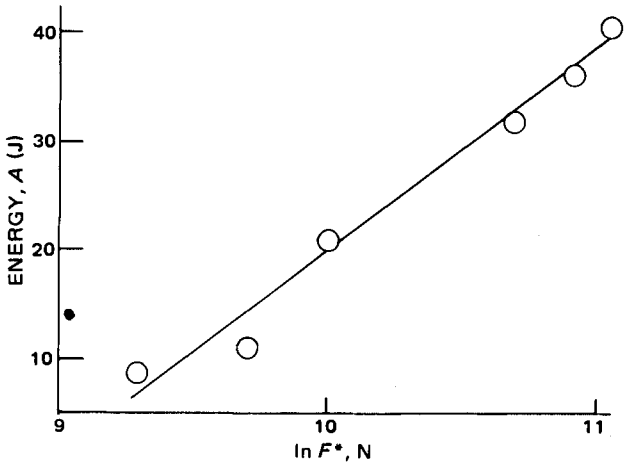

Figure 5—*Areas from plots of the type in Fig. 3 at six different pressures, plotted as a function of maximal pressure according to Eq. 6.*

I. The last column gives the F_0 value that is the smallest force necessary for actual displacement beyond the rearrangement step. It is seen from Table I that, for example, for "excipient + copolymer (50%)" (the case shown in Fig. 3), F_0b ln F_0 = 168 J and F_0b = 18.8 J. In other words, ln F_0 = 168/18.8 = 8.94, or F_0 = 6610 N. This value corresponds to an ordinate of 0.66 in Fig. 3, which corresponds to a "recorded" abscissa value (with arbitrary zero) of 4.5 mm.

Since F_0b = 18.8, it follows that b = 18.8/F_0 = 0.0028 m = 2.8 mm. Hence, in Fig. 3, the final asymptote would be at a recorded abscissa value of 4.5 + 2.8 = 7.3 mm (again recalling that the zero is somewhat arbitrary). At this point, the distance between the upper and lower punch would be 2.4 mm[6]. If the solid achieves its true density at b, then the true density can be deduced. The die has a diameter of 12 mm; *i.e.*, the volume between the upper and lower punch is equal to [π (1.2^2/4) 0.24] = 0.27 cm^3.

[6] This value is obtained from knowledge of the lower punch position in relation to the arbitrary zero and does not account for possible compression of the presumably static lower punch.

The weight of the solids is 500 mg, so the true density would have a value of 0.5/0.27 = 1.84 g/cm^3, which is reasonable. Since compression can possibly (and presumably does) produce densities that are higher than the true densities at atmospheric pressure, the calculated figure only has interest in that it is of a reasonable magnitude. Furthermore, Eq. 8 deals with the energy input and assumes that the relaxation trace in Fig. 3 (the recovery) is vertical. This approximation becomes poorer the more pronounced the recovery after upper punch release.

SUMMARY

The present study shows that the Parmentier–Führer equation for the value of the upper punch force as a function of displacement is useful and can be used to deduce mathematically energy *versus* maximum force relationships for energy input. These relationships were validated experimentally. The plots provide an estimate of the point in the displacement of the upper punch where the powder or granulation was rearranged to its closest packing.

REFERENCES

(1) W. Parmentier, Ph.D. thesis, Technical University Carolo-Wilhemina zu Braunschweig, Braunschweig, West Germany, 1974, pp. 1–90.

(2) C. Führer, *Dtsch. Apoth.-Ztg.*, **105**, 1150 (1965).

(3) P. Toure, Ph.D. thesis, University of Paris-Sud, Chatenay-Malabry, France, 1979, p. 102.

(4) P. Toure, D. Duchene, F. Puisieux, and J. T. Carstensen, *Powder Technol.*, **26**, 213 (1980).

(5) J. T. Carstensen: "Rate Processes and Mechanical Properties in Solid Pharmaceutics," Academic, New York, N.Y., 1980, pp. 187–214.

(6) W. M. Long, *Powder Metall.*, **6**, 73 (1960).

(7) S. Leigh, J. E. Carless, and B. W. Burt, *J. Pharm. Sci.*, **56**, 888 (1967).

(8) J. J. Windheuser, J. Misra, S. P. Eriksen, and T. Higuchi, *ibid.*, **52**, 767 (1963).

