Synthesis of 3-Hydroxy-3-cyclohexylbutyric Acid Derivatives. 2. Cyclic Analogues of Mevalonic Acid

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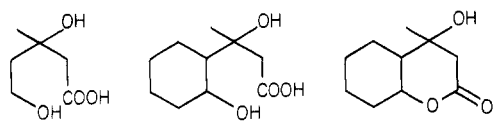
The sodium salt of (*Z*)-3-hydroxy-3-(2-hydroxycyclohexyl)butyric acid (I) and its lactone (II) were prepared through the corresponding *tert*-butyl ester by hydrogenation, over Rh/Al_2O_3 catalyst, of the phenyl ring of *tert*-butyl 3-hydroxy-3-(2-hydroxyphenyl)butyrate (III). (*Z*)-3-Hydroxy-3-(2-methoxycyclohexyl)butyric acid was prepared similarly. (*Z*)-4-Methyloctahydro-2*H*-1-benzopyran-2-one was prepared by hydrogenation, over Rh/Al_2O_3 catalyst, of 4-methylcoumarine, prepared in turn from III by a one-pot procedure comprising hydrolysis, lactonization, and dehydration. The above compounds inhibit acetate incorporation in cholesterol and fatty acids in rat liver slices at 5×10^{-3} M, but they lack specific inhibitory activity on HMG-CoA reductase.

Mevalonic acid (MVA) is a key intermediate of cholesterol biosynthesis.[1] A number of papers deal with the possibility of controlling blood lipid levels by means of MVA analogues and isosters acting as inhibitors or antimetabolites.[2] Here we report the synthesis and biological evaluation of some alicyclic MVA analogues: 3-hydroxy-



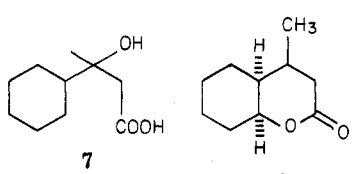
MVA I II

3-(2-hydroxycyclohexyl)butyric acid (I), its lactone (II), and some related compounds. Compounds I and II are related to cyclic homologues of HMG reported in the preceding paper in this issue.

Chemistry. The *tert*-butyl 3-hydroxy-3-phenylbutyrates (**2**) were obtained from the corresponding acetophenones (**1**) by the procedure first described by Hauser[3] and Sisido,[4] while the classical Reformatsky method failed.

Synthesis of *Z* derivatives **3** as a mixture of diastereoisomers *Z*-(3*R*) and *Z*-(3*S*) was attempted (Scheme I) by reducing the phenyl ring of the aromatic analogues **2** catalytically over Rh/Al_2O_3.[5] Hydrogenation of **2** occurred in good yield at low pressure, without any of the cleavage at the benzylic position which is known to occur with other platinum metal catalysts.[6] Treatment of **3a,b** with methanolic KOH gave, respectively, **4** and the lactone **5**, which was in turn opened to **6** by alkaline hydrolysis with aqueous NaOH.

In order to determine the role of the hydroxyl groups in the biological activity of the compounds, we prepared and tested compounds **7** and **8**. Compound **7** was pre-



