oration of the ethanol, was dissolved in 30 ml. of chloroform, cooled in ice and treated for 5 min. with a stream of dry ammonia. The precipitate was filtered, washed with chloroform, and the filtrate evaporated *in vacuo*. The oily residue was allowed to stand in a pressure bottle for 72 hours with 100 ml. of methanol saturated with ammonia gas at 0°. Evaporation of methanol gave 1.31 g. of crystalline product. To a solution of this in 4 ml. of ethanol was added 15 ml. of ether. Some ammonium chloride separated and was removed by filtration. The filtrate was evaporated and the product crystallized from 5 ml. of ether; large prisms, m.p. 57–58°. The yield was 0.6 g. (30%). After another crystallization from ether and sublimation at 0.03 mm. and 70–100°, the melting point was raised to 58–59.5°.

Anal. Calcd. for C_5H_9NO: C, 60.58; H, 9.15; N, 14.13. Found: C, 60.06; H, 9.20; N, 13.70.

URBANA, ILL.

[CONTRIBUTION FROM THE ROCKEFELLER INSTITUTE]

Antimetabolites of Mevalonic Acid[1]

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Antimetabolites of mevalonic acid (MVA) were envisioned as useful agents for suppression of sterol and other isoprenoid biosynthesis. A number of close relatives of MVA were synthesized by the Reformatsky reaction and tested for anti-MVA activity in bacteria, yeast and mice. Several active compounds were found, the best of which was 4-methyl MVA. The relationship of chemical structure to anti-MVA activity in bacteria was studied with a series of analogs.

Mevalonic acid (3,5-dihydroxy-3-methylvaleric acid, MVA)[2,3] has been identified as the common precursor in the biosynthesis of sterols[4] and other isoprenoid compounds.[5] This finding has made it possible to investigate systematically the modification of isoprenoid biosynthesis by applying the principles of antimetabolites, because antimetabolites of MVA would be expected to suppress specifically the biosynthesis of isoprenoids. From such interference, much might be learned of the effects of a specific deficiency of cholesterol and steroid hormones on the animal organism. Such studies also might well lead eventually to clinically useful substances.

An attempt has been made to test this hypothesis by examining a number of structural analogs of MVA for anti-MVA activity. The compounds prepared and tested were hydroxy acids which were homologs or position isomers of MVA, and derivatives of these. The compounds were prepared by condensation of the appropriate acetoxy ketones with ethyl bromoacetate or ethyl 2-bromopropionate by means of the Reformatsky reaction. The esters thus obtained were hydrolyzed to the desired acids. These compounds represented one of the general transformations which could be expected to yield antimetabolites.[6] Another method for making antimetabolites is the conversion of a carboxylic acid to a phenyl ketone. Therefore, a number of phenyl ketones bearing a formal resemblance to MVA also were prepared and assayed.

Since the object of this investigation was to influence MVA utilization in the intact mammalian organism, the plan was to examine the effect of the analogs on cholesterol synthesis by young mice. However, the analogs were first assayed for their ability to inhibit ergosterol synthesis in growing yeast, since the mouse assay was not well adapted to the rapid testing of a large number of compounds. Since the normal turnover of MVA is so great in systems synthesizing a large amount of sterol, it is apparent that either of these assay methods would reveal anti-MVA activity only if (A) the compound being tested were present in a relatively great amount, (B) the compound were extremely potent, or (C) the antagonism could not be reversed by the metabolite. Therefore, a preliminary assay was needed to detect anti-MVA activity in compounds of low potency, in order better to direct the synthetic work. Certain lactobacilli, although they do not synthesize sterol, do have a growth requirement for MVA. *Lactobacillus heterohiochii*[3] has an absolute requirement for MVA, and *L. acidophilus*[7] will grow on either MVA or acetate. Consequently, the analogs were examined for their ability to inhibit the growth of *L. acidophilus* in competition with MVA, by the method of Wright.[7]

While this work was in progress, two reports of anti-MVA activity appeared. Tamura, *et al.*,[8] by the use of *L. heterohiochii*, found 2-methyl- and 2-ethyl-MVA to have reversible anti-MVA activity. Wright[7] showed that the growth of *L. acidophilus* was inhibited by a number of compounds, the most active being the sesquiterpene, farnesinic acid, the action of which was overcome by MVA.

