Hypocholesterolemic Agents III: Inhibition of β-Hydroxy-β-methylglutaryl Coenzyme A Reductase by Half Acid Esters of 1-(4-Biphenylyl)pentanol

KENNETH E. GUYER *, SHARON G. BOOTS, PAUL E. MARECKI, and MARVIN R. BOOTS ˣ

Abstract □ A series of half acid esters of 1-(4-biphenylyl)pentanol was synthesized and assayed for inhibition of rat liver β-hydroxy-β-methylglutaryl coenzyme A reductase. The number of methylenes separating the carboxyl and ester groups was varied from zero to six. A minimum of one methylene was required for reasonable activity. Further separation of the carboxyl and ester groups produced small changes in activity. Investigation of several isomeric (*cis* and *trans*) half acid esters indicated that activity was independent of configuration. Modification of the acid portion of the glutarate analog by incorporating a 3-hydroxyl group increased activity sixfold.

Keyphrases □ Hypocholesterolemic agents—series of half acid esters of 1-(4-biphenylyl)pentanol synthesized, effect on rat liver β-hydroxy-β-methylglutaryl coenzyme A reductase □ β-Hydroxy-β-methylglutaryl coenzyme A reductase—rat liver, effect of half acid esters of 1-(4-biphenylyl)pentanol □ 1-(4-Biphenylyl)pentanol—half acid ester series, synthesized, effect on rat liver β-hydroxy-β-methylglutaryl coenzyme A reductase

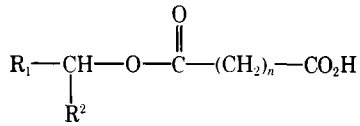
In an earlier report (1), an approach to the design of inhibitors of cholesterol biosynthesis as potential hypocholesterolemic agents was discussed. Specifically, the rationale for the inhibition of the target enzyme, β-hydroxy-β-methylglutaryl coenzyme A reductase, was presented. Previously, a series of arylalkyl hydrogen succinates and glutarates (I) was synthesized and found to be inhibitors of yeast β-hydroxy-β-methylglutaryl coenzyme A reductase. In this study, the alcohol portion of the acid esters was varied; maximum activity was obtained when R_1 = biphenyl and R_2 = *n*-butyl.

This paper describes the synthesis and assay of acid esters of 1-(4-biphenylyl)pentanol. Various diacids were utilized in an effort to determine the importance of the: (*a*) distance separating the free carboxyl and ester groups, (*b*) conformational relationship between the carboxyl and ester groups, and (*c*) hydroxyl group in the 3-position of substituted glutaric acid derivatives.

The recent availability of mammalian β-hydroxy-β-methylglutaryl coenzyme A reductase from rat liver (2) provided an enzyme source for the inhibition studies. Hopefully, these *in vitro* data would be more applicable to the selection of inhibitors for the *in vivo* studies in rats than the *in vitro* data obtained using the yeast reductase.

CHEMISTRY

Various half acid esters of 1-(4-biphenylyl)pentanol (1) were synthesized using five different procedures. Procedure A involved conversion of the diacid to the diacid chloride, followed by treatment of the diacid chloride with 1 equivalent of the alcohol. The reaction mixture was then treated with a sodium bicarbonate solution to hydrolyze the acid chloride present. Although statistical



I: $n = 2$ or 3

yield would only be 50% using this procedure, the actual yields were much lower. This method was later replaced by Procedure D, which was much more versatile and used very mild and essentially neutral reaction conditions.

