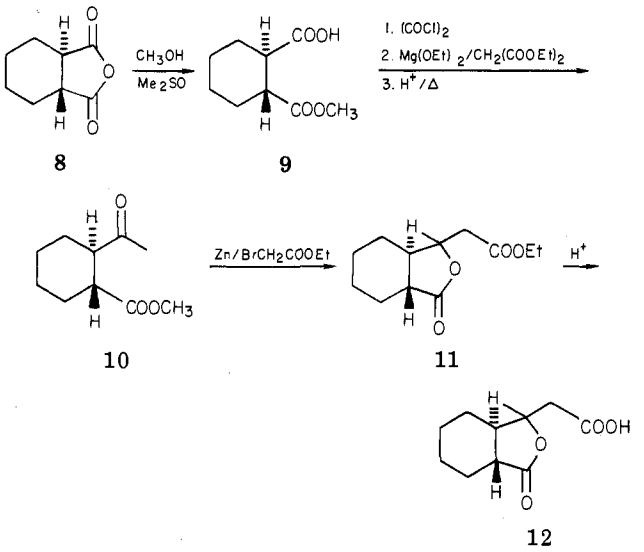


HMG (*Z*,*E*)-I (*Z*,*E*)-II

Chemistry. The *Z* isomer of I was prepared following Scheme I. Lactone ester **2** was obtained from 2-acetylbenzoic acid by the classical Reformatsky procedure with an excess of $Zn/BrCH_2COOEt$. Reduction of the phenyl ring of the aromatic lactone ester **2**, by hydrogenation over Rh/Al_2O_3 catalyst[6] at low pressure and room temperature, gave a mixture of the (*Z*)-lactone ester **3** and its cyclohexenyl analogue **4** in good yield. Compounds **3** and **4** were separated by chromatography. Lactone acids **5**–**7** were obtained from the corresponding ethyl esters by hydrolysis with concentrated hydrochloric acid. The *E* isomer of I was prepared following Scheme II. Lactone ester **11** was obtained by a classical Reformatsky procedure from **10**, which in turn was prepared, as described in the literature,[7] starting from (*E*)-hexahydrophthalic anhydride **8**. In order to assess the possible role of the carboxy group in biological activity, we prepared compounds **13** and **14**, following Scheme III.

An aromatic HMG analogue lacking one carboxy group, penphenone, is reported to have hypolipidemic activity.[8] Disubstituted phthalide **13** was obtained in good yield from 2-acetylbenzoic acid by treatment with an excess of

Scheme II



Scheme III

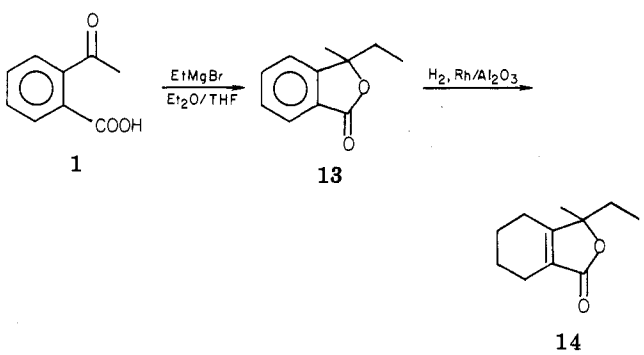


Table I. Inhibition of Cholesterol Biosynthesis in Rat Liver Slices[a]

		% inhibn of [^{14}C]acetate incorp in		
compd	concn, M	CO_2	fatty acids	cholesterol
5	5×10^{-3}	<10	54	48
6	5×10^{-3}	23	34	30
7	5×10^{-3}	45	83	63
12	5×10^{-3}	37	67	42
13	5×10^{-3}	86	99	99
13	5×10^{-4}	<10	29	22
14	5×10^{-3}	63	81	81
HMG	5×10^{-3}	<10	<10	12

[a] The inhibition of [^{14}C]acetate incorporation into CO_2, long-chain fatty acids, and cholesterol was determined by incubation of rat liver slices (250 mg) in mL of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 4 mM of [2-^{14}C]acetate with and without the compound to be assayed. Values are arithmetical means of three determinations.

(4) See the many papers of M. R. Boots and his co-workers; for instance, Boots M. R.; Yeh, Y.-M.; Boots, S. G. *J. Pharm. Sci.* **1980**, *69*, 306, and the references cited there in. Longino, M. A. *Diss. Abstr. Int. B* **1976**, *36*(10), 5047; *Chem. Abstr.* **1976**, *85*, 1651e.

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EtMgBr. Compound 14 was obtained by catalytic hydrogenation over Rh/Al_2O_3 using the conditions described for 4.

