With use of methylene chloride as the reaction solvent and column chromatography with 5% MeOH in $CHCl_3$ as the eluent for purification, a yield of 80% (lit.[11] 61%) and a melting point of 169–170 °C (lit.[11] mp 161–164 °C) were achieved for known compound **1**. For compound **2**, phenylphosphorodichloridate (42.2 g, 0.2 mol) in CH_2Cl_2 (50 mL) was added dropwise under N_2 and with stirring to 1,1-dimethylhydrazine (60.1 g, 1.0 mol) in CH_2Cl_2 (100 mL) at 0–10 °C. The reaction mixture was refluxed for 18 h, cooled to 25 °C, and filtered, and the residue was washed with CH_2Cl_2. The filtrate and washing were evaporated in vacuo to give a residue, which was chromatographed with 5% MeOH in $CHCl_3$ as the eluent to yield 42.3 g (82%) of **2** ($C_{10}H_{19}N_4O_2P$): mp 118–120 °C; IR 3180 (NH), 1600 (C=C), 1200, 1230 (P=O) cm^{-1}; NMR δ 2.5 (s, 12 H, 4 CH_3), 5.75 (s, 1 H, NH), 6.25 (s, 1 H, NH), 7.30 (m, 5 H, arom).

2,2′-(Phenyl- and -phenoxyphosphinylidene)bis(1,1,1-trimethylhydrazinium) Diiodide (3 and 4). An excess of iodomethane (17.0 g, 60 mmol) was added to 30 mmol of **1** (suspension) or **2** (solution) in CH_3CN (120 mL) at 25 °C. The reaction mixtures were heated to 70 °C (**1** went into solution) for 2 h and then to 60 °C for 18 h. The mixtures were cooled to 25 °C and filtered and the residues washed with CH_3CN and Et_2O to yield pure products. For **3** ($C_{12}H_{25}N_4I_2OP$): 12.7 g, 80%; mp 180–182 °C dec; IR 3170 (NH), 1590 (C=C), 1230 (P=O) cm^{-1}; NMR δ 3.61 (s, 18 H, 6 CH_3), 7.63–8.12 (m, 5 H, arom). For **4** ($C_{12}H_{25}N_4I_2O_2P$): 14.4 g, 88%; mp 174–175 °C dec; IR 3030 (NH), 1590, 1600 (C=C), 1170, 1200 (P=O) cm^{-1}; NMR δ 3.68 (s, 18 H, 6 CH_3), 7.43 (m, 5 H, arom).

2,2′-(Phenyl- and -phenoxyphosphinylidene)bis(1,1,1-trimethylhydrazinium) Iodide Inner Salt (5 and 6). Compound **3** or **4** (3.8 mmol) was dissolved in 10% NaOH (1.7 mL) at 25 °C to yield neutral solutions. An additional 1.7 mL of base gave alkaline solutions, an indication that a second molecule of HI was not neutralized. The reaction mixtures were stirred at 25 °C for 1 h, neutralized with 1 N HCl at 5–10 °C, and evaporated in vacuo to dryness at 25 °C. For **5** the residue was extracted several times with CH_2Cl_2, and the extracts were evaporated in vacuo to yield 1.7 g (98%) of **5** ($C_{12}H_{24}N_4IOP \cdot H_2O$): mp 126–128 °C dec; IR 3450 (OH), 3210 (NH), 1590 (C=C), 1200 (P=O) cm^{-1}; NMR δ 3.37 (s, 18 H, 6 CH_3), 5.75 (s, 1 H, NH), 7.39–7.88 (m, 5 H, arom). For **6** the residue was dissolved in acetone and filtered. A solid formed in the filtrate that was collected on a filter, washed with acetone, and dried to yield 2.7 g (86%) of **6** ($C_{12}H_{24}N_4IO_2P$): mp 202–203 °C dec; IR 3200 (NH), 1595 (C=C), 1200, 1230 (P=O) cm^{-1}; NMR δ 3.53 (s, 18 H, 6 CH_3), 7.35 (m, 5 H, arom).

Sympathetic Ganglionic Transmission Testing. Superior cervical ganglia were quickly excised from Sprague–Dawley rats. The ganglia were carefully desheathed while maintained in cold oxygenated (5% CO_2, 95% O_2) Locke's solution (pH 7.4). The ganglia were immersed in this solution in a constant temperature chamber with the pre- and postganglionic (internal carotid) nerves drawn into stimulating and recording suction electrodes. The preganglionic nerve was stimulated supramaximally at 0.3 Hz. Postganglionic compound action potentials were recorded with a capacity-coupled preamplifier and the amplified potentials were displayed on an oscilloscope and permanent records were made on photographic paper or film. Ganglia were selected for study only when the postganglionic action potential was stable for at least 30 min. Solutions of test compounds were added to the perfusion fluid and changes in amplitude of the action potential used as indices of effect on transmission. After the steady-state condition was attained, 8–10 "control" consecutive action potentials were recorded. Dose–response relationships were obtained by testing increasing, cumulative concentrations of the compounds on the action potential. Each new concentration was left in the bath for 5–10 min before recording of the action potential. Change in ganglionic transmission is expressed as the percent change in amplitude in the action potential.

