to be effective in the isolated heart in rats, guinea pigs, rabbits, cats, and dogs and in the *in situ* heart in dogs and cats.

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Hypocholesterolemic Agents V: Inhibition of β-Hydroxy-β-methylglutaryl Coenzyme A Reductase by Substituted 4-Biphenylylalkyl Carboxylic Acids and Methyl Esters

SHARON G. BOOTS [*], **MARVIN R. BOOTS** [*x], **KENNETH E. GUYER** [‡§], and **PAUL E. MARECKI** [*]

Abstract [] Eleven substituted 4-biphenylylalkyl carboxylic acids and three methyl esters were synthesized and assayed for inhibition of rat liver β-hydroxy-β-methylglutaryl coenzyme A reductase. Five of the acids were analogs, resulting from various isosteric replacements of the carbonyl and ether oxygens of the previously described reversible inhibitor 1-(4-biphenylyl)pentyl hydrogen succinate. No significant change in activity was noted, except upon introduction of an amide linkage where a decrease in inhibition was found. Six carboxylic acids and three methyl esters, all containing the 4-biphenylyl radical but lacking the *n*-butyl side chain found in 1-(4-biphenylyl)pentyl hydrogen succinate, also were inhibitors of the reductase.

Keyphrases [] β-Hydroxy-β-methylglutaryl coenzyme A reductase—effect of 4-biphenylylalkyl carboxylic acids, rat liver microsomes [] 4-Biphenylylalkyl carboxylic acids—synthesis, effect on β-hydroxy-β-methylglutaryl coenzyme A reductase, rat liver microsomes [] Enzymes—β-hydroxy-β-methylglutaryl coenzyme A reductase, effect of 4-biphenylylalkyl carboxylic acids, rat liver microsomes [] Hypocholesterolemic agents, potential—4-biphenylylalkyl carboxylic acids synthesized, effect on β-hydroxy-β-methylglutaryl coenzyme A reductase, rat liver microsomes [] Structure–activity relationships—4-biphenylylalkyl carboxylic acids synthesized, effect on β-hydroxy-β-methylglutaryl coenzyme A reductase

In previous reports (1, 2), an approach to the design of inhibitors of cholesterol biosynthesis as potential hypocholesterolemic agents was discussed. The rationale for the inhibition of the enzyme, β-hydroxy-β-methylglutaryl coenzyme A reductase, was presented (1). These studies led to the discovery that maximum inhibition was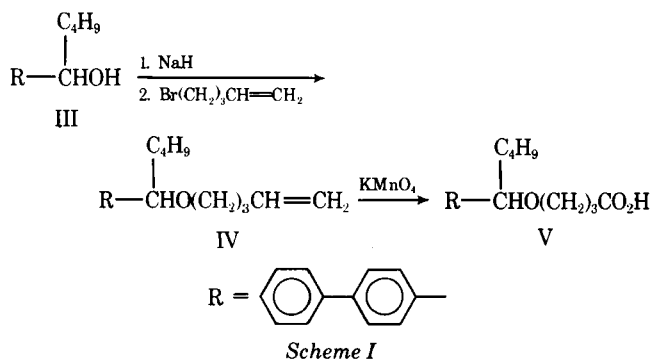 obtained in a series of arylalkyl hydrogen alkanedioates (I), where R_1 = biphenyl, R_2 = *n*-butyl, and *m* = 1–4.
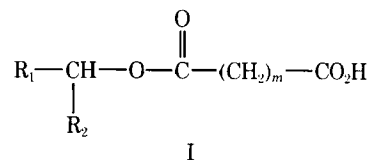
Modification of the acid portion of the glutarate analog of I by the incorporation of a β-hydroxy-β-methyl moiety provided II. Analog II, which closely resembles the acid portion of the substrate, β-hydroxy-β-methylglutaryl coenzyme A, was seven times more active than the glutarate analog of I and is the most active inhibitor found thus far.
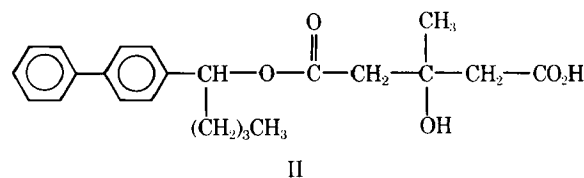
This paper describes the synthesis and assay of compounds where isosteric replacements of the various atoms of the ester function of I were made. In addition, reports by Eades *et al.* (3, 4) indicated that a series of 4-substituted biphenyl derivatives inhibited the incorporation of 1-^{14}C-acetate into cholesterol in the *in vitro* rat liver homogenate system and decreased serum

Scheme I

$$R—CHOH \xrightarrow[\text{2. Br(CH}_2)_3\text{CH=CH}_2]{\text{1. NaH}}$$

with C_4H_9 on the CHOH carbon, labeled III

$$R—CHO(CH_2)_3CH=CH_2 \xrightarrow{KMnO_4} R—CHO(CH_2)_3CO_2H$$

with C_4H_9 groups, labeled IV and V

$$R = \text{(biphenylyl)}$$

Scheme I

Scheme III

$$R—CHOH \xrightarrow{[O]} R—C=O \xrightarrow{NH_2OCH_3} R—C=NOCH_3 \xrightarrow{B_2H_6}$$

with C_4H_9 groups, labeled III, X, XI

$$RCHNH_2 \xrightarrow{\text{succinic anhydride}} RCHNHC(CH_2)_2CO_2H$$

with C_4H_9 groups and O, labeled XII and XIII

$$R = \text{(biphenylyl)}$$

Scheme III

cholesterol levels in rats. These compounds were interesting in that they did not possess either an *n*-butyl group or an ester function situated positionally as in I. Therefore, several of these compounds (as well as similar compounds) were synthesized and assayed as inhibitors of rat liver β-hydroxy-β-methylglutaryl coenzyme A reductase.

DISCUSSION

4-[1-(4-Biphenylyl)pentyloxy]butanoic acid (V) was prepared *via* a two-step synthesis (Scheme I). Conversion of the substituted pentanol (III) to the ether olefin (IV) was readily effected in high yield by treatment of III with sodium hydride, followed by the addition of 5-bromo-1-pentene. The oxidation of the terminal olefin to the desired carboxylic acid (V) proved to be an extremely capricious reaction. Ozonolysis, followed by an oxidative workup, provided only high melting acidic material, which appeared to result from concomitant oxidation of the aromatic ring system. Potassium permanganate (using standard procedures) provided V in low and nonreproducible yields.

Fortunately, an article appeared (5) describing the use of tricaprylylmethylammonium chloride[1] as a phase-transfer agent. The presence of the phase-transfer catalyst allowed one to carry out the oxidation of IV to V with potassium permanganate in a 54% yield.