Experimental

All melting and boiling points are uncorrected. Melting points were determined in a capillary. Infrared spectra were measured with a Perkin–Elmer model 137 spectrophotometer and were determined on films for liquids and on Nujol mulls for solids. We thank Miss Edith Young and Monica Gallagher for technical assistance, and Mr. Theodore Bella for microanalyses.

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TABLE I

PRODUCTS FROM THE REFORMATSKY REACTION

	Compound	Yield, %	B.p., °C. (μ)	Analyses, % Calcd. C	Calcd. H	Found C	Found H	Infrared bands, μ OH	C=O	C=C
I	Ethyl 5-acetoxy-3-hydroxy-3,4-dimethylvalerate[a]	49	80–82 (100[b]) 90–92 (100)	56.9	8.7	57.3	8.5	2.8	5.75	
IV	Ethyl 5-acetoxy-3-hydroxy-2,3,4-trimethylvalerate[c]	48	76 (100)	58.5	9.0	58.3	8.8	2.8	5.75	
VI	*cis*-5-Hydroxy-3,5-dimethyl-2-hexenoic acid lactone[d]	47	50 (100)	68.5	8.6	68.3	8.6[e]		5.87	6.1
VIII	Ethyl 4-acetoxy-3-hydroxy-3-methylvalerate[f]	66	56 (50)	55.0	8.3	55.4	8.2	2.87	5.6, 5.78	
XIV	Ethyl 4-acetoxy-3-hydroxy-3-methylbutyrate[g]	33	68–70 (5)	52.9	7.9	53.0	8.0	2.88	5.6, 5.77	
XVI	Ethyl 4-acetoxy-3-hydroxy-2,3-dimethylbutyrate[h]	53	55–58 (100)	55.0	8.3	55.6	7.9	2.81	5.6, 5.74	
XIX	Ethyl 4-acetoxy-2-benzyl-3-hydroxy-3-methylbutyrate[i]	45	120 (4)	65.3	7.5	64.3	6.4[j]	2.8	5.6, 5.75	

[a] Prepared from 1-acetoxy-2-methyl-3-butanone[9] and ethyl bromoacetate. [b] Two isomers obtained in equal yield. Analysis and infrared identical. [c] Prepared from 1-acetoxy-2-methyl-3-butanone[9] and ethyl 2-bromopropionate. [d] This lactone was formed directly in the reaction of 2-acetoxy-2-methyl-4-pentanone[10] with ethyl bromoacetate. [e] Saponification value: calcd., 140.2; found, 139.8. [f] Prepared from 2-acetoxy-3-butanone[11] and ethyl bromoacetate. [g] Prepared from acetoxy acetone and ethyl bromoacetate. [h] Prepared from acetoxyacetone and ethyl 2-bromopropionate. [i] Prepared from acetoxy acetone and ethyl 2-bromo-3-phenylpropionate.[15,16] By-product ethyl hydrocinnamate obtained in 20% yield. [j] Although not pure, both the unsaturated and hydroxy lactones could be obtained from this ester upon saponification.

Acetylation of Hydroxy Ketones.—Hydroxy ketones were acetylated by the procedure of Hoffman, *et al.*,[2] except that the temperature was kept below 35° during the acetylation, and the catalyst was neutralized with barium carbonate before distillation of the product *in vacuo* through a Vigreux column. In those cases where the boiling point of the acetoxy ketone was too close to that of acetic anhydride to allow convenient separation, the product was allowed to stand overnight with excess absolute ethanol before distillation.