Toxicity Testing. The brine shrimp assay procedure reported by Meyer et al.[12] was employed with two modifications. Filter paper was not used in the sample preparation; instead the sample of test solutions were pipetted directly into the sample vials prior to drying in vacuo and the percent mortalities were determined after 12 h, instead of 6 and 24 h. LD_{50} values were calculated by using the probit analysis method described by Finney.[13] Correlation coefficients (R^2) values of 0.998 and 0.994 were obtained for **5** and **6**, respectively, by linear regression analysis.

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3-Alkyl-3-hydroxyglutaric Acids: A New Class of Hypocholesterolemic HMG CoA Reductase Inhibitors. 1[1]

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Derivatives of 3-hydroxy-3-methylglutaric acid (HMG), a portion of the substrate for HMG CoA reductase, were prepared and tested for their inhibitory action against rat liver HMG CoA reductase and for their hypocholesterolemic activity. Structure-dependent competitive inhibition was observed. Optimal structures had a free dicarboxylic acid with an alkyl group of 13–16 carbons at position 3. 3-*n*-Pentadecyl-3-hydroxyglutaric acid (**3j**) (IC_{50} = 50 μM) reduced serum cholesterol in the Triton-treated rat and HMG CoA reductase activity in the 20,25-diazacholesterol-treated rat.

The loss of feedback regulation of HMG CoA reductase (HMGR) by a deficiency of function in the LDL receptor leads to an inefficient disposal of plasma cholesterol and premature atherosclerosis in man.[3] Compactin and mevinolin, inhibitors of HMG CoA reductase, lower serum total cholesterol levels in normal and familial hypercholesterolemic patients.[4] Hypolipidemic properties of 3-hydroxy-3-methylglutaric acid (HMG) have been reported for rats, rabbits, and man.[5] HMG, as the coenzyme A

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Table I. Chemical Properties and in Vitro HMGR Inhibition of β-Alkyl-β-hydroxyglutaric Acids and Their Derivatives

compd	R	X	Y	mp, °C	recrystn solvent	in vitro inhibn of HMGR @ 10^{-3} M	in vitro inhibn of HMGR IC_{50},[a] μM
3a	CH_3					0	
3b	CH_3CH_2			95–97	Et_2O–CH_2Cl_2	29	
3c	$CH_3(CH_2)_2$			103–105	Me_2CO–$CHCl_3$	37	
3d	$(CH_3)_2CH$			86	Me_2CO–$CHCl_3$	10	
3e	$CH_3(CH_2)_5$			81–83	Me_2CO–$CHCl_3$	5	
3f	$CH_3(CH_2)_9$			65–67	benzene	86	100
3g	$CH_3(CH_2)_{12}$			90–91	benzene	97	50
3h	$CH_3(CH_2)_{13}$			75–76	benzene	95	130
3j	$CH_3(CH_2)_{14}$			74–76	benzene	99	50
3k	$CH_3(CH_2)_{15}$			101–103	benzene	90	100
3l	$CH_3(CH_2)_{16}$			80–81	benzene	85	100
3m	$CH_3(CH_2)_{18}$			93–94	benzene	86	350
4a	CH_3	OC_2H_5	OC_2H_5	liq		0	
4b	CH_3	OCH_3	NH_2	amorph		0	
4c	$CH_3(CH_2)_9$	OC_2H_5	OC_2H_5	liq		0	
4d	$CH_3(CH_2)_{14}$	OCH_3	OCH_3	liq		−1	
4e	$CH_3(CH_2)_{14}$	OC_2H_5	OC_2H_5	liq		36	>1000
4f	$CH_3(CH_2)_{14}$	OH	OC_2H_5	71–72	Me_2CO–hexane	81	900
4g	$CH_3(CH_2)_{14}$	OH	$O(CH_2)_2N(CH_3)_2$	65–67	MeOH	0	
5a	CH_3	O	H			0	
5b	CH_3	NH	H	163–165	EtOAc	50	
5c	$CH_3(CH_2)_{14}$	O	H	94–95	AcOH–H_2O	80	250
5d	$CH_3(CH_2)_{14}$	O	$COCH_3$	75–77	AcOH–H_2O	−9	
6	$CH_3(CH_2)_{14}$			110–111	hexane	0	
7a	$CH_3(CH_2)_9$			amorph		0	
7b	$CH_3(CH_2)_{14}$			66–68		0	
8a	$CH_3(CH_2)_9$	0		amorph.		57	
8b	$CH_3(CH_3)_{14}$	0		58–59		82	750
8c	$CH_3(CH_2)_{14}$	H,OH		60–62		50	1000
compactin						100	1

[a] IC_{50} is the concentration in μM required to inhibit HMG CoA reductase by 50% of control.