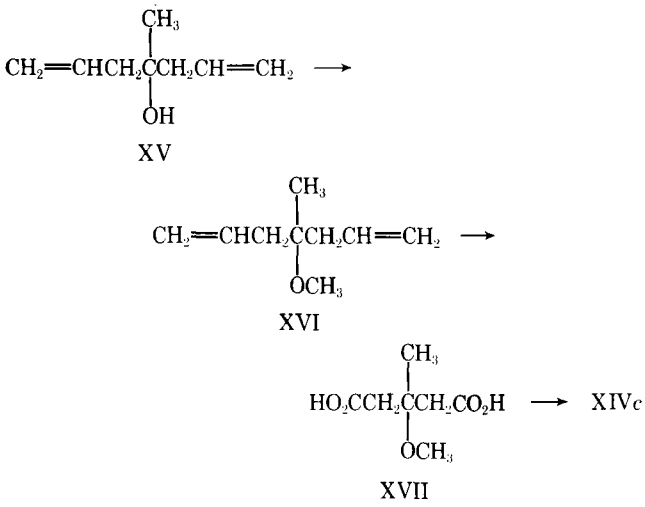
Procedure B involved the conversion of the diacid chloride to the diester using 2 equivalents of the substituted pentanol, followed by partial saponification. This method was also abandoned as a useful synthetic tool and replaced by Procedure D.

Procedure C, originally described by Palazzo *et al.* (3) and modified (1), involved treatment of the acid anhydride with the pentanol in pyridine (heated at reflux). This method gave low yields in many cases and was replaced by either Procedure D or E.

Procedure D, described originally by Büchi *et al.* (4), proved to be extremely useful. It often provided the desired monoester when other methods failed to give any of the desired product. It is the method of choice, especially when other functional groups are present in the starting diacid. This procedure involved the treatment of a diacid in chloroform at 25° with the substituted pentanol in the presence of *N,N*-dimethylformamide dineopentyl acetal.

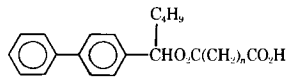
Procedure E, originally described by Steglich and Höfle (5), also proved to be useful. The anhydride was treated with the substituted pentanol in dimethylformamide at 25° in the presence of 4-dimethylaminopyridine and triethylamine.

Synthesis of the monoester of 3-methoxy-3-methylglutaric acid was more involved because the starting diacid was not commercially available. A synthesis was devised starting with the known 4-hydroxy-4-methyl-1,6-heptadiene (XV, Scheme I) (6). Methylation of the hydroxyl group was effected by treatment with sodium hydride in dimethyl sulfoxide, followed by methyl iodide. Ozonolysis at −78°, followed by an oxidative workup using hydrogen peroxide in acetic acid, provided the methoxy diacid (XVII) in a 50% yield. Difficulty was encountered in the synthesis of the monoester (XIVc), and the only method that provided a respectable yield was Procedure D.



Scheme I

Table I—Properties of Monoesters of Dicarboxylic Acids



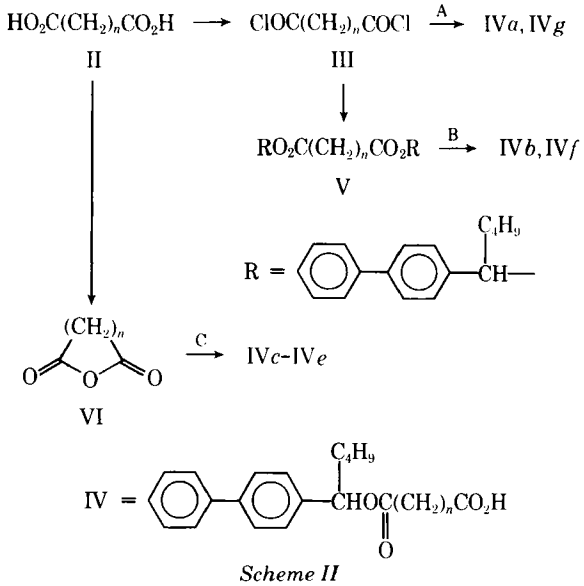
Compound	n	Procedure	Yield, %	Melting Point	Melting Point (Salt)	Formula	Analysis, %[a] Calc.	Found
IV*a*	0	A	19	Oil	169–170°	$C_{27}H_{30}N_2O_4S$	C 67.7	67.7
							H 6.3	6.4
							N 5.9	6.0
IV*b*	1	B	25	65–68°	—	$C_{20}H_{22}O_4$	C 73.6	73.8
							H 6.8	6.8
IV*c*	2	C	68	77–79°[b]	—	—	—	—
IV*d*	3	C	53	Oil[b]	122–123°	—	—	—
IV*e*	4	C	30	Oil	130–131°	$C_{31}H_{38}N_2O_4S$	C 69.6	69.5
							H 7.1	7.2
							N 5.2	5.2
IV*f*	5	B	21	Oil	126–127°	$C_{32}H_{40}N_2O_4S$	C 70.0	70.0
							H 7.3	7.4
							N 5.1	5.3
IV*g*	6	A	22	Oil	130–132°	$C_{33}H_{42}N_2O_4S$	C 70.5	70.3
							H 7.5	7.4
							N 5.0	5.2