6-(4-Biphenylyl)decanoic acid (IX) was prepared *via* a two-step synthesis (Scheme II). Treatment of the known keto acid (VI) with *n*-butylmagnesium bromide afforded the desired addition product (VII) in a 70% yield. Also, the abnormal reaction occurred, affording the reduction product (VIII) in a yield of 22%. Hydrogenolysis of the hydroxyl group in VII occurred readily to afford IX.
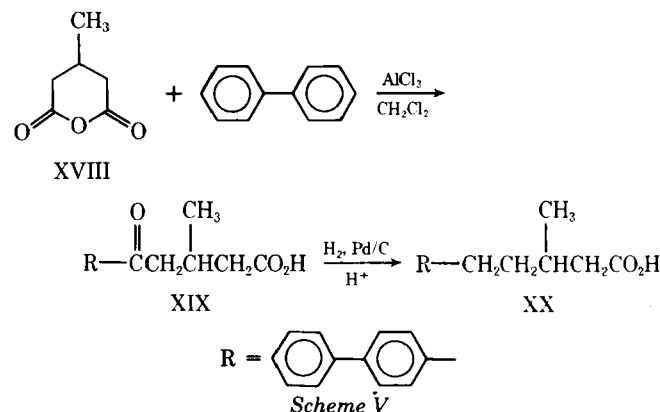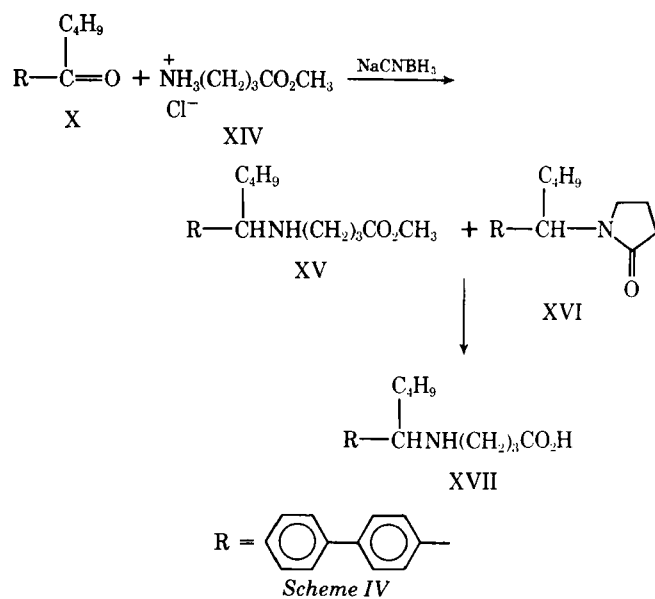
Synthesis of *N*-[1-(4-biphenylyl)pentyl]succinamic acid (XIII) was performed *via* the pathway illustrated in Scheme III. Jones oxidation
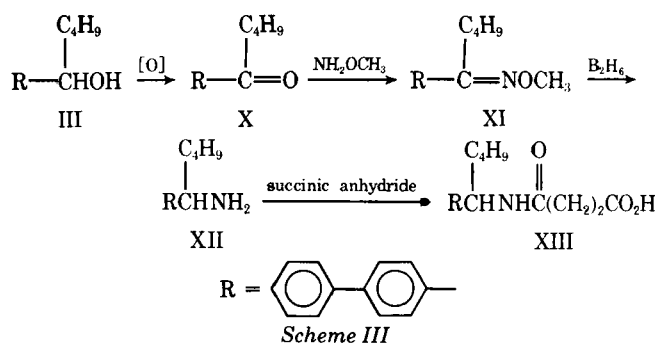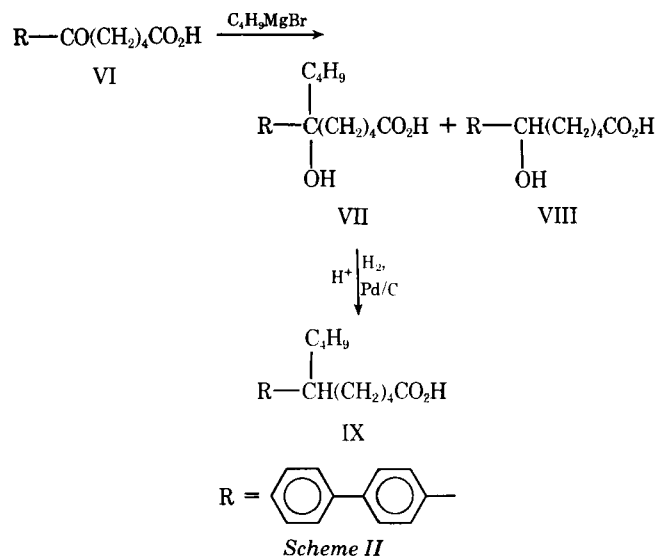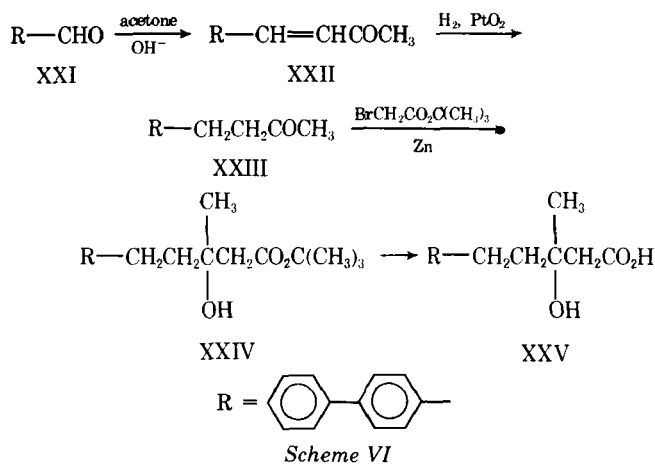
of the alcohol (III) readily afforded the ketone (X), which was subsequently converted to the O-methyloxime (XI) in an overall yield of 78%. Reduction of XI with diborane yielded the amine (XII), which was isolated and purified as the hydrochloride salt. Treatment of XII with succinic anhydride readily yielded XIII.

Synthesis of the amino acid (XVII) (Scheme IV) was effected by condensation of the ketone (X) with methyl 4-aminobutanoate hydrochloride (XIV) in the presence of sodium cyanoborohydride in methanol. Initially, the reaction was attempted in the absence of sodium acetate, which afforded an "apparent pH" of 5–6; X was recovered in essentially quantitative yield. When sufficient sodium acetate was added to raise the apparent pH to 6–7, the desired condensation–reduction occurred. A mixture of the ester (XV) and the lactam (XVI) was produced as well as some recovered X. No attempts were made to optimize the yield of this reaction. Saponification of the mixture of XV and XVI led to XVII.