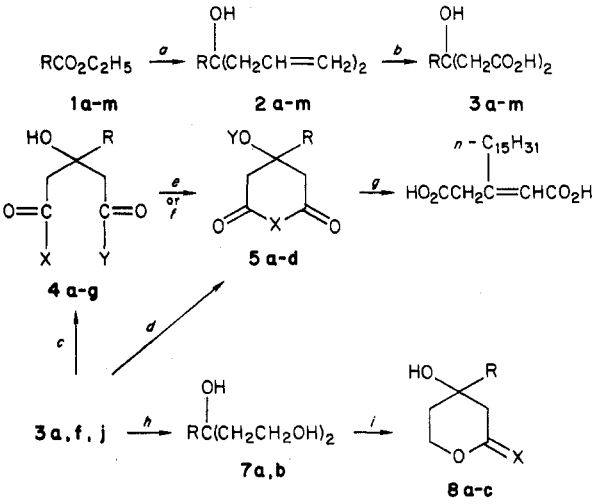
derivative, is the substrate for HMGR. In this paper, we report the synthesis and biological activity of a new class of HMGR inhibitors, the 3-*n*-alkyl-3-hydroxyglutaric acids (alkyl, n = 10–19).

Chemistry. The synthesis of the 3-alkyl-3-hydroxyglutaric acids began with addition of 2 equiv of allylmagnesium bromide to the alkanoic acid ethyl esters **1**, yielding the diallylcarbinols **2** (see Scheme I). Oxidation of **2** with ozone, followed by decomposition of the ozonide with hydrogen peroxide in the presence of sulfuric acid, gave good to moderate yields of the glutaric acids **3**.

The carboxylic acids **3a**, **3f**, and **3j**, when treated with methyl or ethyl iodide and potassium carbonate in dimethylformamide or methanol solution, yielded the corresponding diesters, **4a**, **4c**, **4d**, and **4e**.

In the presence of dicyclohexylcarbodiimide at 20 °C or acetic anhydride at 40 °C, **3j** formed its anhydride **5c**, but at elevated temperatures with acetic anhydride, it gave the acetate anhydride **5d**. The reaction of the anhydride **5c** with *N*,*N*-dimethylethanolamine and **5c** with ethanol in the presence of pyridine gave the esters **4g** and **4f**, respectively. The anhydride **5a**,[9] treated with ammonia

Scheme I



[a] Allylmagnesium bromide. [b] O_3; H_2O_2, H^+. [c] K_2CO_3–MeI or EtI, DMF. [d] DCCD or Ac_2O, Δ. [e] $(CH_3)_2NCH_2CH_2OH$ or EtOH, Py. [f] NH_3; MeI–K_2CO_3. [g] Py, water. [h] LAH. [i] CrO_3.

followed by reaction with methyl iodide in the presence of potassium carbonate, provided a mixture yielding the imide **5b** and amide ester **4b**.

When **5d** was heated with pyridine and water, it was hydrolyzed with loss of the acetoxy group and yielded **6**. Reduction of **3f** and **3j** with lithium aluminum hydride gave the diols **7a** and **7b**, which, upon oxidation with chromic acid, led to the lactones **8a** and **8b**, respectively.

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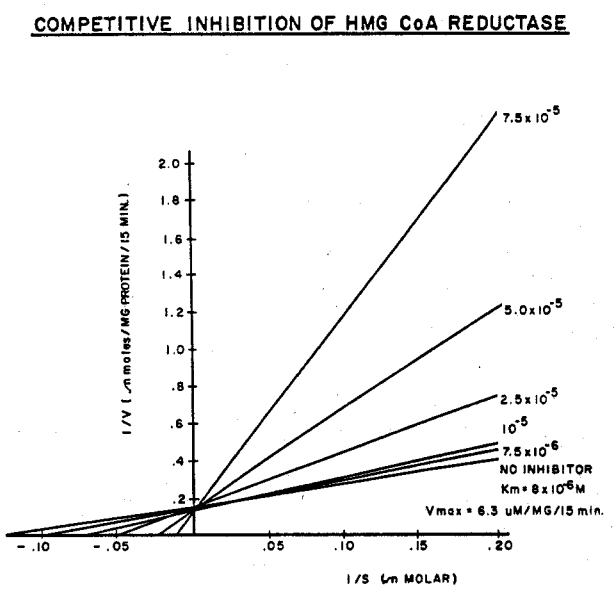

Figure 1. Lineweaver–Burk reciprocal plot for **3j**.