[a] Analytical data were obtained on the solid monoesters, while the oils were characterized as their solid *S*-benzylthiuronium salts (1). [b] Described previously (1).

EXPERIMENTAL[1]

Reagents—The following were used: oxalyl chloride[2], malonyl chloride[3], fumaryl chloride[2], pimeloyl chloride[4], suberoyl chloride[5], *trans*-1,2-cyclobutanedicarboxylic acid chloride[3], maleic acid[3], β-hydroxy-β-methylglutaric acid[6], adipic anhydride (7), *cis*-1,2-cyclobutanedicarboxylic anhydride[3], *N,N*-dimethylformamide dineopentyl acetal[3], 4-dimethylaminopyridine[3], $NADP^+$[7], glucose 6-phosphate[7], torula yeast glucose 6-phosphate dehydrogenase[7], and β-hydroxy-β-methylglutaric-3-^{14}C acid[8].

Monoesters of Diacids—*Procedure A, Partial Esterification Using Diacid Chlorides: Monoesters IV*a, *IV*g, *X*b, *and XI*a—This example typifies the method (Schemes II and III) used to prepare these monoesters (Tables I and II). To a solution of 1.1 ml



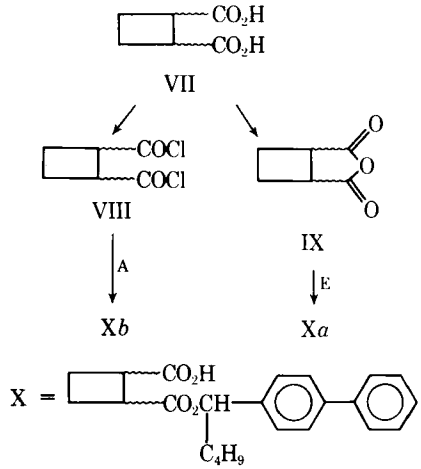
Scheme II

(0.01 mole) of fumaryl chloride in 10 ml of ether was added dropwise, with stirring, a solution of 2.4 g (0.01 mole) of 1-(4-biphenylyl)pentanol (1) in 20 ml of ether. The yellow solution was stirred at 25° for 21 hr, and then 10 ml of a 5% sodium bicarbonate solution was added. The mixture was stirred at 25° for 1.5 hr. Additional ether was added, and the aqueous phase was adjusted to pH 12 with a 5% sodium hydroxide solution.

The basic, aqueous extract was acidified to pH 2 with an ice-cold concentrated hydrochloric acid solution and then extracted with ether. The organic phase was washed with water and then with a saturated sodium chloride solution. The organic phase was dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure to afford 1.2 g of a beige solid.

Chromatography on 50 g of silicic acid (8) afforded 1.02 g (33%) of XI*a* as a white solid. An analytical specimen of XI*a*, mp 100–104°, was prepared by recrystallization from ethyl acetate–petroleum ether (bp 60–75°); IR ($CHCl_3$): 1710 (broad, acid and ester carbonyls) and 1642 (C=C) cm^{-1}; NMR ($CDCl_3$): δ 0.65–2.10 [broad, 9H, $CH_3(CH_2)_3$], 5.90 (t, J = 6 Hz, 1H, HCO_2C—), 6.90 (s, 2H, HC=CH), 7.24–7.70 (m, 9H, aromatic), and 8.85 (s, 1H, CO_2H) ppm.