(9) A. Obiorah, *Int. J. Pharm.*, **1**, 249 (1978).

(10) K. Marshall, "The Physics of Tablet Compression, 12th Arden House Conference, Feb. 2, 1977, Conference Notes," Colorcon, West Point, Pa., pp. 15, 16.

Mevalonic Acid Analogs as Inhibitors of Cholesterol Biosynthesis

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Abstract □ A series of 20 mevalonic acid analogs was synthesized and tested for their ability to inhibit cholesterol biosynthesis from [2-^{14}C]-mevalonate in rat liver homogenates. Removal of the 5-hydroxyl group from mevalonic acid produced an active inhibitor, 3-hydroxy-3-methylpentanoic acid. Removal of the 3-hydroxyl group, addition of an aromatic group in the 3-position, or insertion of a double bond reduced inhibitory activity. Compounds with an aromatic group or halide on the 5-position were active inhibitors. The most active inhibitor was 5-phenylpentanoic acid, with 50% inhibition at 0.064 m*M*.

Keyphrases □ Mevalonic acid—analogs synthesized as potential cholesterol biosynthesis inhibitors □ Cholesterol—inhibitors, synthesis and testing of mevalonic acid analogs □ Structure–activity relationships—mevalonic acid analogs as inhibitors of cholesterol biosynthesis

Considerable progress has been made in understanding the biosynthesis of cholesterol (1, 2). Mevalonic acid, the product of the major rate-limiting reaction of cholesterol biosynthesis, is converted to isopentenyl pyrophosphate by three enzymes, each requiring Mg^{2+} and ATP. The first step is phosphorylation at position 5 by mevalonate kinase (EC 2.7.1.36). Next, 5-pyrophosphomevalonate is formed by phosphomevalonate kinase (EC 2.7.4.2). This product is decarboxylated and dehydrated by pyrophosphomevalonate decarboxylase (EC 4.1.1.33), possibly involving 3-phospho-5-pyrophosphomevalonate as an intermediate.

Interest has focused on these reactions for various reasons. For example, the role of cholesterol in atherosclerosis prompted a search for inhibitors of these reactions with the prospect of decreasing cholesterol synthesis (3–7). It also has been suggested that the decreased brain cholesterol levels seen in phenylketonuria may be due to inhibition of these steps by elevated levels of phenylalanine

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and its deaminated metabolites (8, 9). Furthermore, the development of specific inhibitors could be used to study the mechanism of these reactions and their importance as a secondary regulatory site of cholesterol synthesis. In addition, such inhibitors could help elucidate the mechanism and control of the recently observed nonsterol metabolic pathway of mevalonic acid (10, 11).

This paper describes the synthesis and testing of mevalonic acid analogs for their ability to inhibit the incorporation of [2-^{14}C]mevalonate into cholesterol by rat liver homogenates.

EXPERIMENTAL[1]

All mevalonic acid analogs were either prepared in this laboratory or obtained commercially[2]. The 3-hydroxy esters were prepared by the Reformastki reaction (12). The products were purified by fractional vacuum distillation through a Vigreux column. The structures were verified by NMR spectroscopy. Elemental analyses were performed on those compounds for which there were no analytical data in the literature for comparison.

Ethyl 3-(4-Chlorophenyl)-3-hydroxypentanoate (E-8)—Compound E-8 was prepared by the careful dropwise addition of *p*-chloropropiophenone (15 g, 89 mmoles) and ethyl bromoacetate (15.5 g, 93 mmoles) in ether (20 ml) and benzene (20 ml) to granular zinc (11 g, 168 mmoles) while stirring mechanically. After the exothermic reaction subsided, the mixture was stirred and refluxed for 3 hr, cooled, acidified to pH 3 with 10% HCl, and extracted twice with chloroform.

The organic portions were combined, washed with 5% $NaHCO_3$, and dried over anhydrous magnesium sulfate, and the solvents were removed under reduced pressure. The residue was distilled, yielding 20 g (88%) of a colorless liquid, bp 111–112.5°/0.15 mm Hg; NMR ($CDCl_3$): δ 0.74 (t, 3H, CH_3), 1.09 (t, 3H, CH_3-ester), 1.78 (q, 2H, CH_2), 2.85 (s, 2H, CH_2=O), 4.08 (q, 2H, $COOCH_2$), 4.37 (s, 1H, OH), and 7.42 (s, 4H, aromatic) ppm.