Table II. Reduction of Serum Cholesterol and HMGR in Rats

	Triton-treated rat		diazasterol-treated rat	
compd	dose (ig)	% cholesterol[a] reduction	dose, mg/kg per day (ig)	% decrease in HMGR act.[b]
3j	300	22	100	40
	200	17	50	31
	100	4	20	27
4d	300	2		
	200	4		
	100	4		
4e	200	3		
compactin	50	17[c]	10	60
	80	28[c]		

[a] $p < 0.01$. [b] $p < 0.05$. [c] See ref 11.

A byproduct of the oxidation of the diol **7b** was the lactol **8c**.

Biological Activity. In vitro inhibition of HMGR occurs in the 3-alkyl-3-hydroxyglutaric acids only when the alkyl chain length is sufficiently lengthened (see Table I). 3-*n*-Pentadecyl-3-hydroxyglutaric acid (**3j**) is the most active compound in the series, exhibiting competitive inhibition with a K_m value of 8×10^{-6} M and a V_{max} value of 6.3 μM/mg per 15 min (see Figure 1).

In vivo studies show that, at doses of 300 and 200 mg/kg per day, the reductase inhibitor **3j** lowers serum cholesterol 22% and 17%, respectively, in the Triton-treated rat (see Table II). In rats pretreated with diazasterol, **3j** inhibits the rise in reductase activity from 27% to 40% at doses of 20, 50, and 100 mg/kg per day. In this assay, compactin lowers HMGR activity 60% at a dose of 10 mg/kg per day. Like compactin, the inhibitor **3j** was observed not to reduce serum cholesterol levels in the normal rat.[6]

Discussion

Reduction of HMG to mevalonic acid by HMGR is considered the rate-limiting step in cholesterol production in eucaryotic cells. The reductase is bound to endoplasmic reticulum and is highly regulated by protein metabolism and perhaps phosphorylation. Control by inhibition of the catalytic reaction should also reduce substrate flow but may increase the actual level of the enzyme. The results demonstrate the inhibition of reductase by substrate analogues that act as competitive inhibitors. A similar type of inhibition has been reported for a different structural class based on compactin.[4] Carbon flow can be retarded by these compounds as indicated by reduction in serum total cholesterol.

Results also indicate some properties required in a substrate analogue for maximal activity. A compound should be a dicarboxylic acid with an alkyl substituent in the 3-position having 13–16 carbon atoms.[7] Functionalization of the carboxyl group or loss of the 3-hydroxyl group reduces or eliminates activity. Like the relatively lipophilic [(mercaptoethyl)amino]pantothenic acid amide moiety of coenzyme A in HMG CoA, the space-filling quality of the C_{15} side chain in **3j** very likely plays a significant role in binding to the reductase.

In addition to reducing cholesterol levels in the Triton-treated rat,[11] the inhibitor **3j** reduces the increase in HMGR activity induced by 20,25-diazacholesterol in the rat, a consequence that may be important in the design of a drug for the treatment of hypercholesterolemia. Counsell has observed that in the rat 20,25-diazacholesterol induces an increase in the activity of HMGR, a result of the inhibition of the conversion of desmosterol to cholesterol and loss of feedback inhibition by cholesterol.[12] The condition in this rat model mimics, in part, the loss of feedback regulation and increased HMGR activity observed in human familial hypercholesterolemia.

In conclusion, the 3-*n*-alkyl-3-hydroxyglutaric acids represent a class of new HMGR inhibitors that bear further investigation of their structure and activity relationships.

Experimental Section

Melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. NMR spectra were taken in deuteriochloroform on a Varian A-60A spectrometer with tetramethylsilane used as an internal standard. Microanalytical results reported by the symbols of the elements were within ±0.4% of theory.

For column chromatography, a ratio by weight of 20–30:1 Brinkman silica gel 60 (70–270 mesh) to compound was used. Compounds were visualized on silica gel plates with 5% phosphomolybdic acid in ethanol.