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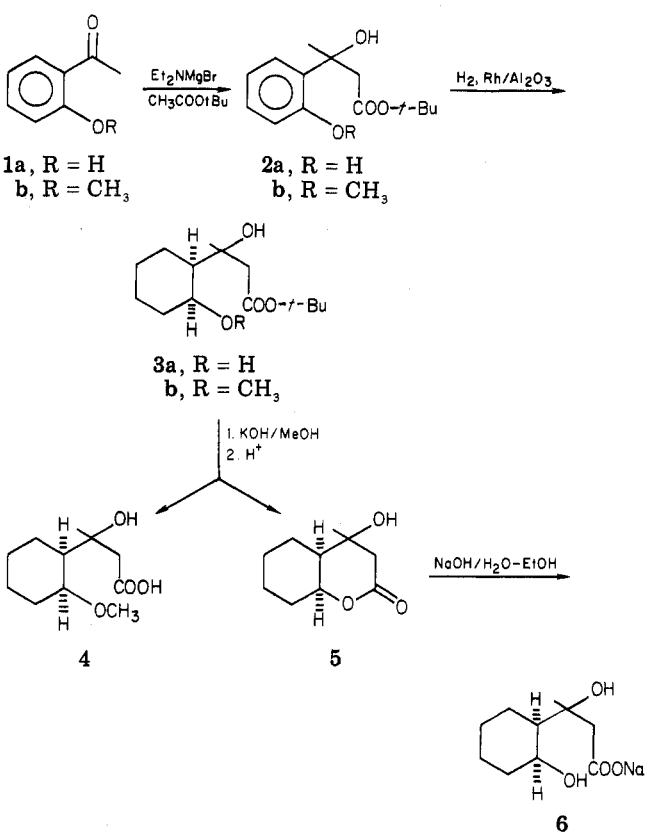
pared by alkaline hydrolysis of the corresponding ethyl ester obtained from acetylcyclohexane following the classical Reformatsky procedure. Compound **8** was prepared by catalytic hydrogenation of 4-methylcoumarine over Rh/Al_2O_3. The latter compound was in turn obtained from **2a** through a one-pot procedure comprising hydrolysis, lactonization, and dehydration, in good yield.[4,7]

Scheme I



Pharmacology. Compounds **4–8** were tested in vitro as inhibitors of cholesterol biosynthesis and of HMG-CoA

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

no.	concn, M	% inhibn of [^{14}C]acetate incorp in CO_2	fatty acids	cholesterol
4	5×10^{-4}	39	57	25
5	5×10^{-4}	10	10	10
	5×10^{-3}	37	47	22
6	5×10^{-4}	10	10	10
	5×10^{-3}	62	68	50
7	5×10^{-4}	62	72	32
8	5×10^{-4}	10	10	10
	5×10^{-3}	24	62	39

[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 4 mL of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 4 mM [2-^{14}C]acetate with and without the compound to be assayed. Values are arithmetical means of three determinations.

reductase, the rate-limiting enzyme in the cholesterol synthetic pathway. As shown in Table I, the compounds did inhibit cholesterol synthesis, but this effect was associated with a significant depression of acetate incorporation in fatty acids and CO_2.

These data indicate action at different sites of the cellular metabolism. in vitro the compounds caused no inhibition of HMG-CoA reductase activity.

Experimental Section

Chemistry. Melting points were determined in open glass capillaries with 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 9385).

***tert*-Butyl 3-Hydroxy-3-(2-hydroxyphenyl)butyrate (2a).** A solution of diethylamine (4.4 g, 0.06 mol) in dry ether (10 mL) was added dropwise under stirring at 0–5 °C to a Grignard reagent prepared from ethyl bromide (6.5 g, 0.06 mol) and magnesium (1.5 g, 0.062 mol) in dry ether (10 mL). After refluxing for 30 min, the mixture was cooled to −5 °C, and a solution of 2′-hydroxyacetophenone (2.7 g, 0.02 mol) and *tert*-butyl acetate (4.6 g, 0.04 mol) in dry ether (20 mL) was added dropwise under vigorous stirring. The reaction mixture was stirred for 2 h, keeping the temperature under 0 °C, and then decomposed with a saturated solution of NH_4Cl in water (100 mL). The organic layer was separated, and the aqueous phase was extracted with ether. The combined organic layer was washed (5% H_2SO_4, H_2O, 5% $NaHCO_3$, H_2O), dried ($MgSO_4$), and evaporated under vaccum, giving 4.8 g of yellow oil. The crude product was chromatographed on silica gel (eluant $CHCl_3$), giving 3.1 g (61%) of **2a** as a colorless oil. Anal. ($C_{14}H_{20}O_4$) C, H.