*Procedure B, Partial Saponification of Diesters: Monoesters IV*b *and IV*f—This example typifies the method (Scheme II) used to prepare these monoesters. To a solution of 7.2 g (0.03 mole) of 1-(4-biphenylyl)pentanol (1) and 3.75 ml (0.03 mole) of *N,N*-dimethylaniline in 50 ml of ether was added, with stirring and cooling, a solution of 1.46 ml (0.015 mole) of malonyl chloride in 25 ml of ether. After the addition was complete, the reaction mixture was allowed to warm to 25°; it was heated at reflux for 2 hr and then



Scheme III

[1] Melting points were determined with a Thomas-Hoover capillary melting-point apparatus and are uncorrected. The elemental analyses were obtained from Galbraith Laboratories, Knoxville, Tenn. The IR spectra were determined using a Perkin-Elmer model 237 spectrophotometer. The NMR spectra were determined using a Perkin-Elmer model R-24 spectrometer in deuterochloroform, with tetramethylsilane as the internal reference.

[2] Eastman Organic Chemicals.

[3] Aldrich Chemical Co.

[4] Fisher Scientific Co.

[5] K and K Laboratories.

[6] Schwarz/Mann.

[7] Sigma Chemical Co.

[8] New England Nuclear.

Table II—Properties of Monoesters of Rigid Dicarboxylic Acids

Compound	Isomer	Procedure	Yield, %	Melting Point	Formula	Analysis, % Calc.	Found
X*a*	*cis*	E	78	126–128°	$C_{23}H_{26}O_4$	C 75.4 H 7.1	75.4 7.2
X*b*	*trans*	A	16	Oil	$C_{23}H_{26}O_4$	C 75.4 H 7.1	75.2 7.2
XI*a*	*trans*	A	33	100–104°	$C_{21}H_{22}O_4$	C 74.6 H 6.5	74.6 6.9
XI*b*	*cis*	D	72	109–112°	$C_{21}H_{22}O_4$	C 74.6 H 6.5	74.3 6.8

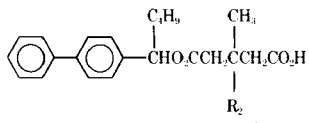


Table III—Properties of Monoesters of 3,3-Disubstituted Glutaric Acids

Compound	R_2	Procedure	Yield, %	Melting Point	Melting Point (Salt)	Formula	Analysis, %[a] Calc.	Found
XIV*a*	H	C	49	Oil	126–128°	$C_{31}H_{38}N_2O_4S$	C 69.6 H 7.1 N 5.2	69.7 7.0 5.3
XIV*b*	OH	D	29	Oil	108–109°	$C_{31}H_{38}N_2O_5S$	C 67.3 H 6.9 N 5.1	67.8 6.8 5.1
XIV*c*	OCH_3	D	59	Oil	94.5–96°	$C_{32}H_{40}N_2O_5S$	C 68.0 H 7.1 N 5.0	68.2 7.1 5.1

[a] The monoesters were characterized as their solid *S*-benzylthiuronium salts (1).

stirred at 25° for 17 hr. The resulting mixture was poured onto ice and filtered, and the organic phase was washed with a 5% hydrochloric acid solution, a 5% sodium hydroxide solution, water, and a saturated sodium chloride solution.

The organic phase was dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure to give 8.4 g of the diester as a yellow oil. A mixture of 3.2 g (0.006 mole) of the diester, 50 ml of 1,2-dimethoxyethane, 15 ml of water, and 1.7 ml of a 3.54 *N* potassium hydroxide solution was stirred at 25° for 17 hr. The resulting solution was extracted with ether, and the aqueous phase was acidified with a 5% hydrochloric acid solution. The resulting acidic aqueous extract was extracted with ether. The organic phase was washed with water and then with a saturated sodium chloride solution and was dried over anhydrous sodium sulfate. Then the solvent was removed under reduced pressure to afford 0.49 g (25%) of IV*b* as a yellow oil, which crystallized on standing.