General Procedure A. Addition of Allylmagnesium Bromide to the Alkanoic Acid Ester 1. To an excess of magnesium metal (0.85 mol) stirred in 250 mL of dry THF under a nitrogen atmosphere were added 2 mL each of allyl bromide and allylmagnesium bromide (1 M in diethyl ether) and a catalytic amount of iodine. After the reaction began, a solution of alkanoic acid ester **1** (0.34 mol) and allyl bromide (0.75 mol) in dry THF (700 mL) was added dropwise to the reaction vessel. The reaction mixture was heated at reflux for 1 h, after which the reaction was quenched with methanol. The mixture was diluted with ether and washed with saturated ammonium chloride. The solution was dried, filtered, and concentrated in vacuo to an oil. Drying the oil at reduced pressure (2 mmHg, 40 °C) gave the diallylcarbinol **2** in yields > 90%; NMR δ 4.0–4.4 (m, 2 H, $=CH_2$), 4.65–5.25 (m, 1 H, $=CH$). The carbinols were generally used in procedure B without purification. The carbinols **2d**, **2f**, and **2j** were purified by distillation; bp (mm) 43–45 °C (1.2), 108–112 °C (0.2), 164–170 °C (0.1), respectively.

General Procedure B. Oxidative Ozonolysis of the Diallylcarbinols 2 to the Corresponding 3-Alkyl-3-hydroxyglutaric Acids 3.[8] The diallylcarbinol **2** (~0.03 mol), prepared by the method of procedure A, was dissolved in a mixture of 100 mL each of dichloromethane and ethyl acetate and the mixture was cooled to −60 to −30 °C. Ozone was bubbled into the solution until a blue color persisted (about 1 h). The solution was purged

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with oxygen and then added dropwise, with stirring, to 60 mL of cold AcOH, utilizing a protective safety shield. The solution was heated gently to distill off CH_2Cl_2 and was allowed to cool. A solution of 10 mL of water, 20 mL of 10% aqueous H_2SO_4, 20 mL of 30% aqueous H_2O_2, and 30 mL of HOAc was added dropwised with stirring. The reaction mixture was heated to 85 °C and then at reflux for 2 h. The solution was concentrated in vacuo and then diluted with 800 mL of cold water, producing an oily precipitate. The crude product was extracted into ether. The ether extract was washed with aqueous $NaHSO_3$ and water, dried ($MgSO_4$), and distilled to dryness. The crude product was purified by crystallization from ether–CH_2Cl_2, acetone–$CHCl_3$, or benzene. When the crude product failed to crystallize by trituration, from a solvent, it was purified by column or pressure chromatography, eluting with hexane–EtOAc–HOAc (80:19:1). Yields of pure **3** ranged from 20% to 60% on the basis of crude **2**; NMR δ 0.88 (3 H, CH_3), 1.25 (br, 2 H, CH_2), 2.66 (2 H, CH_2CO). Anal. ($C_nH_{2n-2}O_5$, n = 7, 8, 11, 15, 18–22, 24) C, H.

General Procedure for the Preparation of Esters 4a–e. A mixture of 500 mg of the desired amide acid or dicarboxylic acid, 2.76 g of potassium carbonate, 3.22 g of the appropriate iodide, and 40 mL of DMF was stirred at 20 °C for 16 h. In the preparation of the diesters, the mixture was diluted with water and extracted with EtOAc. The extract was washed with water, aqueous NaCl solution, dried (Na_2SO_4), and distilled to dryness at 20 °C under reduced pressure. The yields of product were >88%. The diesters could be purified by chromatography by eluting with EtOAc–benzene (1:6).

In the procedure, methanol could be used in place of DMF. Then, the reaction mixture was heated at reflux and the methanol was removed by distillation at reduced pressure to yield the crude product. Anal. ($C_{10}H_{18}O_5$, $C_{19}H_{36}O_5$, $C_{22}H_{42}O_5$, $C_{24}H_{46}O_5$) C, H; ($C_7H_{13}NO_3$, $C_{24}H_{45}NO_5$) C, H, N.

3-Hydroxy-3-methylglutaric Acid Mono(methyl ester) Amide 4b and 3-Hydroxy-3-methylglutarimide (5b). Gaseous anhydrous NH_3 was passed into a solution of 7.3 g (0.051 mol) of 3-hydroxy-3-methylglutaric acid anhydride (**5a**)[9] for 15 min. The solution was distilled to dryness under reduced pressure, and the residue was dissolved in 150 mL of MeOH and 100 mL of EtOAc and concentrated to 150 mL. An additional 120 mL of MeOH and 80 mL of EtOAc was added and the solution concentrated to 50 mL. The solution was distilled to dryness under reduced pressure. To the residue were added 500 mL of MeOH, 30 mL of MeI, and 14 g of anhydrous K_2CO_3. The mixture was stirred for 18 h and filtered. the filtrate was distilled to dryness under reduced pressure and the residue was dissolved in 250 mL of EtOAc and separated from any insoluble K_2CO_3 by filtration. The oil (1.2 g) was purified by chromatography by elution with $CHCl_3$–MeOH (9:1) to yield 890 mg (11%) of **4b**. Anal. ($C_7H_{13}NO_4$) C, H, N.