Anal.—Calc. for $C_{13}H_{17}ClO_3$: C, 60.82; H, 6.67; Cl, 13.81. Found: C, 60.69; H, 6.74; Cl, 13.69.

Ethyl 5-Chloro-3-hydroxy-3-phenylpentanoate (E-9)—Compound E-9 was prepared by the slow addition of β-chloropropiophenone (5 g, 30 mmoles) and ethyl bromoacetate (4.95 g, 30 mmoles) in ether (13 ml) and benzene (13 ml) to zinc dust (1.9 g, 60 mmoles) while stirring mechanically. After the exothermic reaction subsided, the mixture was refluxed overnight and worked up as already described. Vacuum distillation yielded 4.0 g (52%) of a faint-yellow liquid (E-9), bp 120–122°/0.3 mm Hg; NMR ($CDCl_3$): δ 1.03 (t, 3H, CH_3), 2.27 (t, 2H, CH_2), 2.88 (s, 2H, CH_2C=O), 3.34 (m, 2H, CH_2Cl), 4.00 (q, 2H, $COOCH_2$), 4.54 (s, 1H, OH), and 7.33 (s, 5H, aromatic) ppm.

Anal.—Calc. for $C_{13}H_{17}ClO_3$: C, 60.82; H, 6.67; Cl, 13.81. Found: C, 60.51; H, 6.75; Cl, 13.88.

Ethyl 3-Hydroxy-3-methylpentanoate (E-1)—Methyl ethyl ketone (69 g, 0.96 mole) and ethyl bromoacetate (167 g, 1.0 mole) in ether (450 ml) were added to zinc dust (120 g, 1.84 moles) with mechanical stirring. The mixture was refluxed overnight and worked up as already described. Vacuum distillation yielded 108 g (70%) of a colorless liquid (E-1), bp 38–39°/0.015 mm Hg [lit. (13) bp 83–88°/13 mm].

Ethyl 3-Hydroxy-3-phenylpentanoate (E-7)—Propiophenone (33.5 g, 0.25 mole) and ethyl bromoacetate (42 g, 0.25 mole) in ether (50 ml) and benzene (50 ml) were added to zinc dust (33 g, 0.51 mole) with mechanical stirring. The mixture was refluxed overnight and worked up as already described. Distillation resulted in 35 g (63%) of a faint-pink liquid (E-7), bp 86.5–88°/0.15 mm Hg [lit. (14) bp 104°/0.5 mm].

Ethyl Δ^2-3-Methylpentenoate (E-2)—The Emmons modification (15) of the Wittig reaction was used to prepare E-2. Ethyl bromoacetate (83.5 g, 0.5 mole) was added dropwise to a stirred solution of triethyl phosphite (83.1 g, 0.5 mole). After complete addition, the temperature was increased, and ethyl bromide began to distill off. As the liberation of ethyl bromide decreased, the temperature was increased slowly, finally remaining at 170–175° for 1 hr. The cooled solution was added dropwise to sodium ethoxide formed from the addition of absolute ethanol (23 g, 0.5 mole) to sodium ribbon (11.5 g, 0.5 mole) in tetrahydrofuran (300 ml) under nitrogen. Then, methyl ethyl ketone (36 g, 0.5 mole) was added slowly at a rate to keep the temperature below 35°.

After addition of the ketone, the mixture was stirred and refluxed overnight, cooled, and filtered. The precipitate was washed with ether, ethanol, and finally water. The aqueous phase was washed twice with ether. The combined organic phases were dried over anhydrous magnesium sulfate, and the solvents were removed under reduced pressure. Distillation yielded 48 g (68%) of a colorless liquid (E-2), bp 72–74°/23 mm Hg [lit. (16) bp 68°/14 mm]; NMR ($CDCl_3$): δ 1.06 (t, 3H, CH_3), 1.25 (t, 3H, CH_3-ester), 2.10 (m, 2H, CH_2C=), 2.16 (s, 3H, CH_3C=), 4.14 (q, 2H, $COOCH_2$), and 5.65 (s, 1H, HC=C) ppm.