Recrystallization from ether–petroleum ether (bp 60–75°) afforded an analytical specimen of IV*b* as a white solid, mp 65–68°; IR ($CHCl_3$): 1733 (broad, acid and ester carbonyls) cm^{-1}; NMR ($CDCl_3$): δ 0.70–2.20 [broad, 9H, $CH_3(CH_2)_3$], 3.48 (s, 2H, O=CCH_2C=O), 5.85 (t, J = 6 Hz, 1H, HCO_2C—), 7.25–7.75 (m, 9H, aromatic), and 10.10 (s, 1H, CO_2H) ppm.

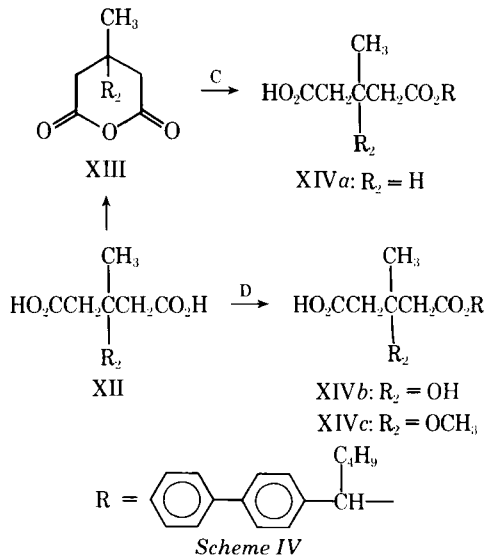
Procedure C, Opening of Anhydrides in Pyridine: Monoesters IVc–IVe and XIVa—This procedure was described previously (1).

Procedure D, Partial Esterification Using Diacids: Monoesters XIb, XIVb, and XIVc—This example typifies the method (Scheme IV) used to prepare these monoesters (Table III). The method of Büchi *et al.* (4) was used. To a suspension of 0.870 g (0.0075 mole) of maleic acid in 40 ml of chloroform was added 2.25 g (0.0098 mole) of *N,N*-dimethylformamide dineopentyl acetal. A colorless solution resulted and was stirred at 25° for 10 min. To this solution was added 1.8 g (0.0075 mole) of 1-(4-biphenylyl)pentanol (1), and the resulting yellow solution was stirred at 25° for 84 hr. The solvent was removed under reduced pressure, and then the residue was added to ether.

The organic phase was washed with water and then twice with a 5% sodium hydroxide solution. The basic, aqueous extract was adjusted to pH 2 with a 5% hydrochloric acid solution and then was extracted with ethyl acetate. The organic phase was washed with water and then with a saturated sodium chloride solution. The organic phase was dried over anhydrous sodium sulfate, and then the solvent was removed under reduced pressure to afford 1.82 g (72%) of XI*b* as a white solid, mp 105–108°.

Recrystallization from ethyl acetate–petroleum ether (bp 60–75°) afforded an analytical specimen of XI*b* as white prisms, mp 109–112°; IR ($CHCl_3$): 1730 (broad, acid and ester carbonyls) and 1630 (C=C) cm^{-1}; NMR ($CDCl_3$): δ 0.70–2.20 [broad, 9H, $CH_3(CH_2)_3$], 5.85 (t, J = 6 Hz, 1H, HCO_2C—), 6.32 (s, 2H, HC=CH), 7.25–7.65 (m, 9H, aromatic), and 10.97 (s, 1H, CO_2H) ppm.

Procedure E, Opening of Anhydrides in the Presence of N,N-*Dimethylaminopyridine: Monoester Xa*—This example typifies the method (Scheme III) used to prepare this monoester. The procedure of Steglich and Höfle (5) was used. To a solution of 0.378 g (0.003 mole) of *cis*-1,2-cyclobutanedicarboxylic anhydride, 0.720 g (0.003 mole) of 1-(4-biphenylyl)pentanol (1), and 0.366 g (0.003 mole) of 4-dimethylaminopyridine in 10 ml of dimethylformamide (distilled from barium oxide) was added 0.6 ml (0.0045 mole) of



Scheme IV

triethylamine, and the solution was stirred at 25° for 24 hr. The mixture was poured on an ice–hydrochloric acid mixture and then extracted with ether.