The remaining material was dissolved in EtOAc. The EtOAc solution was concentrated slowly by distillation to 15 mL. When the solution cooled, the product crystallized to yield 4.3 g (59%) of **5b**. Anal. ($C_6H_9NO_3$) C, H, N.

3-Hydroxy-3-*n*-pentadecylglutaric Acid Anhydride (5c). A mixture of 10 g (0.028 mol) of **3j**, 36 mL of HOAc, 100 mL of benzene, and 15 mL of acetic anhydride was heated at 35–40 °C for 16 h, concentrated to 50 mL, and cooled to 4 °C. The crystalline material, which was collected by filtration and air-dried, weighed 6.1 g (64%). Anal. ($C_{20}H_{36}O_4$) C, H.

The anhydride could also be prepared in almost quantitative yields by treating **3j** with an equivalent amount of dicyclohexylcarbodiimide in THF. After separation of the urea by filtration, the filtrate was distilled to dryness to yield the product.

3-Acetoxy-3-*n*-pentadecylglutaric Acid Anhydride (5d). When the procedure for the preparation of **5c** was repeated by heating the reaction solution at 90 °C or replacing toluene for benzene, the acetate **5d** was obtained in 60% yield; NMR δ 1.28 (28 H, CH_2), 1.95 (3 H, CH_3CO), 2.70, 2.97 (2 H, CHCOO), 3.27, 3.53 (2 H, CHCOO). Anal. ($C_{22}H_{38}O_5$) C, H.

Mono[(dimethylamino)ethyl] 3-Hydroxy-3-*n*-pentadecylglutarate (4g). A solution of 90 mL of (dimethylamino)ethanol and 30 mL of benzene was concentrated by distillation to 90 mL. Then 4.6 g (0.0134 mol) of **5c** was added. The solution was stirred for 48 h and distilled to dryness and cooled. The crystalline solid was triturated with ether and separated by filtration to yield 3.8 g (66%) of **4g**. Anal. ($C_{24}H_{45}NO_5$) C, H, N.

Monoethyl 3-Hydroxy-3-*n*-pentadecylglutarate (4f). A solution of 2.0 g (0.0058 mol) of **5c**, 300 mL of anhydrous EtOH, and 10 mL of pyridine was allowed to stand for 18 h and concentrated to dryness under reduced pressure. The residue was dissolved in toluene and distilled to dryness under reduced pressure at 45 °C. Purification of the crude product (2.5 g) by chromatography by elution with EtOAc–toleuene (3:7) yielded 1.74 g (78%) of **4f**. Anal. ($C_{21}H_{42}O_5$) C, H.

3-*n*-Pentadecylglutaconic Acid (6). A solution of 5 g (0.013 mol) of **5d**, 100 mL of CH_2Cl_2, 200 mL of pyridine, and 50 mL of water was stirred at 20 °C for 16 h and then distilled to dryness at 100 °C under reduced pressure. The solid that remained was crystallized from hexane to yield 1.5 g (34%) of **6**; NMR δ 0.92 (3 H, CH_3), 2.06–2.40 (m, 2 H, CH_2C=), 3.73 (2 H, CH_2COO), 5.80 (H, CH=). Anal. ($C_{20}H_{36}O_4$) C, H.

3-(2-Hydroxyethyl)-*n*-octadecane-1,3-diol (7b) and 3-(2-Hydroxyethyl)-*n*-tridecane-1,3-diol (7a). To a mixture of 40 mL of THF and 4.25 g (0.11 mol) of LAH at 0 °C was added dropwise a solution of 5 g (0.014 mol) of **3j** in 20 mL of THF. The reaction mixture was stirred for 16 h and quenched at −5 °C with water (1 mL) and 15% aqueous NaOH (1 mL). Ether was added and the mixture was separated by filtration. The ether solution was washed with water, dried (Na_2SO_4), and distilled to dryness. The crude product was purified by chromatography by elution with EtOAc–toluene (1:4) to yield 880 mg (19%) of **7b**. Compound **7a** was also prepared in 87% yield from **3f** by the same procedure. However, it was used for the synthesis of **8a** without further purification. Anal. ($C_{20}H_{42}O_3$) H: calcd, 12.81; found, 13.24.