Pharmacology. Compounds 5–7, 12–14, and HMG were tested in vitro as inhibitors of cholesterol biosynthesis and HMG-CoA reductase, the rate-limiting enzyme in the cholesterol synthetic pathway. Table I shows that the compounds (except HMG) did inhibit cholesterol synthesis, but this effect was associated with a significant depression of acetate incorporation in fatty acids and CO_2.

These results indicate activity at different sites of cellular metabolism but not specific inhibition of cholesterol biosynthesis. When the compounds were tested at 5×10^{-3} M in vitro on HMG-CoA reductase, no inhibition was observed; in this test, HMG at 5×10^{-3} M caused 34% inhibition of enzymatic activity. No clear conclusion can be drawn about possible structure–activity relationships; alicyclic *Z* and *E*, aromatic, and cyclohexenyl analogues show a similar profile of activity.

Experimental Section

Chemistry. Melting points were determined in open glass capillaries using a Büchi Mel-Temp apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 683 instrument; frequencies are expressed in reciprocal centimeters. NMR spectra were recorded on a Bruker HX 90 instrument. Chemical shifts are reported in parts per million with Me_4Si as the internal reference. Elemental analyses were performed on a Carlo Erba 1106 instrument and were within ±0.4% of the calculated values. Column chromatographic separations were performed by flash technique on 40–60 μm silica gel (Merck no. 9385).

3-Methyl-3-[(ethoxycarbonyl)methyl]-1(3*H*)-isobenzofuranone (2). A solution of 2-acetylbenzoic acid (4 g, 0.024 mol) and ethyl α-bromoacetate (6.44 mL, 0.058 mol) in dry tetrahydrofuran (30 mL) was added dropwise to a vigorously stirred suspension of zinc (3.8 g, 0.058 mol) in dry tetrahydrofuran (50 mL). The mixture was stirred for 3 h and then cooled, treated with 2 N H_2SO_4, and extracted with diethyl ether. The organic layer was washed (H_2O, 5% $NaHCO_3$, H_2O), dried ($CaCl_2$), and evaporated under reduced pressure to give 4.5 g (80%) of 2 as a colorless oil: IR (neat liquid) 1760 (C=O lactone), 1730 (C=O ester) cm^{-1}; NMR ($CDCl_3$) δ 1.08 (t, 3 H, CH_2CH_3), 1.80 (s, 3 H, CH_3), 3.05 (s, 2 H, CH_2COO), 4.02 (q, 2 H, CH_2CH_3), 7.45–7.91 (m, 4 H, aromatic protons). Anal. ($C_{13}H_{14}O_4$) C, H.

(*Z*)-3-Methyl-3-[(ethoxycarbonyl)methyl]hexahydro-1(3*H*)-isobenzofuranone (3) and 3-Methyl-3-[(ethoxycarbonyl)methyl]-5,6,7,8-tetrahydro-1(3*H*)-isobenzofuranone (4). A mixture of 2 (2.4 g, 0.01 mol) and 5% rhodium on alumina catalyst (1.25 g) in 95% ethanol (50 mL) was hydrogenated for 24 h at room temperature in a Parr low-pressure apparatus at an initial pressure of 50 psi. Filtration of the suspended catalyst and removal of the solvent under vacuum gave a crude mixture, as a colorless oil, which was chromatographed on silica gel (eluant *n*-hexane/EtOAc, 7:3). The purification furnished two different reduction products, 3 and 4. Compound 3: yield 0.8 g (32.5%); the component at higher Rf; colorless oil; NMR ($CDCl_3$) δ 1.27 (t, 3 H, CH_2CH_3), 1.46 and 1.50 (2 s, 3 H, CH_3), 1.05–1.96 (m, 9 H, cyclohexane protons), 2.74 (d, 3 H, CH_2COO), 2.99 (m, 1 H, CHCOO), 4.13 (q, 2 H, CH_2CH_3). Anal. ($C_{13}H_{20}O_4$) C, H. Compound 4: yield 1 g (43%); colorless oil; IR 1770 (C=O lactone), 1730 (C=O ester) cm^{-1}; NMR ($CDCl_3$) δ 1.20 (t, 3 H, CH_2CH_3), 1.48 (s, 3 H, CH_3), 1.59–1.90 (m, 4 H, CH_2CH_2), 2.05–2.41 (m, 4 H, allylic CH_2), 2.78 (d, 2 H, CH_2COO), 4.06 (q, 2 H, CH_2CH_3). Anal. ($C_{13}H_{18}O_4$) C, H.

3-Methyl-3-(carboxymethyl)-1(3*H*)-isobenzofuranone (5). A solution of 2 (2.34 g, 0.01 mol) in 37% HCl (30 mL) was stirred overnight at room temperature. The reaction mixture was diluted with H_2O (100 mL) and extracted with ether. The organic layer was extracted with 5% aqueous $NaHCO_3$. The aqueous extract was acidified with 6 N HCl to pH 3 and extracted with ether. The solvent was removed under reduced pressure, and the crude product, recrystallized from ether/petroleum ether (40–70 °C) (1:1), gave 1.5 g (73%) of 5, mp 87–88 °C. Anal. ($C_{11}H_{10}O_4$) C, H.