The organic phase was washed with water and then twice with a 5% sodium hydroxide solution. The basic, aqueous extract was acidified with a 5% hydrochloric acid solution and then was extracted with ether. The organic phase was washed with water and a saturated sodium chloride solution and then was dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to afford 0.860 g (78%) of a yellow oil, which crystallized on standing.

Recrystallization from ethyl acetate–petroleum ether (bp 60–75°) afforded an analytical specimen of X*a* as a white solid, mp 126–128°; IR ($CHCl_3$): 1720 (broad, acid and ester carbonyls) cm^{-1}; NMR ($CDCl_3$): δ 0.54–2.40 [broad, 9H, $CH_3(CH_2)_3$], 3.30 (broad, s, 2H, $RO_2CCHCHCO_2$), 5.80 (t, J = 6 Hz, 1H, HCO_2C—), 7.20–7.70 (m, 9H, aromatic), and 11.20 (s, 1H, CO_2H) ppm.

Four-Step Synthesis of Monoester of 3-Methoxy-3-methylglutaric Acid (XIV*c*)—The method of Tschesche and Machleidt (6) was used for the synthesis of 4-hydroxy-4-methyl-1,6-heptadiene (XV), 89%, bp 51° (15 mm) [lit. (6) bp 50–51° (12 mm)].

The method of Anderson and Cree (9) was used for the preparation of 4-methoxy-4-methyl-1,6-heptadiene (XVI). To a suspension of 3.7 g (0.088 mole) of 57% sodium hydride in 50 ml of dry dimethyl sulfoxide was added dropwise at 25°, with stirring, 7.76 g (0.062 mole) of XV in 25 ml of dimethyl sulfoxide. The mixture was stirred until gas evolution ceased (2.5 hr). The suspension was cooled in an ice bath, and 26 g (0.18 mole) of methyl iodide was added in a dropwise manner. The mixture was allowed to warm to 25° and was stirred at 25° for 18 hr. Then it was poured into ether and water.

The organic phase was washed with water and a saturated sodium chloride solution and then was dried over anhydrous sodium sulfate. Removal of the solvent under reduced pressure afforded a brown liquid, which was distilled to give 5.64 g (65%) of XVI as a clear, colorless liquid, bp 149–151° (760 mm); IR (liquid film): 1645 (strong, C=C) cm^{-1}; NMR ($CDCl_3$): δ 1.10 (s, 3H, CH_3C), 2.25 [d, J = 7 Hz, 4H, $(C{=}CCH_2)_2$], 3.20 (s, 3H, CH_3O), 4.83–5.16 [m, 4H, $(C{=}CH_2)_2$], and 5.50–6.16 [m, 2H, $(CH{=}C)_2$] ppm.

Anal.—Calc. for $C_9H_{16}O$: C, 77.1; H, 11.5. Found: C, 77.2; H, 11.6.

The method of Tschesche and Machleidt (6) (Scheme I) was used for the preparation of 3-methoxy-3-methylglutaric acid (XVII). A solution containing 7.36 g (0.053 mole) of XVI, 7.0 ml of acetic acid, and 85 ml of methylene chloride was cooled in an acetone–dry ice bath. Ozone was passed through the solution for 3.5 hr, and the resulting blue solution was allowed to warm to 25°. To the clear, colorless solution was added 50 ml of acetic acid, and then the methylene chloride was removed under reduced pressure. To the residue were added 50 ml of acetic acid and 50 ml of 30% hydrogen peroxide; then the solution was heated at reflux for 8 hr.