Methyl Δ^3-3-Methylpentenoate (E-3)—Kon and Linstead (17) claimed that ethyl Δ^3-3-methylpentenoate could be selectively prepared by the dehydration of E-1 by heating in the presence of potassium hydrogen sulfate. However, this procedure resulted in a mixture of the Δ^2- and Δ^3-esters, as confirmed by NMR. Thus, the more complex procedure of Wagner (13) had to be utilized, which would specifically locate the double bond in the Δ^3-position and result in the *trans*-isomer. Briefly, the procedure involved four steps: (*a*) aldol condensation of acetaldehyde and methyl ethyl ketone in the presence of base to yield 4-hydroxy-3-methylpentanone, (*b*) dehydration of the β-hydroxyketone to 3-methyl-3-penten-2-one, (*c*) formation of 3,4-dibromo-3-methyl-2-pentanone by the addition of bromine to the unsaturated ketone, and (*d*) Favorskii rearrangement of the α,β-dibromoketone in the presence of sodium methoxide to form methyl Δ^3-3-methylpentenoate (E-3) (25% yield), bp 47–50°/11 mm Hg [lit. (13) bp 74°/50 mm]; NMR ($CDCl_3$): δ 1.5 [m, 6H, (CH_2C=)$_2$], 3.0 (d, 2H, CH_2), 3.65 (s, 3H, OCH_3), and 5.42 (m, 1H, CH=C) ppm.

Saponification of Esters—Two general procedures similar to those of Stewart and Woolley (4) were used.

Procedure 1—To a stirred solution containing an ester (40 mmoles) in 80% ethanol (30 ml) at 60°, 40 ml of sodium hydroxide (1 *N*, 40 mmoles) was added dropwise over 90 min. After the mixture was stirred at 60° for 18 hr, the solvent was removed under reduced pressure. The remaining solution was extracted twice with ether, and the combined ether extractions were backwashed once with 5% $NaHCO_3$. The combined aqueous portion was acidified to pH 3 with 1 *N* HCl.

If the acid was a solid at room temperature, it precipitated out of the acidic solution and was collected by filtration and purified by recrystallization. If no precipitate formed, the aqueous solution was extracted twice with ether and twice with ethyl acetate. The combined organic extracts were washed with water until neutral, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was vacuum distilled.

Procedure 2—Because dehydration or isomerization was a problem during the saponification of E-1, E-2, E-3, a gentler procedure was used. The ester (40 mmoles) in 80% ethanol (60 ml) was cooled in an ice bath while 40 ml of sodium hydroxide (1 *N*, 40 mmoles) was added over 24 hr. The solution was stirred at room temperature for 1–2 days and the product was worked up as already described.

3-(4-Chlorophenyl)-3-hydroxypentanoic Acid (A-8)—Compound E-8 was hydrolyzed according to Procedure 1 producing A-8 (93%), which was recrystallized from benzene–hexane (1:1), mp 97–98°; NMR ($CDCl_3$): δ 0.71 (t, 3H, CH_3), 1.78 (q, 2H, CH_2), 2.91 (s, 2H, CH_2C=O), and 7.40 (s, 6H, aromatic, OH, COOH) ppm.

Anal.—Calc. for $C_{11}H_{13}ClO_3$: C, 57.79; H, 5.73; Cl, 15.50. Found: C, 57.67; H, 5.71; Cl, 14.88.

3-Hydroxy-3-phenylpentano-5-lactone (E-10)—Hydrolysis of E-9 by Procedure 1 unexpectedly resulted in a lactone, E-10. Recrystallization in ethyl acetate gave white crystals (56%), mp 133–135°; NMR ($CDCl_3$): δ 2.16 (broad, 2H, CH_2), 2.84 (s, 2H, $COCH_2$), 3.08 (s, 1H, OH, not present with D_2O), 4.44 (m, 2H, OCH_2), and 7.34 (s, 5H, aromatic) ppm.

Anal.—Calc. for $C_{11}H_{12}O_3$: C, 68.74; H, 6.29. Found: C, 68.48; H, 6.40; Cl, none.

3-Hydroxy-3-phenylpentanoic Acid (A-7)—Hydrolysis of E-7 by Procedure 1 resulted in A-7. Recrystallization from benzene gave white crystals (71%), mp 122–123° [lit. (14) mp 120–121°].

Δ^2-3-Ethoxybutenoic Acid (A-4)—Hydrolysis of E-4 by Procedure 1 resulted in A-4. Recrystallization from absolute ethanol gave white crystals (31%), which decomposed on melting at 139–141° [lit. (18) mp 140°].