***tert*-Butyl 3-hydroxy-3-(2-methoxyphenyl)butyrate (2b)** was prepared in the same manner, starting from 2′-methoxyacetophenone: yield 54%; oil. Anal. ($C_{15}H_{22}O_4$) C, H.

(*Z*)-*tert*-Butyl 3-Hydroxy-3-(2-hydroxycyclohexyl)butyrate (3a). A mixture of **2a** (4.3 g, 17 mmol) and 5% rhodium on alumina catalyst (1 g) in 95% ethanol (60 mL) was hydrogenated for 10 h at room temperature in a Parr low-pressure apparatus at an initial pressure of 50 psi. Filtration of the suspended catalyst, followed by distillation under reduced pressure, yielded 4.1 g (93%) of the crude ester **3a** as a colorless oil: NMR ($CDCl_3$) δ 1.11–2.48 (m, 9 H, cyclohexane protons), 1.26 and 1.32 (2 s, 3 H, CH_3), 1.45 (s, 9 H, *tert*-butyl), 2.58 (d, 2 H, CH_2COO), 4.10 (m, 1 H, C*H*OH), 4.38 (br s, 2 H, COOH + OH). Anal. ($C_{14}H_{26}O_4$) C, H.

(*Z*)-*tert*-Butyl 3-hydroxy-3-(2-methoxycyclohexyl)butyrate (3b) was prepared in the same manner as **3a**, starting from **2b**: yield 91%; colorless oil; IR (neat liquid) 3490 (OH), 1725 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 1.15–1.72 (m, 9 H, cyclohexane protons), 1.27 and 1.32 (2 s, 3 H, CH_3), 1.47 (s, 9 H, *tert*-butyl), 2.48 (d, 2 H, CH_2COO), 3.31 (s, 3 H, OCH_3), 3.85 (m, 1 H, C*H*OH), 4.15 (br s, 1 H, OH). Anal. ($C_{15}H_{28}O_4$) C, H.

(*Z*)-3-Hydroxy-3-(2-methoxycyclohexyl)butyric Acid (4). Ester **3b** (1.8 g, 6.6 mmol) was dissolved in N/2 methanolic potassium hydroxide (150 mL). The solution was kept at 50 °C for 2 h and then cooled, washed with ether, acidified with cold 2 N H_2SO_4, and extracted with ethyl acetate. The organic portion was washed (H_2O) and dried (Na_2SO_4); removal of the solvent yielded 1.25 g (87.5%) of colorless oil. Anal. ($C_{11}H_{20}O_4$) C, H.

(*Z*)-4-Hydroxy-4-methyloctahydro-2*H*-1-benzopyran-2-one (5) was obtained from **3a** using the same hydrolytic conditions as for compound **4**, intramolecular lactonization occurring during the reaction: yellow oil; yield 79%; IR ($CHCl_3$) 2600 (free OH), 3420 (ass. OH), 1720 (C=O lactone) cm^{-1}; NMR ($CDCl_3$) δ 1.05–2.31 (m, 9 H, cyclohexane protons), 1.23 and 1.35 (2 s, 3 H, CH_2), 2.48 (m, 2 H, CH_2COO), 3.91 (br s, 1 H, OH), 4.30 and 4.81 (2 m, 1 H, $W_{1/2H}$ = 7 Hz, CHO). Anal. ($C_{10}H_{16}O_3$) C, H.

Sodium (*Z*)-3-Hydroxy-3-(2-hydroxycyclohexyl)butyrate (6). Lactone **5** (0.3 g, 1.6 mmol) was dissolved in 50% aqueous ethanol (100 mL) and treated with 0.4 N NaOH (4 mL, 1.6 mmol). After stirring at 35 °C for 24 h, the solvent was evaporated under vacuum, and the residue, treated with anhydrous ethanol (3 × 50 mL), gave 0.34 g (95%) of **6** as a white, hygroscopic powder. Anal. ($C_{10}H_{17}NaO_4$) C, H, Na.