Preparation of Hydroxy Esters by the Reformatsky Reaction.—The procedure of Hoffman, *et al.*,[2] was used, and the products were distilled *in vacuo* through a Vigreux column. The following compounds and those of Table I were prepared by this method:

Ethyl 4-Acetoxy-3-hydroxy-3-methyl-4-phenylvalerate (X).—2-Acetoxy-2-phenyl-3-butanone[12,13] reacted with ethyl bromoacetate to give a product boiling at 72–80° (0.001 mm.). Both infrared and elemental analysis showed that this was a mixture of the hydroxy and unsaturated esters. Infrared bands: OH, 2.85; C=O, 5.6, 5.72; C=C, 6.1 μ.

Ethyl 4-Acetoxy-3,4-dimethyl-2-pentenoate (XII).—Condensation of 2-acetoxy-2-methyl-3-butanone[12,14] and ethyl bromoacetate gave a mixture of the desired hydroxy ester and the corresponding unsaturated ester, as shown by infrared and analysis. Although neither the hydroxy nor the unsaturated ester was obtained analytically pure, hydrolysis of the crude ester gave pure unsaturated lactone (XIII). Upon distillation of the ester (b.p. 68° (0.1 mm.)), the last fractions crystallized in the receiver, and were recrystallized from ether–hexane; m.p. 49–51°. *Anal.* Found: C, 57.9; H, 7.5.

Diethyl 3,5-Dihydroxy-3,5-dimethylsuberate.—The condensation of 1 mole of 2,5-hexanedione with 2 moles of ethyl bromoacetate gave as a product a viscous oil, which could not be distilled without decomposition even at a pressure of 5 μ. During the reaction, the zinc salt precipitated so extensively that 500 ml. of benzene was added to facilitate stirring. The crude ester was saponified without further purification.

Saponification of Esters.—Both the 3-hydroxylactones and the corresponding unsaturated lactones were formed by saponification of the esters of Table I with sodium hydroxide at 60°. Usually dehydration to the unsaturated lactone could not be entirely prevented, even by saponification at room temperature, although the percentage of unsaturated lactone was lower under those conditions. In all of the products bearing a 4-hydroxyl group, the unsaturated lactone (crotonolactones) formed in alkaline solution during the saponification, and could be extracted into ether free of hydroxylactone, except in the case of XI, where some hydroxylactone also was extracted. This cyclization in alkaline solution did not occur with compounds having the hydroxyl group in position 5 instead of 4. In both cases, the subsequent acidification of the hydrolysate and extraction with ethyl acetate yielded a mixture of hydroxy and unsaturated lactones. Isolation of the hydroxy lactone free of the unsaturated derivative frequently was difficult, and with some of the compounds it was only accomplished by countercurrent distribution.

Two general procedures for saponification were followed.

Procedure A.—A solution of 0.25 mole of the acetoxy ester from the Reformatsky reaction in 300 ml. of 80% ethanol was stirred at 60° while a solution of 23 g. of sodium hydroxide in 75 ml. of water was added at such a rate as just to keep the solution strongly alkaline. After the addition was complete, stirring was continued for 30 min. The ethanol was evaporated under reduced pressure below 30°, and the residual solution diluted with water and extracted three times with ether. The 4-crotonolactones were recovered from this ether extract. The aqueous solution was acidified with hydrochloric acid and extracted four times with ethyl acetate. The ethyl acetate was washed with water, dried with magnesium sulfate and evaporated below 30°. In some cases much additional product could be obtained by evaporating the aqueous phase to dryness *in vacuo*, redissolving the residue in water and evaporating several times to remove hydrogen chloride and extracting the residual salt with ethyl acetate.

Procedure B.—The saponification was done in aqueous ethanol at room temperature for 24 hours, using 40 g. of sodium hydroxide for each 0.25 mole of acetoxy ester. The product was worked up as above.