3-Hydroxy-3-methylpentanoic Acid (A-1)—Hydrolysis of E-1 by Procedure 2 resulted in A-1 (52%), bp 78–81°/0.02 mm Hg [lit. (3) bp 93–95°/0.03 mm].

Δ^2-3-Methylpentenoic Acid (A-2)—Hydrolysis of E-2 by Procedure

[1] Melting points were determined on a Fisher-Johns apparatus and are uncorrected. NMR spectra were determined using a Varian A-60A spectrometer with tetramethylsilane as an internal standard. Elemental analyses were performed by Bristol Laboratories, Syracuse, N.Y.

[2] Compounds E-5, A-11, A-12, A-13, and A-14 were from Aldrich Chemical Co., and E-4 and A-6 were from Frinton Laboratory.

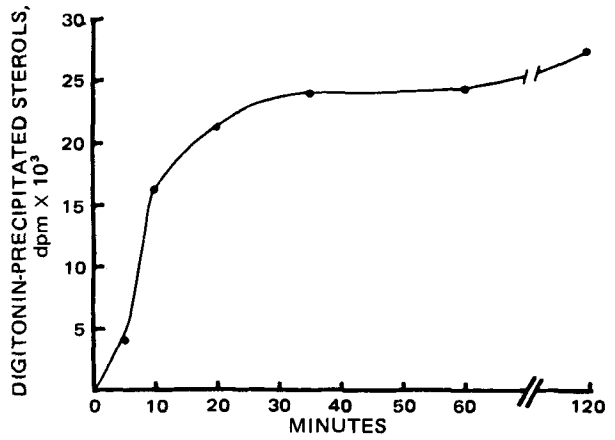


Figure 1—*Incorporation rate of [2-^{14}C]mevalonate into digitonin-precipitated sterols in rat liver homogenate. Each point represents the average of two determinations.*

2 resulted in A-2 (51%), which separated on standing into the *trans*-isomer (23%), mp 43–45° [lit. (19) mp 46–47°], and the *cis*-isomer (77%), bp 61°/0.55 mm Hg [lit. (20) bp 96°/5 mm]. Since complete separation could not be effected, a mixture of roughly equal parts of the isomers was used for testing in the homogenate system.

***trans*-Δ^3-3-Methylpentenoic Acid (A-3)**—Hydrolysis of E-3 by Procedure 2 resulted in A-3 (97%), bp 51–53°/0.015 mm Hg [lit. (13) mp 35°]; NMR: δ 1.70 [m, 6H, $(CH_3C{=})_2$], 3.06 (d, 2H, CH_2), 5.45 (m, 1H, CH=C), and 11.56 (s, 1H, COOH) ppm.

***In Vitro* Assay of Cholesterol Biosynthesis**—The rat liver homogenates from mature male Wistar rats were prepared following the general procedures described previously (21). The homogenization medium consisted of 100 m*M* potassium phosphate, 30 m*M* nicotinamide, 4 m*M* magnesium chloride, 5 m*M* glutathione, and 1 m*M* ethylenediaminetetraacetic acid at pH 7.4. The final cofactor concentration[3] in each incubation flask was 2 m*M* ATP, 1 m*M* NAD, 1 m*M* NADP, 8 m*M* glucose-6-phosphate, and 2 units of glucose-6-phosphate dehydrogenase (baker's yeast, type XV).

To each 25-ml incubation flask were added 2 ml of homogenate, 0.1 ml of cofactor solution, and 0.1 ml of solution containing the compound to be tested. The acids were added as the potassium salt in water at pH 7.4, while the esters were added in 50% ethanol. Every compound was tested in duplicate. The appropriate controls, either water or 50% ethanol, also were run in duplicate in every assay. The assay was started by the addition of 0.1 ml of water containing the potassium salt of [2-^{14}C]mevalonate (0.1 μCi, 0.0124 μmole) prepared from the dibenzylethylene ammonium salt[4]. Incubation continued for 20 min at 37° in a Dubnoff shaker at 100 oscillations/min. The incubation was terminated by the addition of 2 ml of 11% KOH (w/v) in ethanol.