5-Oxo-5-(4-biphenylyl)-3-methylpentanoic acid (XIX) was prepared using the method of Goldschmidt (6) from 3-methylglutaric anhydride and biphenyl in the presence of aluminum chloride (Scheme V). Hydrogenolysis occurred readily, using a palladium-

Scheme II

$$R—CO(CH_2)_4CO_2H \xrightarrow{C_4H_9MgBr}$$

labeled VI

$$R—C(CH_2)_4CO_2H + R—CH(CH_2)_4CO_2H$$

with C_4H_9 and OH groups, labeled VII and VIII

$$\xrightarrow[\text{H}^+]{\text{H}_2, \text{Pd/C}}$$

$$R—CH(CH_2)_4CO_2H$$

with C_4H_9, labeled IX

$$R = \text{(biphenylyl)}$$

Scheme II

Scheme IV

$$R—C=O + \overset{+}{NH_3}(CH_2)_3CO_2CH_3 \xrightarrow{NaCNBH_3}$$

with C_4H_9, Cl⁻, labeled X and XIV

$$R—CHNH(CH_2)_3CO_2CH_3 + R—CH—N$$

with C_4H_9 groups, labeled XV and XVI

$$R—CHNH(CH_2)_3CO_2H$$

with C_4H_9, labeled XVII

$$R = \text{(biphenylyl)}$$

Scheme IV

Scheme V

3-methylglutaric anhydride (with CH_3) + biphenyl, labeled XVIII

$$\xrightarrow[\text{CH}_2\text{Cl}_2]{\text{AlCl}_3}$$

$$R—CCH_2CHCH_2CO_2H \xrightarrow[\text{H}^+]{\text{H}_2, \text{Pd/C}} R—CH_2CH_2CHCH_2CO_2H$$

with O and CH_3 groups, labeled XIX and XX

$$R = \text{(biphenylyl)}$$

Scheme V

[1] Aliquat 336, General Mills Chemicals.

Scheme VI

$$R\text{—CHO} \xrightarrow[\text{OH}^-]{\text{acetone}} R\text{—CH}=\text{CHCOCH}_3 \xrightarrow{\text{H}_2,\ \text{PtO}_2}$$

$$\underset{\text{XXI}}{} \qquad \underset{\text{XXII}}{}$$

$$R\text{—CH}_2\text{CH}_2\text{COCH}_3 \xrightarrow[\text{Zn}]{\text{BrCH}_2\text{CO}_2\text{C(CH}_3)_3}$$

XXIII

$$\overset{\displaystyle CH_3}{R\text{—CH}_2\text{CH}_2\overset{|}{\underset{|}{C}}\text{CH}_2\text{CO}_2\text{C(CH}_3)_3} \longrightarrow \overset{\displaystyle CH_3}{R\text{—CH}_2\text{CH}_2\overset{|}{\underset{|}{C}}\text{CH}_2\text{CO}_2\text{H}}$$

$$\underset{\text{OH}}{} \qquad\qquad \underset{\text{OH}}{}$$

XXIV   XXV
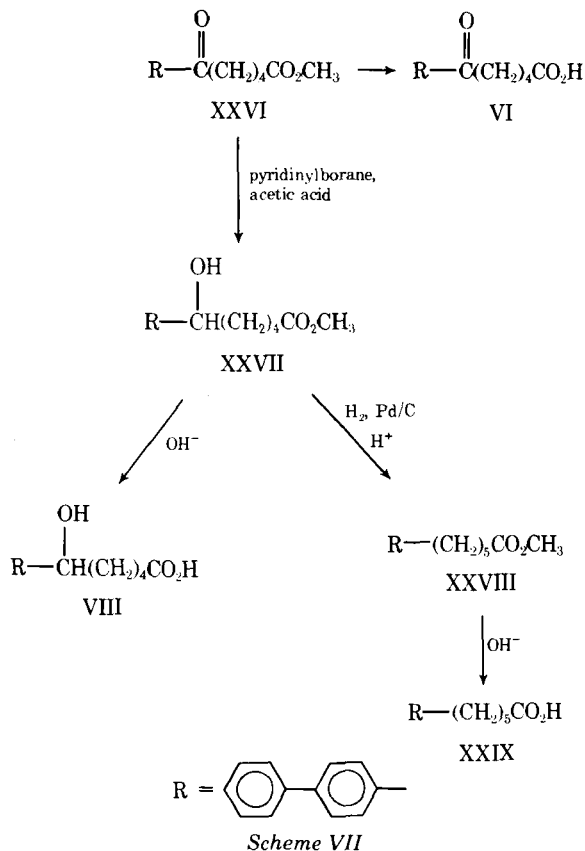
R = [biphenylyl]

Scheme VI

on-charcoal catalyst in the presence of hydrochloric acid, to afford the desired deoxy acid (XX).

5-(4-Biphenylyl)-3-hydroxy-3-methylpentanoic acid (XXV) was prepared via a four-step synthesis (Scheme VI). The initial condensation of 4-biphenylcarboxaldehyde (XXI) and acetone, to yield the α,β-unsaturated ketone (XXII), was described previously (7). The subsequent catalytic hydrogenation of XXII to the saturated ketone (XXIII) was effected as described previously (8). The Reformatsky reaction of the ketone with *tert*-butyl bromoacetate and zinc proceeded readily to afford the β-hydroxy ester (XXIV). The ester was not purified but was hydrolyzed directly with *p*-toluenesulfonic acid monohydrate in benzene to yield XXV.

The keto ester (XXVI) (Scheme VII) and the corresponding keto acid (VI) were prepared by the method of Morand *et al.* (9). The hydroxy ester (XXVII) was readily prepared from XXVI by reduction of the keto group with pyridinylborane in acetic acid. The corresponding hydroxy acid (VIII) was obtained in a quantitative yield from XXVII using a standard saponification procedure.

Compound XXVII also was used to prepare the ester, methyl 6-(4-biphenylyl)hexanoate (XXVIII), and the corresponding acid

$$\overset{\displaystyle O}{R\text{—}\overset{\|}{C}\text{(CH}_2)_4\text{CO}_2\text{CH}_3} \longrightarrow \overset{\displaystyle O}{R\text{—}\overset{\|}{C}\text{(CH}_2)_4\text{CO}_2\text{H}}$$

XXVI   VI

↓ pyridinylborane, acetic acid

$$\overset{\displaystyle OH}{R\text{—CH(CH}_2)_4\text{CO}_2\text{CH}_3}$$

XXVII

(left) /OH⁻   (right) H₂, Pd/C \ H⁺

$$\overset{\displaystyle OH}{R\text{—CH(CH}_2)_4\text{CO}_2\text{H}}$$

VIII

$$R\text{—(CH}_2)_5\text{CO}_2\text{CH}_3$$

XXVIII

↓ OH⁻

$$R\text{—(CH}_2)_5\text{CO}_2\text{H}$$

XXIX

R = [biphenylyl]

Scheme VII

(XXIX). Hydrogenolysis of the hydroxyl group, as already described, readily afforded XXVIII, which, upon saponification, yielded XXIX.

EXPERIMENTAL[2]

5-[1-(4-Biphenylyl)pentyloxy]-1-pentene (IV)—Sodium hydride (50% dispersion in oil), 1.2 g (0.025 mole), was rinsed thoroughly with dry benzene. Dry dimethylformamide (25 ml) was added. To this suspension was added dropwise, with stirring and cooling, a solution of 2.4 g (0.01 mole) of 1-(4-biphenylyl)pentanol (III) (1) in 25 ml of dry dimethylformamide. The resulting yellow suspension was stirred for 2 hr at 25° after the addition was completed.