Removal of the solvent under reduced pressure afforded 7.39 g of a colorless oil, which crystallized on standing. Recrystallization from ether–petroleum ether (bp 60–75°) gave 7.08 g (76%) of XVII as a white solid, mp 78–80°; IR ($CHCl_3$): 2500–2700 (broad, OH) and 1710 (CO_2H) cm^{-1}; NMR (acetone-d_6): δ 1.40 (s, 3H, CH_3C), 2.76 [s, 4H, $(CH_2)_2$], 3.26 (s, 3H, CH_3O), and 9.93 [s, 2H, $(CO_2H)_2$] ppm.

Anal.—Calc. for $C_7H_{12}O_5$: C, 47.7; H, 6.9. Found: C, 47.7; H, 7.0.

Procedure D (Scheme IV) was used to prepare the monoester XIV*c* from the synthesized diacid.

Preparation of Rat Liver Microsomes—Active rat liver microsomes containing β-hydroxy-β-methylglutaryl coenzyme A reductase activity were prepared according to Shapiro and Rodwell (10). Male Sprague–Dawley rats, ~100–130 g, were housed in an animal room in which light was provided from 7 am to 7 pm. Food[9] and water were available *ad libitum*. Between 12:05 and 12:30 am, the rats were stunned and decapitated.

The livers were excised into 0°, pH 6 homogenization buffer (30 m*M* ethylenediaminetetraacetic acid, 70 m*M* sodium chloride, and 10 m*M* β-mercaptoethanol). Then the livers were forced through a tissue press into 2.5 ml of 0° homogenization buffer per gram of liver prior to homogenization in a Potter–Elvehjem homogenizer with a pestle[10]. The homogenate was centrifuged twice for 15 min at 12,000×*g*, and the pellets were discarded. The supernate was centrifuged for 1 hr at 48,000×*g*; the resulting pellets were frozen in a dry ice–isopropyl alcohol bath. The tubes containing the frozen pellets were sealed[11] and stored at −60° for up to 3 weeks.

[9] Purina Laboratory Chow.

Table IV—Inhibition of β-Hydroxy-β-methylglutaryl Coenzyme A Reductase

Compound	*n*	Activity I/S[a]	Activity Inhibition, %
IV*a*	0	50[b]	36
IV*b*	1	6	50
IV*c*	2	11	50
IV*d*	3	11	50
IV*e*	4	7	50
IV*f*	5	20	50
IV*g*	6	20	50
X*a*		5[c]	50
X*b*		5[c]	50
XI*a*		3	50
XI*b*		5	50
XIV*a*		9	50
XIV*b*		1.5	50
XIV*c*		10	50

[a] The inhibition index, I/S, equals the ratio of the micromolar concentration of the inhibitor to the micromolar concentration of the substrate. At least two sets of duplicate determinations were used. [b] Insoluble at higher concentrations. [c] The assay mixture was saturated at these concentrations.

Assay of Enzyme Activity and Inhibition—Literature procedures (10–12) were modified. The rat liver microsomal pellet (obtained from about 3–4 g of minced liver) from one tube (described under *Preparation of Rat Liver Microsomes*) was suspended in 1.7 ml of homogenization buffer, pH 6.8. The assay system consisted of $NADP^+$ (9.7 μmoles), glucose 6-phosphate (59.7 μmoles), torula yeast glucose 6-phosphate dehydrogenase (5 units), 0.15 ml of the microsomal suspension containing about 2 mg of protein[12], and 0.1 ml of ethylene glycol monoethyl ether containing the inhibitor in a total volume of 2.9 ml.

Enzymatic activity was started by the addition of 0.1 ml of a solution containing 94 nmoles of *dl*-β-hydroxy-β-methylglutaryl-3-^{14}C coenzyme A (specific activity 890 dpm/nmole) (13). After 30 min of shaking incubation at 37°, the reaction was stopped by the addition of 0.2 ml of concentrated hydrochloric acid. Fifty microliters of an acetone solution containing 7×10^5 dpm (24 μmoles) of *dl*-mevalonolactone-5-^{3}H was added to the incubation mixture and allowed to stand overnight. The mixture was saturated with anhydrous sodium sulfate and then extracted three times with 10-ml portions of ether. The ether was evaporated at 30° under a nitrogen stream, and the residue was dissolved in 0.2 ml of acetone–water (9:1).