The contents of each incubation flask were poured into 16 × 150-mm screw-capped tubes. Each flask was washed twice with 2-ml portions of 11% KOH (w/v) in ethanol, and the washings were added to the appropriate tube. Each sample was capped and saponified in a boiling water bath for 30 min. When the sample was cool, 7.5 ml of water and 2 ml of ethanol were added. The nonsaponifiable fraction containing the cholesterol was removed by three extractions with 9-ml portions of petroleum ether. This extraction procedure removed >99% of the cholesterol.

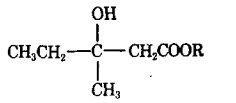
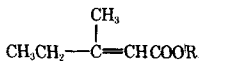
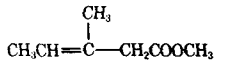
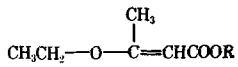
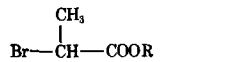
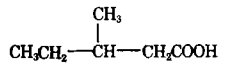
The procedure for the precipitation of cholesterol by digitonin was based on the method of Zak *et al.* (22). The digitonin precipitates were dissolved by warming in 2.0 ml of absolute methanol. A 1-ml aliquot was added to a toluene-based scintillation cocktail containing 2,5-diphenyloxazole (3.92 g/liter) and *p*-bis(*o*-methylstyryl)benzene (0.08 g/liter)[5].

RESULTS AND DISCUSSION

The incorporation rate of [2-^{14}C]mevalonate into the digitonin-precipitated sterols by rat liver homogenates is shown in Fig. 1. Although digitonin precipitates all 3-β-hydroxysterols, this is almost entirely cholesterol in the liver. The incorporation rate was rapid for the first 20 min of incubation and leveled off by 30 min. By 120 min, ~25% of the biologically available optical isomer of mevalonic acid was incorporated into cholesterol. A 20-min incubation period was chosen for testing the inhibitory compounds because this point was at the end of the period of maximal sterol synthesis.

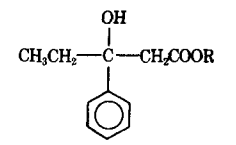
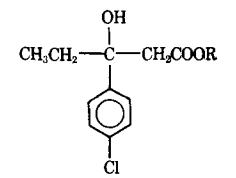
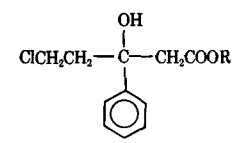
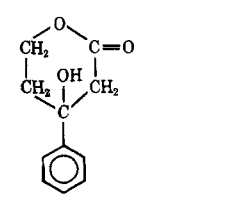
The effects of 20 mevalonic acid analogs on cholesterol biosynthesis from [2-^{14}C]mevalonate in rat liver homogenates were studied. All

Table I—Effect of Aliphatic Mevalonic Acid Analogs on the Incorporation of [2-^{14}C]Mevalonate into Cholesterol by Rat Liver Homogenates

Compound[a]	Structure	Inhibition at 1 m*M*, % Ester[b]	Acid[c]
1		9.6[d]	29.3
2		21.0	15.0
3		0	0
4		41.1	9.5
5		12.0	—
6		—	20.8

[a] In the text, compounds are identified with a number preceded by an E for an ester or an A for an acid. [b] R = $-CH_2CH_3$. [c] R = H. [d] Each number represents the average of at least two determinations.

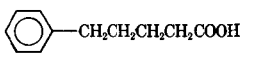
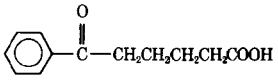
Table II—Effect of Aromatic Mevalonic Acid Analogs on the Incorporation of [2-^{14}C]Mevalonate into Cholesterol by Rat Liver Homogenates

Compound[a]	Structure	Inhibition at 1 m*M*, % Ester[b]	Acid[c]
7		7.9[d]	29.5[e]
8		44.0	11.0
9		28.2	—
10		0[f]	—

[a] In the text, compounds are identified with a number preceded by E for an ester or an A for an acid. [b] R = $-CH_2CH_3$. [c] R = H. [d] Each number represents the average of at least two determinations. [e] Percent stimulation. [f] Lactone.

[3] All cofactors were obtained from Sigma Chemical Co.
[4] New England Nuclear Corp.
[5] Radioactive samples were counted on a Packard Tri-Carb liquid scintillation spectrometer, model 2425.