The acids and lactones prepared by these procedures are described in Table II, with these explanatory comments:

3-Hydroxy-3,4-dimethyl-5-valerolactone (II) and *cis*-5-Hydroxy-3,4-dimethyl-2-pentenoic Acid Lactone (III).—Separation of these two lactones produced by saponification of ester I was not practical by distillation, but a 50-tube countercurrent distribution in an ether–water system gave an effective purification (hydroxylactone centered in tube 8, unsaturated lactone centered in tube 24). The material from the tubes containing the hydroxylactone was combined, the solvent evaporated *in vacuo* below 30°, and the liquid lactone analyzed directly. This compound has been prepared by another method by Tamura.[17] The unsaturated lactone, also a liquid, was further purified by distillation.

4-Hydroxy-3-methyl-4-phenyl-2-pentenoic Acid and Lactone (XI).—Saponification of ester X by procedure A gave in the ether extract of the alkaline hydrolysate a 75% yield of an oil shown by its infrared spectrum to be a mixture of the hydroxy and unsaturated lactones (OH, 2.86; C=O, 5.7, 5.82; C=C, 6.1 μ). Distillation did not separate the mixture. The analysis found was intermediate between the values calculated for the hydroxy and unsaturated lactones.

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TABLE II

ACIDS AND LACTONES

	Compound	Prepared from	Method	Yield, %	B.p., °C. (μ)	M.p., °C.	Analyses, % Calcd. C	Calcd. H	Found C	Found H	Infrared bands, μ OH	C=O	C=C
II	3-Hydroxy-3,4-dimethyl-5-valerolactone	I	B	50[a]	[b]	....	58.3	8.4	58.4	8.4	2.86	5.8	[c]
III	*cis*-5-Hydroxy-3,4-dimethyl-2-pentenoic acid lactone	I	A	75[d]	34 (3)	....	66.6	8.0	66.3	8.0	..	5.80	6.08[e]
V	3-Hydroxy-2,3,4-trimethyl-5-valerolactone	IV	B	99	75 (70)	....	60.7	8.9	60.9	8.8	2.85	5.80	..
VI	*cis*-5 Hydroxy-3,5-dimethyl-2-hexenoic acid lactone	See Table I			....	....							
VII	*cis*-5-Hydroxy-3,5-dimethyl-2-hexenoic acid	VI	A[f]	99	....	96–98[g]	60.7	8.9	60.3	8.8[h]	3.1,3.9	6.0	6.1
IX	3-Hydroxy-3-methyl-4-valerolactone	VIII	A	77	93–96 (100)	....	55.4	7.8	55.6	7.8	3.2[i]	5.85	..
XIII	*cis*-4-Hydroxy-3,4-dimethyl-2-pentenoic acid lactone	XII	A[j]	99	....	44.5–46.5[k]	66.6	8.0	66.2	8.0	..	5.75	6.1
XV	3-Hydroxy-3-methyl-4-butyrolactone	XIV	A	98	90 (1)	....	51.7	6.9	51.4	7.0	2.95	5.70	..
XVII	3-Hydroxy-2,3-dimethyl-4-butyrolactone	XVI	B	80[m]	....	....	55.4	7.8	55.2	7.8	2.85	5.70	..
XVIII	*cis*-2,3-Dimethyl-4-crotonolactone	XVI	A[j]	50[n]	....	36–38[k]	64.3	7.2	64.3	7.3	..	5.70	5.94
XX	2-Benzyl-3-hydroxy-3-methyl-4-butyrolactone	XIX	B	40[p]	75 (60)	....	69.8	6.8	69.5	6.9	2.85	5.68	..
XXI	*cis*-2-Benzyl-3-methyl-4-crotonolactone	XIX	A[j]	30[q]	113 (2)	....	76.6	6.4	76.2	6.4[r]	..	5.70	5.94
XXX	3,5-Dihydroxy-3,5-dimethylsuberic acid		A	15[s]	....	167–168.5[t]	51.3	7.8	51.1	7.8[u]	2.8,3.75	5.84	..