Ethyl 3-Hydroxy-3-cyclohexylbutyrate. A solution of acetylcyclohexane (12.6 g, 0.1 mol) and ethyl α-bromoacetate (20.3 g, 0.12 mol) in anhydrous benzene (50 mL) was added dropwise, while heating gently, to a vigorously stirred suspension of zinc (7.8 g, 0.12 g atom) in anhydrous benzene (50 mL). The mixture was refluxed for 2 h and then cooled, treated with dilute H_2SO_4, and extracted with ether. The organic layer was washed (H_2O), dried ($CaCl_2$), and evaporated to dryness. The crude product was distilled under reduced pressure (18 mmHg); the fraction boiling at 143–144 °C was collected, giving 16 g of pure ester (75%). Anal. ($C_{12}H_{22}O_3$) C, H.

3-Hydroxy-3-cyclohexylbutyric Acid (7). A solution of ethyl 3-hydroxy-3-cyclohexylbutyrate (3.96 g, 18 mmol) and KOH (1.7 g, 30 mmol) in 95% ethanol (40 mL) was refluxed for 3 h. The solvent was evaporated under vacuum, water (50 mL) was added, and the solution, acidified with 8% HCl, was extracted with ether. The organic layer was washed (H_2O), dried ($CaCl_2$), and evaporated to dryness to give 3 g (92%) of **7**, mp 49–51 °C. Anal. ($C_{10}H_{18}O_3$) C, H.

(*Z*)-4-Methyloctahydro-2*H*-1-benzopyran-2-one (8) was obtained by reduction of 4-methylcoumarine by the procedure already described for **3a** and **3b**: yield 55%; yellow oil; IR (neat liquid) 1730 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 0.75–2.31 (m, 9 H, cyclohexane protons), 0.90 and 0.98 (2 d, 3 H, CH_3), 2.28 (m, 2 H, CH_2COO), 4.4 (m, 1 H, CHO). Anal. ($C_{10}H_{16}O_2$) C, H.

4-Methylcoumarine. A solution of ester **2a** (3 g, 11.9 mmol) in N/2 methanolic KOH (150 mL) was kept for 8 h at 40 °C. The solvent was evaporated to dryness under reduced pressure. The residue, taken up with water, was acidified with 2 N HCl and extracted with ethyl acetate. After removal of the solvent, the crude product was chromatographed on silica gel (eluant *n*-hexane/ethyl acetate, 3:1), giving 1.3 g (68%) of white solid, mp 79–81 °C (lit.[7] mp 81–82 °C).

Pharmacology. The compounds were tested in vitro as described in the preceeding paper in this issue.[8-10]

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Registry No. **2a**, 86994-33-4; **2b**, 86994-34-5; **3a** (isomer 1), 86994-35-6; **3a** (isomer 2), 87038-36-6; **3b** (isomer 1), 86994-36-7; **3b** (isomer 2), 87038-37-7; **4**, 86994-37-8; **5**, 86994-38-9; **6**, 86994-39-0; **7**, 17692-20-5; **8**, 86994-40-3; 2′-hydroxyacetophenone, 118-93-4; 2′-methoxyacetophenone, 579-74-8; *tert*-butyl acetate, 540-88-5; acetylcyclohexane, 823-76-7; ethyl α-bromoacetate, 105-36-2; ethyl 3-hydroxy-3-cyclohexylbutyrate, 28811-84-9; 4-methylcoumarine, 607-71-6; cholesterol, 57-88-5.