Table III—Effect of 5-Substituted Mevalonic Acid Analogs on the Incorporation of [2-^{14}C]Mevalonate into Cholesterol by Rat Liver Homogenates

Compound[a]	Structure	Inhibition of Acid at 1 m*M*, %
11		92.4[b]
12		42.8
13	$ClCH_2CH_2CH_2CH_2COOH$	15.2
14	$BrCH_2CH_2CH_2CH_2COOH$	21.0

[a] In the text, compounds are identified with a number preceded by an A for acid. [b] Each number represents the average of at least two determinations.

compounds were initially tested at a concentration of 1 m*M*. Table I shows the series of analogs with a methyl group at position 3. Removal of the 5-hydroxyl group resulted in an effective inhibitor, 3-hydroxy-3-methylpentanoic acid (A-1). These data agree, in part, with those of Klimov *et al.* (23, 24), which showed that, in a series of mevalonic acid analogs with aromatic groups in position 2 or 3, the absence of a hydroxyl group in the 5-position increased the ability of the compound to inhibit cholesterol biosynthesis from [^{14}C]mevalonate in rat liver homogenates.

For bacterial and yeast assay systems, Stewart and Woolley (4, 5) concluded that the hydroxyl group at position 3 was necessary for highest antimevalonic activity. Table I shows that this conclusion also applies to the rat liver homogenate since several compounds (A-2, A-3, and A-6) that have similar structures to the active 3-hydroxyl inhibitor (A-1), but lack the 3-hydroxyl group, all showed lower activity.

Table I also shows that alterations of the backbone structure by introduction of a double bond at position 2 or 3 (A-2 or A-3) reduced inhibitory activity. These results confirm earlier work (3) that was questionable because the synthetic procedures used would not necessarily lead to the pure isomers and the structures were not proven adequately. On the other hand, Klimov *et al.* (23) found that a double bond at position 2 increased the activity compared to a saturated 3-hydroxyl analog. However, these data are not directly comparable since all compounds also contained an aromatic group at position 2 or 3.

The substitution of an aromatic group for the 3-methyl group produced inactive acids (A-7 and A-8) (Table II). In contrast, Klimov *et al.* (23) found increased activity of 3-hydroxyl compounds by adding a phenyl group to position 2 and/or a tolyl group to position 3. However, these data are not directly comparable since different aromatic groups were used.

In contrast to the acids, the ethyl esters of these 3-hydroxy-3-aromatic compounds showed considerable inhibitory activity. In general, there was little relationship between the activity of a compound as an ester and its activity as an acid. Compound E-10, resembling mevalonolactone with a phenyl ring replacing the 3-methyl group, was inactive.

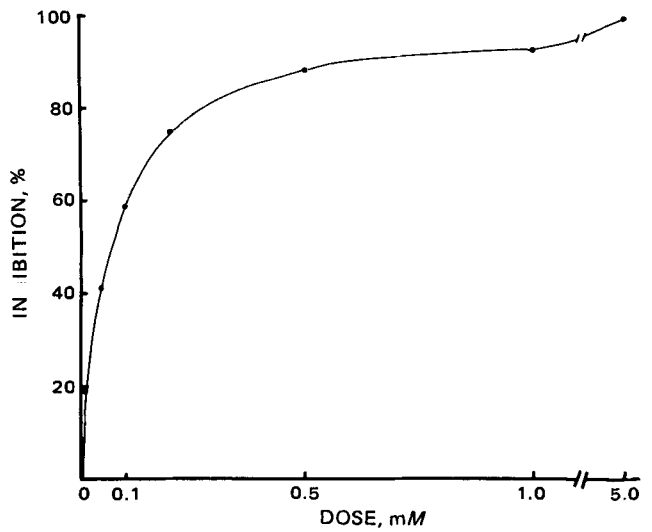


Figure 2—*Inhibition by 5-phenylpentanoic acid (A-11) of incorporation of [2-^{14}C]mevalonate into cholesterol by rat liver homogenates.*

Table III shows several compounds with an aromatic group or halide on the 5-carbon, all of which caused inhibition. The most active inhibitor was 5-phenylpentanoic acid (A-11), with 92.4% inhibition of cholesterol biosynthesis. Of the two 5-halogenated pentanoic acids, the bromo derivative was more inhibitory. Not all modifications of the 5-position yield inhibitors. Humber *et al.* (25) replaced the 5-hydroxyl group of mevalonic acid with a methoxy, ethoxy, or dimethylamino group and found that none of the compounds inhibited cholesterol biosynthesis in rat liver homogenates at concentrations of 1 m*M*.