A solution of 7.1 g (0.05 mole) of 5-bromo-1-pentene[3] in 10 ml of dry dimethylformamide then was added with stirring and cooling over 5 min. The mixture was stirred at 0–5° for an additional 1 hr and then at 25° for 24 hr. The resulting yellow solution was poured onto an ice–water mixture and extracted with ether. The organic phase was washed with water and with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to a yellow liquid (2.94 g). Petroleum ether (bp 60–68°) was added (∼10 ml), and the mixture was stored at 5° for 4 days.

The resulting white precipitate (identified by spectral and physical properties as the starting alcohol) was removed by filtration. The petroleum ether was then removed under reduced pressure to afford 2.44 g (80%) of IV as a yellow liquid. Since attempted distillation led to extensive decomposition, no analytical specimen could be prepared; NMR (CDCl₃): δ 0.70–2.30 (m, 13H, C₄H₉, CH₂CH₂), 3.30 (t, J = 6 Hz, 2H, OCH₂), 4.25 (t, J = 7 Hz, 1H, HCO), 4.80–5.20 (m, 2H, =CH₂), 5.48–6.17 (m, 1H, HC=C), and 7.20–7.80 (m, 9H, aromatic) ppm.

4-[1-(4-Biphenylyl)pentyloxy]butanoic Acid (V)—The procedure of Starks (5) was used. To a solution of 1.8 g (0.0056 mole) of IV in 30 ml of benzene containing 0.280 g (0.56 mmole) of tricaprylylmethylammonium chloride[1] was added dropwise, over 45 min with stirring and cooling, a solution of 3.5 g (0.022 mole) of potassium permanganate in 60 ml of water. The mixture was stirred at 26° for an additional 2 hr and then was added to 60 ml of a freshly prepared 10% sodium sulfite solution.

Sulfuric acid (55 ml, 3 N) was added and a colorless two-phase system resulted. Ether was added, and then the organic phase was washed with water and with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to a yellow oil (2.2 g). This oil was chromatographed on 100 g of silicic acid.

Elution with 10% ether–petroleum ether (bp 60–68°) afforded 0.990 g (54%) of V as a colorless viscous oil. The oil was characterized analytically as the white S-benzylthiuronium salt (1), mp 116–118°; IR (CHCl₃): 1710 (acid C=O) cm⁻¹; NMR (CDCl₃): δ 0.65–2.55 (m, 13H, C₄H₉, CH₂CH₂), 3.30 (t, J = 6 Hz, 2H, OCH₂), 4.22 (t, J = 7 Hz, 1H, HCO), 7.20–7.70 (m, 9H, aromatic), and 8.76 (s, 1H, CO₂H) ppm.

Anal.—Calc. for C₂₁H₂₆O₃·C₈H₁₀N₂S: C, 70.7; H, 7.3; N, 5.7. Found: C, 70.6; H, 7.4; N, 5.5.

Treatment of 6-Oxo-6-(4-biphenylyl)hexanoic Acid (VI) with *n*-Butylmagnesium Bromide—The method of Morand *et al.* (9) was used for the preparation of VI, mp 160–161° [lit. (9) mp 162–163°]. To a mixture of 0.246 g (0.01 mole) of magnesium turnings and 2 ml of dry tetrahydrofuran was added dropwise, with stirring, 1.2 ml (0.011 mole) of *n*-butyl bromide in 5 ml of dry tetrahydrofuran. To this solution was added dropwise, with stirring, 1.06 g (0.0375 mole) of VI in 17 ml of dry tetrahydrofuran. The addition rate was regulated so that a gentle reflux was maintained without the necessity of warming or cooling.

After the addition was complete, the mixture was stirred at 25° for 17 hr. The reaction mixture was then poured onto an ice–water mixture, and concentrated hydrochloric acid was added until a pH of 2 was reached. The mixture was then extracted with ether. The organic phase was washed with water and with saturated sodium chloride

solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to a viscous yellow oil (1.24 g).

Chromatography on silicic acid afforded 0.900 g (70%) of the hydroxy acid VII as a viscous yellow oil (eluted with 50% ether–petroleum ether, bp 60–68°). Compound VII was characterized analytically as the white S-benzylthiuronium salt (1), mp 147–148°; IR (CHCl₃): 1708 (acid C=O) cm⁻¹; NMR (CDCl₃): δ 0.60–2.50 [m, 18H, C₄H₉, (CH₂)₄, ≥CH], 6.45 (s broad, 2H, CO₂H, OH), and 7.20–7.70 (m, 9H, aromatic) ppm.

Anal.—Calc. for C₂₂H₂₈O₃·C₈H₁₀N₂S: C, 71.2; H, 7.5; N, 5.5. Found: C, 70.9; H, 7.6; N, 5.5.

Further elution with the same solvent mixture afforded 0.231 g (22%) of the hydroxy acid VIII as a white solid, mp 130–133°. A different route (described later) was used to synthesize VIII on a preparative scale. The physical and spectral properties are described under the method used to prepare it quantitatively.

6-(4-Biphenylyl)decanoic Acid (IX)—A mixture of 0.420 g (0.00124 mole) of VII, 0.100 g of a 10% palladium-on-charcoal catalyst, 0.1 ml of concentrated hydrochloric acid, and 50 ml of absolute ethanol was hydrogenated in a Parr apparatus at 25° and 40 psi for 6 hr. The mixture was filtered, the solvent was removed under reduced pressure, and the residue was dissolved in ether.

The mixture was washed with water, with 5% sodium hydroxide solution, with water, and with saturated sodium chloride solution. It was then dried over anhydrous sodium sulfate and concentrated under reduced pressure to a colorless oil (0.277 g). This oil was the ethyl ester of IX. The resulting ester was saponified by treatment with a mixture of 6 ml of methanol and 6 ml of a 5% sodium hydroxide solution at 25° for 17 hr. The colorless solution was added to ether and water. The basic, aqueous extract was acidified to pH 2 with an ice–concentrated hydrochloric acid mixture and then extracted with ether.

The organic phase was washed with water and with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to 0.195 g (76%) of IX as a colorless oil, which crystallized slowly on standing. Two recrystallizations from petroleum ether (bp 38–52°) afforded an analytical specimen of IX as white prisms, mp 50–50.5°; IR (CHCl₃): 1710 (acid C=O) cm⁻¹; NMR (CDCl₃): δ 0.65–2.75 (m, 18H, aliphatic), 7.10–7.70 (m, 9H, aromatic), and 10.68 (s, 1H, CO₂H) ppm.

Anal.—Calc. for C₂₂H₂₈O₂: C, 81.5; H, 8.6. Found: C, 81.9; H, 8.6.