The solution was streaked on a silica gel thin-layer sheet[2] and developed with acetone–benzene (1:1). The area of the chromatogram containing the mevalonolactone, which corresponded to an R_f value between 0.6 and 1.0, was scraped into a liquid scintillation vial. Twenty milliliters of Bray's scintillation solution (15) was added, and ^{3}H and ^{14}C were determined simultaneously in a liquid scintillation counter[13]. Efficiency of counting was determined by the external standard method. Some variability in the inhibition data was noted between tubes of microsomes (even from the same liver preparation). For this reason, a standard inhibitor, 1-(4-biphenylyl)pentyl hydrogen succinate (IV*c*), was utilized routinely.

RESULTS AND DISCUSSION

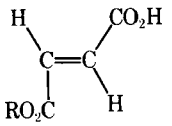
The activity of IV*a*–IV*g*, X*a*, X*b*, XI*a*, XI*b*, and XIV*a*–XIV*c* as inhibitors of rat liver β-hydroxy-β-methylglutaryl coenzyme A reductase is shown in Table IV. To determine if the distance sepa-

[10] Teflon (du Pont).

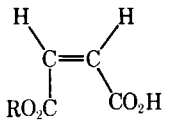
[11] Parafilm.

[12] Protein was precipitated by 5% trichloroacetic acid to remove interference from β-mercaptoethanol, redissolved in 3% sodium hydroxide solution, and determined colorimetrically by the method of Lowry *et al.* (14).

[13] Nuclear Chicago Mark I.



XI*a*



XI*b*

rating the carboxyl and ester groups was critical for activity, a series of monoesters related to I was prepared and assayed. The number (n) of methylene groups between the carboxyl and ester groups was varied from zero to six (IV*a*–IV*g*). While the data indicated that the distance between the groups was not highly critical, a minimum of one methylene group was required for reasonable activity, as shown by the decreased activity of IV*a* compared to IV*b*. Increasing the number of methylene groups from one to four did not alter the activity significantly. Further separation of the carboxyl and ester groups produced a decrease in activity (IV*f* and IV*g*).

The monoesters of several rigid dicarboxylic acids were investigated to determine if activity was dependent on the stereochemical relationship between the carboxyl and ester groups. The monoesters of *cis*- and *trans*-1,2-cyclobutanedicarboxylic acids (X*a* and X*b*) and the monoesters of fumaric and maleic acids (XI*a* and XI*b*) were studied. These rigid analogs were about two to three times more active than the corresponding flexible analog (IV*c*). However, the activity of the inhibitors does not appear to be strongly dependent on the stereochemical relationship between the carboxyl and ester groups.

Modifications of the glutarate analog (IV*d*) were made in an effort to provide inhibitors that resemble the glutaric acid portion of the substrate more closely. This was accomplished by incorporating a 3-methyl group (XIV*a*) and the 3-methyl-3-hydroxyl groups (XIV*b*). Compound XIV*a* was slightly more active than the unsubstituted glutarate analog (IV*d*), whereas XIV*b* was six times more active than XIV*a*. The methoxyl analog (XIV*c*) was investigated in an attempt to determine if the hydroxyl group in XIV*b* was acting as a hydrogen donor or hydrogen acceptor during binding to the enzyme. The decreased activity of XIV*c* compared to XIV*b* suggested that the hydroxyl group in XIV*b* was acting as a hydrogen donor in the binding interaction. However, an alternative explanation for the decreased activity of XIV*c* involves the steric bulk of the methoxyl group. If the steric bulk of the methoxyl group was responsible for the decreased activity, a greater decrease in activity would have been expected (see XIV*a* and XIV*c*).

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* Present address: Department of Biochemistry, Marshall University School of Medicine, Huntington, WV 25701

ˣ To whom inquiries should be directed.