[a] A 45% yield of III also was isolated. [b] Not distilled. Tamura (ref. 17) found b.p. 133–137° (2 mm.). [c] Other bands: 9.52, 9.7, 10.0, 10.47, 10.7, 11.37 μ. [d] A 20% yield of II also was isolated. [e] Other bands: 9.48, 10.1, 11.6 μ. [f] Saponification was abnormally slow. [g] Recrystallized from isopropyl ether. [h] Neutralization equivalent: calcd. 158.2, found 159. [i] Broad. [j] This lactone formed in alkaline solution and was extracted into ether. [k] Recrystallized from ether–hexane. [m] A 10% yield of XVIII also isolated. Compounds XVII and XVIII were separated quantitatively by the alkaline ether extraction procedure. [n] A 40% yield of XVII also isolated. [p] A 55% yield of XXI also isolated. [q] From crude ester. [r] Benzylisothiouronium salt, m.p. 149° (from ethyl acetate–alcohol). *Anal.* Calcd. for $C_{20}H_{24}N_2O_3S$: C, 64.5; H, 6.5; N, 7.5. Found: C, 64.4; H, 6.4; N, 7.6. [s] Over-all from ketone. [t] Recrystallized from tetrahydrofuran. [u] Neutralization equivalent; calcd. 117.1, found 117.

The ethyl acetate extract of the acidified aqueous hydrolysate gave upon evaporation a 25% yield of a viscous oil, shown by infrared to be a mixture of the hydroxy and unsaturated acids, rather than lactones. This fraction was not further purified.

2-Benzyl-3-hydroxy-3-methyl-4-butyrolactone (XX).—The saponification of ester XIX by procedure B gave in the ether extract of the alkaline solution a 30% yield of the unsaturated lactone XXI, and in the ethyl acetate extract of the acidified solution a 60% yield of the hydroxylactone, somewhat contaminated with XXI. The hydroxylactone was purified by a 150-tube countercurrent distribution in the system ethyl acetate:hexane:ethanol:water (1:1:1:1). Although this distribution did not quite give complete separation of the components (hydroxylactone peak, tube 70; unsaturated lactone peak, tube 85), pure hydroxylactone could be obtained.

3,5-Dihydroxy-3,4-dimethylvaleramide (XXII) (Amide of 4-Methyl-MVA).—A solution of 2.4 g. of 3-hydroxy-3,4-dimethyl-5-valerolactone (II) in 20 ml. of liquid ammonia was kept at room temperature in a bomb for four days. Evaporation of the ammonia *in vacuo* left a product shown by nitrogen analysis to be 85% amide. When the reaction was allowed to proceed for seven days, the yield was quantitative. Mixtures of the amide and lactone were separated by a 60-tube countercurrent distribution in the system, ethyl acetate: *n*-butanol:water (1:1:2). The amide peak was centered in tube 15, and the lactone in tube 30. The material from the tubes containing amide was evaporated and the product, which was a gum, dried over phosphorus pentoxide at room temperature. Upon standing at room temperature, it eliminated ammonia and reverted to the lactone, and a sample stored at −20° had a strong odor of ammonia after six days.

Anal. Calcd. for $C_7H_{15}NO_3$: C, 52.2; H, 9.4; N, 8.7. Found: C, 51.9; H, 9.0; N, 8.9. Infrared: OH, NH, 2.93, 3.05; C=O, 6.0; NH_2, 6.2 μ.

3,4-Dihydroxy-3-methylbutyramide (XXVI).—The amide from 3-hydroxy-3-methyl-4-butyrolactone was prepared by the above procedure and purified by a 50-tube countercurrent distribution in ethyl acetate–water. The amide did not move, while the lactone was centered in tube 14. The product was a gum, and decomposed upon standing at room temperature.

Anal. Calcd. for $C_5H_{11}NO_3$: C, 45.1; H, 8.3; N, 10.5. Found: C, 46.0; H, 8.3; N, 10.8. Infrared: OH and NH, 3.0; C=O, 6.0; NH_2, 6.2 μ.