1-(4-Biphenylyl)-1-pentanone (X)—The procedure of Bowers *et al.* (10) was used. To a solution of 2.5 g (0.0104 mole) of 1-(4-biphenylyl)pentanol (III) (1) in 20 ml of acetone was added dropwise, with stirring and cooling, 15 ml (0.0210 mole) of Jones reagent (11). The temperature was maintained below 10° during the addition. The resulting brown mixture was stirred at 0° for 0.5 hr. Isopropyl alcohol (20 ml) was added, the mixture was stirred at 25° for 1 hr, and then ether was added.

The organic phase was washed with water and with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to 2.2 g (87%) of the ketone X as a white solid, mp 75–77°. An analytical specimen was prepared by recrystallization from absolute ethanol, mp 79.5–80.5°; IR (CHCl₃): 1669 (aromatic ketone C=O) cm⁻¹; NMR (CDCl₃): δ 0.80–1.96 (m, 7H, C₃H₇), 2.81 (t, J = 7 Hz, 2H, CH₂CO), and 7.40–8.06 (m, 9H aromatic) ppm.

Anal.—Calc. for C₁₇H₁₈O: C, 85.7; H, 7.6. Found: C, 85.6; H, 7.5.

O-Methyloxime of 1-(4-Biphenylyl)-1-pentanone (XI)—The method of Feuer and Braunstein (12) was used. To a solution of 2.15 g (0.009 mole) of X in 50 ml of absolute ethanol and 50 ml of dry pyridine was added 0.751 g (0.009 mole) of methoxylamine hydrochloride. The solution was heated at reflux for 24 hr and then cooled to 25°. The reaction mixture was poured onto a slurry of ice and 5% hydrochloric acid and then was extracted with ether. The organic phase was washed with water and with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to a viscous yellow oil, which crystallized on standing.

This procedure afforded 2.12 g (88%) of XI as a white solid, mp 42–44°. An analytical specimen was prepared by recrystallization from ether–petroleum ether (bp 60–68°), mp 43.5–44.5°; IR (CHCl₃): 1610 (C=N) cm⁻¹; NMR (CDCl₃): δ 0.67–1.67 (m, 7H, C₃H₇), 2.75 (t, J = 7 Hz, 2H, N=CCH₂), 3.97 (s, 3H, OCH₃), and 7.23–7.90 (m, 9H, aromatic) ppm.

Anal.—Calc. for C₁₈H₂₁NO: C, 80.9; H, 7.9; N, 5.2. Found: C, 80.7; H, 8.1; N, 5.4.

1-(4-Biphenylyl)-1-aminopentane Hydrochloride (XII)—The method of Boots *et al.* (13) was modified. To a mixture of 1.83 g (0.0068 mole) of XI and 0.660 g (0.017 mole) of sodium borohydride in 25 ml of dry dimethoxyethane was added dropwise, with stirring and cooling, 3.2 g (0.023 mole) of boron trifluoride etherate in 20 ml of dimethoxyethane. The mixture was stirred an additional hour at 25° and then heated at reflux for 2 hr. The mixture was cooled, and then 3 ml of water was added slowly, followed by 15 ml of a 5% hydrochloric acid solution.

The resulting solution was heated at reflux for 1 hr and then stirred at 25° for 14 hr. The dimethoxyethane was removed under reduced pressure, and the residue was adjusted to pH 12 with a 5% sodium hydroxide solution and extracted with ether. The organic phase was washed with water and with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to the amine as a viscous oil, which was characterized as the amine hydrochloride.

Addition of an ethereal hydrogen chloride solution to the amine afforded a white solid, 1.60 g (85%), mp 250–253°. Recrystallization from ethyl acetate afforded an analytical specimen of XII, mp 252–253.5°; IR (KBr): 3400 (⁺NH₃) cm⁻¹; NMR (dimethyl sulfoxide-d₆): δ 0.62–1.53 (m, 9H, C₄H₉), 4.72 (s, 3H, ⁺NH₃), and 7.35–7.92 (m, 9H, aromatic) ppm.

Anal.—Calc. for C₁₇H₂₂ClN: C, 74.0; H, 8.0; N, 5.1. Found: C, 74.4; H, 8.2; N, 5.4.

N-[1-(4-Biphenylyl)pentyl]succinamic Acid (XIII)—The method described by Vogel (14) was used. A solution of 0.50 g (0.0018 mole) of XII in 18 ml of an ice-cold 5% sodium hydroxide solution was extracted with 50 ml of benzene. The organic phase was washed with water and with saturated sodium chloride solution and dried over anhydrous sodium sulfate. To the benzene solution of the amine was added 0.200 g (0.002 mole) of succinic anhydride. The solution was stirred at 25° for 3 hr and then was extracted with a 5% sodium hydroxide solution. The basic aqueous phase was adjusted to pH 2 with an ice-cold 5% hydrochloric acid solution and then extracted with ether.

The organic phase was washed with water and with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to 0.573 g of a white solid, mp 165–170°. Recrystallization from ethyl acetate–petroleum ether (bp 60–68°) afforded 0.259 g (40%) of XIII, mp 176.5–178°; IR (KBr): 3250 (NH), 1725 (acid C=O), and 1640 (amide C=O) cm⁻¹; NMR (dimethyl sulfoxide-d₆): δ 0.67–2.00 (m, 9H, C₄H₉), 2.54 (s, 4H, CH₂CH₂), and 7.34–7.84 (m, 9H, aromatic) ppm.

Anal.—Calc. for C₂₁H₂₅NO₃: C, 74.3; H, 7.4; N, 4.1. Found: C, 74.1; H, 7.4; N, 4.2.

4-[1-(4-Biphenylyl)pentylamino]butanoic Acid (XVII)—To a solution of 1.23 g (0.008 mole) of methyl 4-aminobutanoate hydrochloride (XIV), mp 119–120° [lit. (15) mp 121.5–122.5°], in 20 ml of methanol was added 1.0 g (0.012 mole) of sodium acetate. A cloudy solution resulted with an apparent pH of 6–7. To this solution was added a solution of 0.476 g (0.002 mole) of X in 15 ml of ether and 5 ml of methanol. To this mixture were added 2 g of anhydrous sodium sulfate and 0.112 g (0.0018 mole) of sodium cyanoborohydride⁴.

The mixture was stirred at 25° for 72 hr and then poured into ether and water. The organic phase was washed with water and with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to 530 mg of a pale-yellow semisolid, which was chromatographed on 30 g of magnesium silicate⁵ (60–100 mesh). Elution with 5% ether–petroleum ether (bp 60–68°) afforded 0.149 g of X. Elution with 50% ether–petroleum ether (bp 60–68°) through 100% ether afforded 0.233 g of a mixture of the amino ester XV and the lactam XVI as a viscous colorless liquid; IR (CHCl₃): 1758 (ester C=O) and 1665 (lactam C=O) cm⁻¹. The mixture was hydrolyzed directly as described later.