α-Adrenergic Activities of Some Substituted 2-(Aminomethyl)imidazolines

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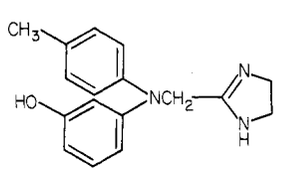
A series of 2-(aminomethyl)imidazolines related to the α-adrenergic antagonist phentolamine was prepared and evaluated for α-adrenergic agonist and antagonist activities in the isolated, field-stimulated rat vas deferens. Affinities for α-adrenergic receptors were determined by displacement of [^{3}H]clonidine and [^{3}H]prazosin from membrane binding sites of calf cerebral cortex. This series provided a variety of α-adrenergic profiles, with some of the (aminomethyl)imidazolines being nonselective α_1- and α_2-adrenergic antagonists like phentolamine, while others were either nonselective α_1- and α_2-agonists or mixed α_1-agonists/α_2-antagonists.

The α-adrenoceptor blocking agent phentolamine (**1**) is



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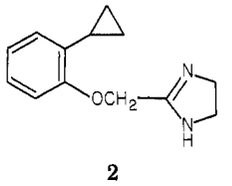

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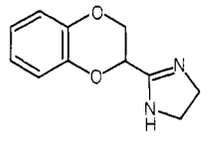

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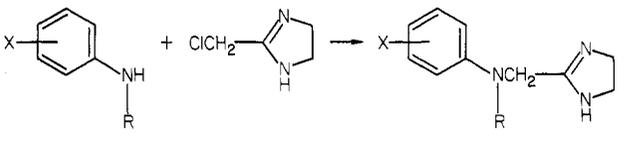
recognized to be a potent, but nonselective, competitive antagonist at both α_1- and α_2-adrenoceptor sites.[1,2] However, other imidazoline derivatives have recently been found to possess different profiles of adrenergic activities. For example, cirazoline (**2**) is described as having α_1-agonist and α_2-antagonist activities,[3] while RX 781094 (**3**) is reported to be a potent and selective α_2-antagonist with weaker α_1-antagonism properties.[4]

It was therefore of interest to characterize those molecular features of phentolamine responsible for α_1- and α_2-adrenoceptor antagonism and to use this information for the synthesis of more selective adrenergic blocking agents.

Chemistry. All of the imidazoline derivatives of Table I were synthesized by reaction of the appropriate arylamine with 2-(chloromethyl)imidazoline (Scheme I).[5,6] The required aniline derivatives have been reported in the literature and are readily available. However, in the case of the *N*-cyclohexyl-*N*-(4-tolyl) analogue **4f**, a two-step reductive alkylation of 4-methylaniline with cyclohexanone proved to be a more convenient preparation of the amine than the previously published procedure.

Testing Methods. Relative affinities of the imidazolines of Table I for central α-adrenergic binding sites were determined by measurement of radioligand displacement from membrane binding sites of calf cerebral cortex. Displacement of [^{3}H]clonidine was used as a measure of interaction with α_2-adrenoceptor binding sites, while

Scheme I



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[^{3}H]prazosin displacement served as an assay for α_1-adrenoceptor affinity.

α-Adrenergic agonist–antagonist profiles for these compounds were determined in the rat, isolated, field-stimulated vas deferens according to protocols described by Lotti et al.[7] In this tissue, presynaptic (α_2) adrenergic agonists characteristically inhibit stimulation-induced contractions, while postsynaptic (α_1) agonists enhance contractions. The α_1- and α_2-adrenergic agonist activities of the test compounds were verified by the ability of prazosin and rauwolscine to completely reverse the contractile enhancement or inhibition, respectively, produced by the test compounds. Antagonistic activities of the imidazolines upon α_1- and α_2-adrenoceptors were determined by blockade of the selective α_1- and α_2-adrenergic agonists methoxamine and clonidine, respectively.

Results and Discussion

Inspection of the rat vas deferens results summarized in Table II shows that phenotolamine remains the most potent α_1/α_2-adrenoceptor antagonist in this series. Replacement of the phenolic hydroxyl group of phentolamine by hydrogen gives **4a**, which, although slightly less potent than phentolamine, still functions as a nonselective α-ad-

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