3,5-Dihydroxy-3-*n*-pentadecylvaleric Acid Lactone (8b) and the Corresponding Lactol 8c. To a solution of 3.55 g (0.01 mol) of **7b** in 250 mL of acetone was added, dropwise at 0 °C, 4.3 mL of 8 N CrO_3/H_2SO_4. The mixture was distilled to dryness under reduced pressure and the residue was dissolved in EtOAc. Insoluble material was removed by filtration. The filtrate was washed with water and aqueous $NaHCO_3$, dried (Na_2SO_4), and distilled to dryness under reduced pressure. The crude product, 2.74 g, was purified by low-pressure chromatography by elution with EtOAc–toluene (15:85). One hundred 20-mL fractions were obtained. Fractions 25–40 yielded 1.26 g (31%) of **8b**; UV λ_{max} 2.77, 5.73 μm. Anal. ($C_{20}H_{38}O_3$) C, H. Fractions 71–92 yielded 520 mg (16%) of **8c**; NMR δ 5.0 (m, 1 H, OCH); IR ($CHCl_3$) no carbonyl. Anal. ($C_{20}H_{40}O_3$) C, H.

3,5-Dihydroxy-3-*n*-decylvaleric Acid Lactone (8a). When 1.19 g (0.0046 mol) of **7a** was oxidized according to the procedure described for the preparation of **8b**, 880 mg (72%) of **8a** was obtained. Anal. ($C_{15}H_{28}O_3$) C, H.

Biological Studies. The in vitro inhibitory activity of hydroxyglutaric acids was evaluated with use of rat liver microsomal HMG CoA reductase as described Edwards et al.[10] The IC_{50} was defined as the concentration required to inhibit the enzyme by 50% of control.

HMG derivatives were added to a preincubation mixture consisting of 0.1 M KPO_4, pH 7.2, 0.02 M glucose 6-phosphate, 2.5 mM NADP, 0.7 unit of glucose-6-phosphate dehydrogenase, 5 mM dithiothreitol, 50 mM mevalonic acid, and approximately 50 μg of microsomal protein. Triplicate samples were preincubated for 15 min at 37 °C in a volume of 1 mL. Incubation was started with 40 μM [^{14}C]HMG CoA (0.1 mL) run for 15 min at 37 °C and stopped with 5 N HCl (0.1 mL). Assay tubes were allowed to set for at least 30 min and then approximately 50 000 dpm of [^{3}H]mevalonic acid was added to provide for extraction efficiency. Mevalonic acid was extracted with ether and the percent [^{14}C]-HMG CoA incorporation determined for concurrent control and test reaction systems. Testing was done (first) with a range finding assay followed by a 4 or 5 point assay to find the IC_{50} value. Coefficient of variation ranged from 5% to 20% with an average value of 14%. Compactin had an IC_{50} value of 1 μM under these conditions.

The in vivo hypocholesterolemic activity was determined in Triton-treated male rats.[11] Compounds were given intragastrically 1 h before Triton and 5 h post-Triton as a split dose. Serum was collected 24 h post-Triton for analysis of total cholesterol.

The procedure used to study the effect of HMG derivatives on HMGR in rats was adapted from a method described previ-

ously.[12] Male CD rats, 180–250 g, were fed Purina certified rodent chow (5002) and water ad libitum. 20,25-Diazacholesterol was given intragastrically as a suspension (0.1% Tween 80/saline) at a daily dose of 5 mg/kg for 7 days. Test compounds were administered, as suspensions, intragastrically over the last 4 days of the test. Rats were killed 2 h after the last treatment, the livers were removed, the microsomal fraction was prepared (6×10^6 G/min), and HMG CoA reductase activity was determined for control and treat group rats on the basis of conversion of [^{14}C]-HMG CoA to [^{14}C]mevalonate.[13] All results are presented as percent reduction from concurrent controls. Means were compared to respective controls by Student's t test.

(13) M. S. Brown, S. E. Dana, and J. L. Goldstein, *J. Biol. Chem.*, **249**, 789 (1974).