To a solution of XV and XVI (0.230 g) in 5 ml of 95% ethanol and 15 ml of a 50% *tert*-butyl alcohol–water mixture was added 0.700 g (0.011 mole) of 85% potassium hydroxide pellets. The colorless solution was heated at reflux for 21 hr. Then the solvent was removed under reduced pressure, and the residue was made up to a total volume of 10 ml with water. The mixture was chilled (5°) and filtered, and the solid was rinsed with 10 ml of water (5°). The filtrate was adjusted to pH 6 with acetic acid, and the resulting white viscous oil was extracted with ethyl acetate.

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⁴ Aldrich Chemical Co.
⁵ Florisil.

The organic phase was washed with water and with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to 0.183 g of a pale-yellow viscous oil, which was triturated with ether and then allowed to stand at 5° for 6 days. A white solid resulted, which proved to be hygroscopic, so no melting point could be obtained. An analytical specimen of XVII was prepared by recrystallization from ethyl acetate–ether; IR (CHCl$_3$): 1715 (broad, acid C=O, unionized) and 1600–1560 (broad, acid C=O, ionized) cm^{-1}; NMR (CDCl$_3$): δ 0.65–3.00 (m, 16H, C$_4$H$_9$, CH$_2$CH$_2$CH$_2$CH), 7.30–7.80 (m, 9H, aromatic), and 8.15 (s, broad, 2H, NH, CO$_2$H) ppm.

Anal.—Calc. for C$_{21}$H$_{27}$NO$_2$·2H$_2$O: C, 69.8; H, 8.6; N, 3.9. Found: C, 70.0; H, 7.9; N, 3.6.

5-Oxo-5-(4-biphenylyl)-3-methylpentanoic Acid (XIX)—The procedure of Goldschmidt (6) was used. To a suspension of 6.5 g (0.049 mole) of aluminum chloride in 60 ml of dry methylene chloride was added dropwise, with stirring at 25°, a solution of 2.49 g (0.02 mole) of 3-methylglutaric anhydride^4 in 20 ml of methylene chloride, followed by a solution of 3.08 g (0.02 mole) of biphenyl in 20 ml of methylene chloride. The reaction mixture was stirred at 25° for 23 hr and then poured onto an ice–concentrated hydrochloric acid mixture. Chloroform was added until a clear two-phase system was obtained, and then the chloroform was removed under reduced pressure. The residue was added to ether and water.

The organic phase was washed with water and with a 5% sodium hydroxide solution. The basic, aqueous extract was acidified to pH 2 with a 5% hydrochloric acid solution and extracted with ether. The organic phase was washed with water and with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to 1.7 g (30%) of XIX as an orange oil, which crystallized on standing. Repeated recrystallization from ethyl acetate–petroleum ether (bp 60–68°) afforded an analytical specimen of XIX as cream plates, mp 119–120°; IR (CHCl$_3$): 1708 (acid C=O) and 1676 (ketone C=O) cm^{-1}; NMR (CDCl$_3$): δ 1.05 (d, J = 6 Hz, 3H, CH$_3$), 2.30–3.15 (m, 5H, CH$_2$CHCH$_2$), 7.20–8.18 (m, 9H, aromatic), and 10.05 (s, 1H, CO$_2$H) ppm.

Anal.—Calc. for C$_{18}$H$_{18}$O$_3$: C, 76.6; H, 6.4. Found: C, 76.6; H, 6.3.

5-(4-Biphenylyl)-3-methylpentanoic Acid (XX)—A mixture of 0.500 g (0.0018 mole) of XIX, 0.100 g of a 10% palladium-on-charcoal catalyst, 0.1 ml of concentrated hydrochloric acid, and 50 ml of absolute ethanol was hydrogenated on a Parr apparatus at 25° and 40 psi for 5 hr. The mixture was filtered, and the solvent was removed under reduced pressure to give 0.420 g of a colorless liquid. This mixture of the acid and ethyl ester was treated with a mixture of 10 ml of methanol and 10 ml of a 5% sodium hydroxide solution at 25° for 48 hr. The colorless solution was added to ether and water. The basic, aqueous extract was acidified to pH 2 with an ice–concentrated hydrochloric acid mixture and then extracted with ether.

The organic phase was washed with water and with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to 0.370 g (78%) of XX as a pale-yellow solid. Recrystallization from petroleum ether (bp 60–68°) afforded 0.253 g of white needles, mp 89–91° [lit. (6) mp 92–93°]; IR (CHCl$_3$): 1708 (acid C=O) cm^{-1}; NMR (CDCl$_3$): δ 1.03 (d, J = 6 Hz, 3H, CH$_3$), 1.27–2.80 (m, 7H, CH$_2$CH$_2$CHCH$_2$), 7.14–7.58 (m, 9H, aromatic), and 10.15 (s, 1H, CO$_2$H) ppm.

4-Phenylbenzylideneacetone (XXII)—The procedure of Ayling *et al.* (7) was used for the synthesis of this α,β-unsaturated ketone, mp 126–129° [lit. (7) mp 137°].

4-(4-Biphenylyl)-2-butanone (XXIII)—The method of Cromwell and Cahoy (8) was used for the synthesis of this ketone, mp 71–73° [lit. (8) mp 75–77°].

***tert*-Butyl 5-(4-Biphenylyl)-3-hydroxy-3-methylpentanoate (XXIV)**—The procedure of Cornforth *et al.* (16) was used. To a mixture of 0.295 g (0.0045 mole) of zinc (20 mesh, unactivated) in 5 ml of dry tetrahydrofuran (heated at reflux and maintained under a nitrogen atmosphere) was added dropwise, over 15 min, a solution of 1.00 g (0.0045 mole) of XXIII and 0.7 ml (0.0045 mole) of *tert*-butyl bromoacetate in 7 ml of dry tetrahydrofuran. The mixture was heated at reflux for 3 hr after the addition was completed. Then the mixture was cooled, poured onto ice–5% hydrochloric acid, and rapidly extracted with ether.

The organic phase was washed with a 5% sodium hydroxide solution, with water, and with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to 1.42 g (93%) of a colorless liquid. Attempts to purify XXIV

resulted in extensive decomposition, so it was hydrolyzed directly to the free acid (XXV).

5-(4-Biphenylyl)-3-hydroxy-3-methylpentanoic Acid (XXV)—A mixture of 0.454 g (0.0013 mole) of the ester XXIV, 0.060 g of *p*-toluenesulfonic acid monohydrate, and 10 ml of benzene was heated at reflux for 30 min. The solution was added to ether and water. The organic phase was washed with water and with a 5% sodium hydroxide solution. The basic, aqueous extract was acidified to pH 2 with a 5% hydrochloric acid solution and then extracted with ether.

The organic phase was washed with water and with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to 0.164 g (45%) of XXV as a white solid. Recrystallization from ethyl acetate–petroleum ether (bp 60–68°) afforded an analytical specimen as white needles, mp 91–91.5°; NMR (CDCl$_3$): δ 1.37 (s, 3H, CH$_3$), 1.70–2.03 (m, 2H, CH$_2$ at C-4), 2.60–3.12 (m, 4H, CH$_2$, CH$_2$ at C-2 and C-5), and 7.05–8.20 (m, 11H, aromatic, OH, CO$_2$H) ppm.