Esters 3 and 4 were hydrolyzed by the procedure described above to give, respectively, compounds 6 and 7. **(*Z*)-3-Methyl-3-(carboxymethyl)hexahydro-1(3*H*)-isobenzofuranone (6)**: yield 86%; mp 70–90 °C. Anal. ($C_{11}H_{16}O_4$) C, H. **3-Methyl-3-(carboxymethyl)-5,6,7,8-tetrahydro-1(3*H*)-isobenzofuranone (7)**: yield 77%; mp 99–101 °C. Anal. ($C_{11}H_{14}O_4$) C, H.

(*E*)-3-Methyl-3-[(ethoxycarbonyl)methyl]hexahydro-1(3*H*)-isobenzofuranone (11) was prepared from methyl (*E*)-2-acetylcyclohexanecarboxylate[7] (10) by the Reformatsky procedure described for the synthesis of 2, internal lactonization occurring during the reaction.

The crude product was chromatographed on silica gel (eluant *n*-hexane/EtOAc, 8:2) to give a colorless oil (76%): IR (neat liquid) 1775 (C=O lactone), 1735 (C=O ester) cm^{-1}; NMR ($CDCl_3$) δ 1.25 (t, 3 H, CH_2CH_3), 1.35 and 1.55 (2 s, 3 H, CH_3), 1.11–2.25 (m, 9 H, cyclohexane protons), 2.65 (m, 3 H, CHCOO and CH_2COO), 4.15 (q, 2 H, CH_2CH_3). Anal. ($C_{13}H_{20}O_4$) C, H.

(*E*)-3-Methyl-3-(carboxymethyl)hexahydro-1(3*H*)-isobenzofuranone (12) was obtained from 11 by using the acidic hydrolytic conditions reported above to obtain 6 and 7: yield 81%; mp 186–188 °C. Anal. ($C_{11}H_{16}O_4$) C, H.

3-Methyl-3-ethyl-1(3*H*)-isobenzofuranone (13). A solution of 2-acetylbenzoic acid (4 g, 0.024 mol) in a mixture of dry ether (30 mL) and dry THF (30 mL) was added dropwise under stirring to a Grignard reagent prepared from ethyl bromide (6.32 g, 0.058 mol) and magnesium (1.45 g, 0.06 mol) in dry ether (50 mL). The mixture was stirred for 3 h at 0–5 °C and for 2 h at room temperature, cooled to under 5 °C, and treated with saturated NH_4Cl solution. After removal of the organic solvent under vacuum, the solution was extracted with ether. The organic layer was washed (H_2O), dried ($CaCl_2$), and evaporated to dryness; the crude product was purified by distillation under reduced pressure to give 3.17 g (72%) of 13: bp 85 °C (0.1 mmHg) [lit.[9] bp 154–156 °C (17 mmHg)]. Anal. ($C_{11}H_{12}O_2$) C, H.

3-Methyl-3-ethyl-5,6,7,8-tetrahydro-1(3*H*)-isobenzofuranone (14) was obtained by catalytic reduction of 13 under the conditions described for 4: white powder; yield 71%; mp 39–40 °C; NMR ($CDCl_3$) δ 0.75 (t, 3 H, CH_2CH_3), 1.38 (s, 3 H, CH_3), 1.53–1.95 (m, 6 H, 3 CH_2), 2.05–2.32 (m, 4 H, allylic CH_2). Anal. ($C_{11}H_{16}O_2$) C, H.

Pharmacology. In vitro cholesterol synthesis was determined in liver slices (250 mg), prepared from fed OFA ICO SD (IOPS Caw) male rats. The slices were incubated in 4 mL of Krebs–Ringer bicarbonate buffer, pH 7.4, containing 4 mM [2-^{14}C]acetate, flushed with 95% O_2–5% CO_2.

The compounds were added to give a final concentration of 5×10^{-3} M. The rate of [^{14}C]acetate uptake in CO_2, long-chain fatty acids, and cholesterol was determined according to described methods.[10,11] The compounds were also tested in vitro, at 5×10^{-3} M, for HMG-CoA reductase activity, with a liver microsomal fraction prepared from male rats.[12]

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Registry No. 2, 86954-83-8; 3, 86954-84-9; 4, 86954-85-0; 5, 86954-86-1; 6, 86954-87-2; 7, 86954-88-3; 10, 43143-03-9; 13, 86954-89-4; 14, 86954-90-7; 2-acetylbenzoic acid, 577-56-0; ethyl α-bromoacetate, 105-36-2; cholesterol, 57-88-5.

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