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Registry No. **1d**, 97-62-1; **1f**, 627-90-7; **1j**, 628-97-7; **2d**, 52939-56-7; **2f**, 52939-58-9; **2j**, 52939-55-6; **3b**, 52939-72-7; **3c**, 52939-71-6; **3d**, 52939-65-8; **3e**, 95249-30-2; **3f**, 52939-68-1; **3g**, 95249-31-3; **3h**, 95249-32-4; **3j**, 52939-64-7; **3k**, 95249-33-5; **3l**, 95249-34-6; **3m**, 95249-35-7; **4a**, 73489-84-6; **4b**, 72060-93-6; **4c**, 95249-36-8; **4d**, 95249-37-9; **4e**, 95249-38-0; **4f**, 95249-39-1; **4g**, 95249-40-4; **5a**, 34695-32-4; **5b**, 95249-41-5; **5c**, 95249-42-6; **5d**, 95249-43-7; **6**, 95249-29-9; **7a**, 95249-44-8; **7b**, 95249-45-9; **8a**, 95249-46-0; **8b**, 95249-47-1; **8c**, 95249-48-2; HMGR, 9028-35-7; allyl bromide, 106-95-6.

Effect of Structural Modification of the Hydantoin Ring on Anticonvulsant Activity

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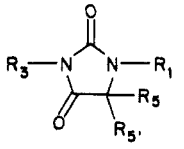
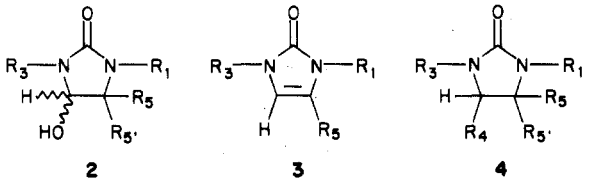
Selectively substituted hydantoins **1** (15 examples), 4-hydroxy-2-imidazolidinones **2** (13 examples), 2-imidazolones **3** (10 examples), 2-imidazolidinones **4** (four examples), vicinal diamines **5** (two examples), and simple amino acid derivatives **6** (four examples) have been prepared and evaluated in the maximal electroshock seizure (MES), subcutaneous pentylenetetrazole seizure threshold (sc Met), and rotorod (Tox) tests. The medium effective doses (ED50) and the medium toxic dose (TD50) for the most active compounds are reported. In general, the most pronounced activity was observed for hydantoins **1** and protected amino acids **6**. Within each series of compounds, enhanced anticonvulsant activity was often noted for compounds containing an aromatic group one carbon removed from a nitrogen atom. Among the most active compounds observed were the amino acid derivative *N*-acetyl-D,L-alanine benzylamide (**6d**) and the two 2-imidazolones 4-methyl-1-(phenylmethyl)-1,3-dihydro-2*H*-imidazol-2-one (**3e**) and 1-phenyl-1,3-dihydro-2*H*-imidazol-2-one (**3g**). Compound **6d** proved to be slightly more potent in the MES test than phenacemide.

Vicinal diamine based substrates form an important set of CNS-active medicinal agents.[2] Among the most important members of this class of compounds are the hydantoins **1**. The effect of structural modification of the hydantoin ring system on biological activity has been a subject of considerable interest.[3] Attention has been focused on the select replacement of the ring atoms and the alteration of the hydrogen bonding properties of the heterocycle.[4]

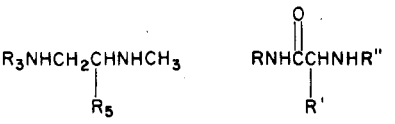


1

In this report, we describe the syntheses, physical properties, and anticonvulsant activities of a select series of hydantoins **1**, 4-hydroxy-2-imidazolidinones **2**, 2-imidazolones **3**, 2-imidazolidinones **4**, vicinal diamines **5**, and amino acid derivatives **6**. This study differed considerably from previous reports in that the basic sequence of atoms (N–C–C–N–C) present in hydantoins **1** has been retained in almost all the substrates examined. Differentiation among the classes of compounds (1–6) evaluated, however, was achieved by altering the oxidation state, basicity, and lipophilicity of the compounds.



2 **3** **4**



5 **6**

Selection of Compounds. Hydantoins **1a–i** served as the parent compounds in this study (Table I). Within this class of compounds we have systematically varied the R_3 substituent from methyl to benzyl to phenyl and the R_5 group from hydrogen to methyl to phenyl. Identical substituent patterns were incorporated into the 4-

(1) (a) Abstracted from Ph.D dissertation of this author. Additional structure proof, discussion, and experimental and spectral data may be found in this reference. (b) On leave from the University of Mary Hardin-Baylor, Belton, TX 76513, 1982. (c) Camille and Henry Dreyfus Teacher–Scholar Grant Recipient, 1977–1982.

(2) For previous studies, see: Kohn, H.; Kohn, B. A.; Steenberg, M. L.; Buckley, J. P. *J. Med. Chem.* **1977**, *20*, 158–160. Arceneaux, J. H.; Kohn, H.; Steenberg, M. L.; Buckley, J. P. *J. Pharm. Sci.* **1978**, *67*, 600–602.

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