The relationship between the dose and the effect of the most active compound, A-11, is shown in Fig. 2. There was 50% inhibition of cholesterol biosynthesis from labeled mevalonate in rat liver homogenates at a 0.064 m*M* concentration.

The results of this study show that A-11 is a very potent inhibitor of cholesterol biosynthesis from mevalonate in rat liver homogenates. Other investigators (3–6, 23, 24) tested mevalonic acid analogs that were effective inhibitors at concentrations of ≥1 m*M*. Compound A-11 showed significant inhibition of 20% at 0.01 m*M*, a 100-fold difference. The only compounds that rival its activity are 3,5-dihydroxy-3-fluoromethylpentanoic acid (26) and 3-hydroxy-3-methyl-6-phosphonohexanoic acid (7), which caused 50% inhibition of mevalonic acid metabolism at 0.07 and 0.145 m*M*, respectively.

REFERENCES

(1) E. D. Beytía and J. W. Porter, *Ann. Rev. Biochem.*, **45,** 113 (1976).

(2) J. R. Sabine, "Cholesterol," Dekker, New York, N.Y., 1977, pp. 79–104.

(3) H. Weiss, E. Schiffman, and E. Titus, *J. Lipid Res.*, **2,** 258 (1961).

(4) J. M. Stewart and D. W. Woolley, *J. Am. Chem. Soc.*, **81,** 4951 (1959).

(5) J. M. Stewart and D. W. Woolley, *Biochemistry*, **3,** 1998 (1964).

(6) F. H. Hulcher, *Arch. Biochem. Biophys.*, **146,** 422 (1971).

(7) G. Popjak, T. S. Parker, V. Sarin, B. E. Tropp, and R. Engel, *J. Am. Chem. Soc.*, **100,** 8014 (1978).

(8) S. N. Shah, N. A. Peterson, and C. M. McKean, *Biochim. Biophys. Acta*, **187,** 236 (1969).

(9) S. Ranganathan and T. Ramasarma, *J. Neurochem.*, **22,** 987 (1974).

(10) J. Edmond and G. Popjak, *J. Biol. Chem.*, **249,** 66 (1974).

(11) A. M. Fogelman, J. Edmond, and G. Popjak, *ibid.*, **250,** 1771 (1975).

(12) M. W. Rathke, *Org. React.*, **22,** 423 (1975).

(13) R. B. Wagner, *J. Am. Chem. Soc.*, **71,** 3214 (1949).

(14) J. A. Barltroop, R. M. Acheson, P. G. Philpott, K. E. McPhee, and J. S. Hunt, *J. Chem. Soc.*, **1956,** 2928.

(15) W. S. Wadsworth and W. D. Emmons, *J. Am. Chem. Soc.*, **83,** 1733 (1961).

(16) G. A. R. Kon and K. S. Nargund, *J. Chem. Soc.*, **1932,** 2461.

(17) G. A. R. Kon and R. P. Linstead, *ibid.*, **1925,** 616.

(18) M. V. S. Sultanbawa and P. Veerevagu, *ibid.*, **1958,** 4113.

(19) R. B. Wagner and J. A. Moore, *J. Am. Chem. Soc.*, **72,** 974 (1950).

(20) G. A. R. Kon, R. P. Linstead, and J. M. Wright, *J. Chem. Soc.*, **1934,** 599.

(21) N. L. R. Bucher and K. McGarnahan, *J. Biol. Chem.*, **222,** 1 (1956).

(22) B. Zak, R. C. Dickerman, E. G. White, H. Burnett, and P. J. Cherney, *Am. J. Clin. Pathol.*, **24,** 1307 (1954).

(23) A. N. Klimov, E. D. Polyakova, A. L. Remizov, and L. A. Petrova, *Biokhimiya*, **32,** 88 (1967).

(24) A. N. Klimov, T. A. Klimova, L. A. Petrova, and E. D. Polyakova, *ibid.*, **36,** 851 (1971).

(25) L. G. Humber, M. Kraml, J. Dubuc, and R. Gaudry, *J. Med. Chem.*, **6,** 210 (1963).

(26) F. M. Singer, J. P. Januszka, and A. Borman, *Proc. Soc. Exp. Biol. Med.* **102,** 370 (1959).

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