Anal.—Calc. for C$_{18}$H$_{20}$O$_3$: C, 76.0; H, 7.1. Found: C, 76.2; H, 6.9.

Methyl 6-Oxo-6-(4-biphenylyl)hexanoate (XXVI)—The method of Morand *et al.* (9) was used for the preparation of this ester, mp 109–110° [lit. (9) mp 108–109°].

6-Oxo-6-(4-biphenylyl)hexanoic Acid (VI)—The method of Morand *et al.* (9) was used for the preparation of this acid, mp 160–161° [lit. (9) mp 162–163°]. This keto acid was also used as an intermediate in the synthesis of IX.

Methyl 6-Hydroxy-6-(4-biphenylyl)hexanoate (XXVII)—The method of Boots^6 was used. To a solution of 1.81 g (0.0061 mole) of XXVI in 15 ml of acetic acid was added dropwise, with stirring at 25°, a solution of 2 ml (0.019 mole) of pyridinylborane^7 in 5 ml of acetic acid. The mixture was stirred at 25° for 4 hr and then poured onto an ice (100 g)–concentrated hydrochloric acid (6 ml) mixture. This mixture was stirred for 30 min and then extracted with ether.

The organic phase was washed with water, with 5% sodium hydroxide solution, with water, and with saturated sodium chloride solution. Then it was dried over anhydrous sodium sulfate and concentrated under reduced pressure to 1.44 g (79%) of a pale-pink oil, which crystallized upon trituration with ether–petroleum ether (bp 60–68°), mp 46–48°. An analytical specimen of XXVII as white prisms was prepared by recrystallization from ether–petroleum ether (bp 60–68°), mp 49–50°; IR (CHCl$_3$): 3450–3600 (broad, OH) and 1725 (ester C=O) cm^{-1}; NMR (CDCl$_3$): δ 1.2–2.5 [m, 9H, (CH$_2$)$_4$, OH], 3.62 (s, 3H, OCH$_3$), 4.72 (t, J = 6 Hz, 1H, CH), and 7.22–7.72 (m, 9H, aromatic) ppm.

Anal.—Calc. for C$_{19}$H$_{22}$O$_3$: C, 76.5; H, 7.4. Found: C, 76.8; H, 7.4.

6-Hydroxy-6-(4-biphenylyl)hexanoic Acid (VIII)—A solution of 0.330 g (0.0011 mole) of the hydroxy ester XXVII, 7.5 ml of methanol, and 5 ml of a 5% sodium hydroxide solution was heated at reflux for 1 hr. The solvent was removed under reduced pressure, and then the residue was added to ether and water. The basic, aqueous extract was acidified to pH 2 with an ice-cold 5% hydrochloric acid solution and then extracted with ethyl acetate.

The organic phase was washed with water and with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to a quantitative yield (0.316 g) of the hydroxy acid VIII as a beige solid, mp 130–131°. Recrystallization from ethyl acetate–petroleum ether (bp 60–68°) afforded an analytical specimen as white prisms, mp 132–133°; IR (mineral oil): 1725 (acid C=O) cm^{-1}; NMR (acetone-d_6): δ 1.10–2.50 [m, 8H, (CH$_2$)$_4$], 4.71 (t, J = 6 Hz, 1H, CH), 6.25 (s, 2H, OH, CO$_2$H), and 7.30–7.75 (m, 9H, aromatic) ppm.

Anal.—Calc. for C$_{18}$H$_{20}$O$_3$: C, 76.0; H, 7.1. Found: C, 75.7; H, 7.4.

Methyl 6-(4-Biphenylyl)hexanoate (XXVIII)—A mixture of 0.600 g (0.002 mole) of XXVII, 0.100 g of a 10% palladium-on-charcoal catalyst, 0.1 ml of concentrated hydrochloric acid, and 50 ml of absolute ethanol was hydrogenated on a Parr apparatus at 25° and 40 psi for 6 hr. The mixture was filtered, and the solvent was removed under reduced pressure. The residue was added to ether and water.

The organic phase was washed with water and with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to 0.490 g (85%) of a cream solid,

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^{6} Manuscript in preparation.
^{7} Callery Chemical Co.

mp 35–38°. Recrystallization from petroleum ether (bp 60–68°) afforded an analytical specimen of XXVIII as white prisms, mp 37–39°; IR (CHCl₃): 1728 (ester C=O) cm⁻¹; NMR (CDCl₃): δ 1.15–2.85 [m, 10H, (CH₂)₅], 3.67 (s, 3H, OCH₃), and 7.20–7.72 (m, 9H, aromatic) ppm.

Anal.—Calc. for $C_{19}H_{22}O_2$: C, 80.8; H, 7.8. Found: C, 80.9; H, 7.8.

6-(4-Biphenylyl)hexanoic Acid (XXIX)—A solution of 0.215 g (0.00076 mole) of the ester XXVIII, 7 ml of methanol, and 5 ml of a 5% sodium hydroxide solution was heated at reflux for 1 hr. The solvent was removed under reduced pressure, and then the residue was added to ether and water. The basic, aqueous extract was acidified to pH 2 with 5% hydrochloric acid and then extracted with ethyl acetate.

The organic phase was washed with water and with saturated sodium chloride solution, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to 0.184 g (91%) of a white solid, mp 95–97°. Recrystallization from ether–petroleum ether (bp 60–68°) afforded an analytical specimen of XXIX as white needles, mp 98–99° [lit. (17) mp 103–105°]; IR (CHCl₃): 1705 (acid C=O) cm⁻¹; NMR (CDCl₃): δ 1.2–2.85 [m, 10H, (CH₂)₅] and 7.15–7.65 (m, 9H, aromatic) ppm.

Assay of Enzyme Activity and Inhibition—Rat liver microsomes containing β-hydroxy-β-methylglutaryl coenzyme A reductase activity were prepared according to Shapiro and Rodwell (18). The assay procedure used was described previously (2). The enzyme incubation mixture consisted of NADP⁺ (9.7 μmoles)[8], glucose 6-phosphate (59.7 μmoles)[8], torula yeast glucose 6-phosphate dehydrogenase (5 units)[8], microsomal protein (2 mg) as determined by a modification of the method of Lowry *et al.* (2, 19), 0.1 ml of ethylene glycol monoethyl ether containing the inhibitor, and sufficient assay buffer (30 mM ethylenediaminetetraacetic acid, 70 mM sodium chloride, and 10 mM β-mercaptoethanol, pH 6.8) to produce a total volume of 2.9 ml.

Enzymatic activity was initiated by the addition of 0.1 ml of a solution containing 94 nmoles (31 μM) of 3-¹⁴C-*dl*-β-hydroxy-β-methylglutaryl coenzyme A prepared (20) from 3-¹⁴C-β-hydroxy-β-methylglutaric acid⁹. After 30 min of incubation, the reaction was stopped by the addition of 0.2 ml of concentrated hydrochloric acid, and 5-³H-*dl*-mevalonolactone⁹ was added. Mevalonolactone was extracted into ether and isolated by TLC, and the radioactivity was determined[10].

RESULTS

Various isosteric replacements of the ester group were made in an effort to determine if the ester function was critical for activity. Also, replacements were made to find compounds that would not contain the acid-labile ester group found in I. A recent review article (21) described a 2-(4-biphenylyl)propyl group as a "super-sensitive, acid-labile protecting group" of carboxylic acids. The close similarity between the alcoholic portion of the ester analogs I and II and this group suggested that the esters could be highly susceptible to acid hydrolysis (which could be important upon oral administration).

Thus, the ether analog V, the carbo analog IX, the amido analog XIII, and the amino analog XVII were evaluated as inhibitors of rat liver β-hydroxy-β-methylglutaryl coenzyme A reductase (Table I). Both V and IX had essentially the same inhibitory activity as I. However, XIII definitely showed less inhibitory activity than I, even though solubility problems were encountered. On the other hand, XVII was about one-half as active as I.

These data show that the ester function is not necessary for significant inhibitory activity. The isosteric replacements involved which afforded the ether and carbo analogs provided compounds with essentially equal inhibitory activity when compared to the ester I.

In 1966, Eades *et al.* (3, 4) reported the evaluation of 36 compounds as inhibitors of the incorporation of 1-¹⁴C-acetate into cholesterol by rat liver homogenates. The close similarity in structure of these compounds to those described here led to the investigation as to whether three of these compounds (XIX, XX, and XXV) were inhibitors of β-hydroxy-β-methylglutaryl coenzyme A reductase. Analog XXV was selected on the basis of its close similarity in structure to our most active inhibitor, the ester II. Compound XX was also evaluated because it was the most active and extensively studied com-

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

Compound	Activity, $(I/S)_{50\%}$[a]
I, $m = 2$ [b]	11
II[c]	1.5
V	10
VII	11
IX	16
XIII	30[d] (18%)
XVII	20
XIX	12
XX	12
XXV	15
VI	3[d] (30%)
XXVI	5[d] (16%)
VIII	15
XXVII	8[d] (26%)
XXIX	3[d] (40%)
XXVIII	3[d] (26%)

[a] The inhibition index, $(I/S)_{50\%}$, equals the ratio of the micromolar concentration of the inhibitor to the micromolar concentration of the substrate required to give 50% inhibition. At least two sets of duplicate determinations were used. [b] A standard inhibitor used routinely to provide comparability of each assay set (2). [c] Described previously (2). [d] The I/S ratio reported produced the parenthetical percentage of inhibition. The inhibitor was insoluble at higher concentrations.

pound by Eades *et al.* Analogs XIX, XX, and XXV exhibited the same degree of inhibition shown by the ester analog I. Comparison of the substituted 3-hydroxy-3-methyl ester (II) with the similar analog XXV showed remarkable dissimilarities in their inhibitory activities. This finding suggests that XXV is binding to the enzyme at a different site or in a different mode than II.

Analogs VI and XXVI–XXIX could not be evaluated quantitatively because of their limited solubility in the test system. Analogs VII and VIII exhibited essentially the same inhibitory activity, demonstrating that the *n*-butyl side chain was not required for activity in this particular series.

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[8] Sigma Chemical Co.
[9] New England Nuclear.
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Physical Characterization and Activity *In Vivo* of Polymorphic Forms of 7-Chloro-5,11-dihydrodibenz[*b,e*][1,4]oxazepine-5-carboxamide, a Potential Tricyclic Antidepressant

IRWIN S. GIBBS ×, **ANTHONY HEALD, HAROLD JACOBSON, DEODATT WADKE, and IRVING WELIKY**

Abstract □ The biological availability in dogs and humans of 7-chloro-5,11-dihydrodibenz[*b,e*][1,4]oxazepine-5-carboxamide, a potential antidepressant drug, was increased when the compound was administered in capsule formulations as micronized drug coated with 1% sodium lauryl sulfate or as a lyophilate with poloxamer 407. This increase with these two formulations had been predicted by dissolution tests *in vitro*. The lyophilized combination with poloxamer 407 was more soluble in 0.1 N HCl than was the untreated compound. Characterization of the lyophilate by differential thermal analysis, X-ray diffraction, and IR spectroscopy indicated that the increase in solubility was attributable to the formation of a polymorphic form. A polymorph of the compound, designated Form B, was prepared. The solubility and dissolution characteristics of the two polymorphic forms, A and B, as well as of the lyophilized combination with poloxamer 407, were determined.

Keyphrases □ Dibenz[*b,e*][1,4]oxazepine, substituted—solubility, dissolution rate, and bioavailability, micronized and surfactant coated compared to lyophilate with copolymer formulation □ Bioavailability—substituted dibenz[*b,e*][1,4]oxazepine, micronized and surfactant coated compared to lyophilate with copolymer formulation □ Polymorphic forms—substituted dibenz[*b,e*][1,4]oxazepine, solid dispersion, solubility, dissolution rate, and bioavailability □ Antidepressants, tricyclic—substituted dibenz[*b,e*][1,4]oxazepine, solubility, dissolution rate, and bioavailability

In preclinical studies for the development of a new drug, its biological availability should be assessed (1). If a drug is incompletely absorbed, efforts are usually made to increase its biological availability.

The common causes of biological unavailability of a drug include poor solubility and slow dissolution in aqueous media, specifically GI fluids (1). When synthesis of a more soluble chemical derivative is not feasible, diminution of the particle size (2, 3) or utilization of another polymorphic form (4–7) or solid dispersions of the drug (8–12) may increase its biological availability.

In preformulation studies of a potential tricyclic antidepressant, 7-chloro-5,11-dihydrodibenz[*b,e*][1,4]-oxazepine-5-carboxamide[1] (I), synthesized by Yale (13), the drug was more readily and more completely absorbed when given to dogs orally as capsules containing a fine powder rather than a coarse powder[2].

The equilibrium solubility of I in aqueous solution at 37° is 0.04 mg/ml, irrespective of pH.

I
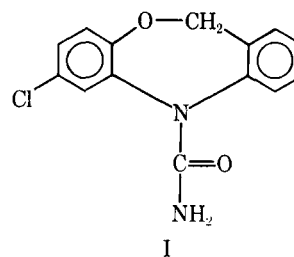
This report describes the methods evolved for increasing the bioavailability of this drug by increasing its dissolution rate and/or solubility. To accomplish these effects, the material was either micronized and combined with a wetting agent or prepared as a solid dispersion. During the preparation of the latter, the existence of a polymorphic form was discovered.

EXPERIMENTAL

Materials—The following was used: poloxamer 407[3], I polymorph Form A[4], sodium lauryl sulfate USP, magnesium stearate USP, an-

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[1] SQ 10,966.

[2] J. Dreyfuss, Department of Drug Metabolism, Squibb Institute for Medical Research, New Brunswick, N.J., unpublished data.

[3] Pluronic F-127, BASF Wyandotte Corp., Wyandotte, Mich.

[4] Squibb lot RR002RC (see Ref. 13).