3,4-Dihydroxy-3-methylbutyranilide (XXVII).—A solution of 4.4 g. of 3-hydroxy-3-methylbutyrolactone in 15 ml. of dry dioxane was added to a cold ethereal solution of anilinomagnesium bromide[18] prepared from 8.8 g. of ethyl bromide, 2.0 g. of magnesium turnings and 7.45 g. of aniline. After refluxing for one hour the solution was poured into ice–dilute hydrochloric acid, and the product was removed by five extractions with ethyl acetate. The ethyl acetate solution was washed with water, 5% sodium hydroxide solution, water, and dried over magnesium sulfate. Evaporation *in vacuo* left 3.6 g. of a brown oil which was subjected to a 50-tube countercurrent distribution in the system ethyl acetate:hexane:water (2:1:3). Lactone (tube 6) and anilide (tube 19) peaks were separated, but both the analysis and the infrared spectrum indicated that the anilide was still contaminated with another compound, most likely the corresponding unsaturated lactone.

Anal. Calcd. for $C_{11}H_{15}NO_3$: C, 60.9; H, 7.7; N, 7.1. Found: C, 60.2; H, 6.9; N, 3.7. Infrared: OH, NH, 2.8, 2.95; C=O, 5.72, 6.0; C=C, 6.1; amide NH, 6.47 μ.

3,5-Dihydroxy-3,4-dimethylvaleranilide (XXIII).—3-Hydroxy-3,4-dimethyl-5-valerolactone was converted to the anilide with anilinomagnesium bromide by the same procedure, except that the lactone was added in ether. A 50-tube countercurrent distribution in the system benzene:water:ethanol (10:10:1) separated the anilide (peak in tube 12) from the unreacted lactone (peak in tube 3). The anilide, a gum, was obtained in 15% yield.

Anal. Calcd. for $C_{12}H_{19}NO_3$: C, 65.8; H, 8.1; N, 5.9. Found: C, 65.3; H, 7.9; N, 5.7. Infrared: OH, NH, 2.85, 3.0; C=O, 6.0; NH, 6.44 μ.

3,5-Dihydroxy-3,4-dimethylvaler-*p*-chloroanilide (XXIV) was prepared from 3-hydroxy-3,4-dimethyl-5-valerolactone

(18) D. V. N. Hardy, *J. Chem. Soc.*, 398 (1936).

and *p*-chloroanilinomagnesium bromide by the same procedure described above and was purified by a 50-tube countercurrent distribution in the system ethyl acetate:hexane:ethanol:water (1:2:1:3) (anilide peak was in tube 32). This anilide was also a gum.

Anal. Calcd. for $C_{13}H_{18}ClNO_3$: C, 57.5; H, 6.7; N, 5.2. Found: C, 56.8; H, 6.9; N, 5.0. Infrared: OH and NH, 3.0; C=O, 6.0; NH, 6.5 μ.

3,4-Dihydroxy-3-methylbutyr-*p*-chloroanilide (XXVIII) was prepared in the same manner, but not purified.

Hydrazide of 3,5-Dihydroxy-3,4-dimethylvaleric Acid (XXV).—To 1.44 g. of 3-hydroxy-3,4-dimethyl-5-valerolactone (II) was added a solution of 1.0 g. of hydrazine hydrate in 10 ml. of ethanol. After standing 24 hours at room temperature, the solution was evaporated under reduced pressure below 35°, and the residue was twice redissolved in ethanol and evaporated. The hydrazide was a highly viscous oil and was pure directly.

Anal. Calcd. for $C_7H_{16}N_2O_3$: C, 47.7; H, 9.2; N, 15.9. Found: C, 47.3; H, 9.5; N, 16.1. Infrared: bands at 2.88, 3.1, 6.02, 6.2, 6.55 μ.

The hydrazide of 3,4-dihydroxy-3-methylbutyric acid (XXIX) was prepared in the same manner from 3-hydroxy-3-methyl-4-butyrolactone and was not purified.

Hydroxyalkylphenones and Precursors.—5′-Hydroxycaprophenone, 5′-hydroxyvalerophenone, 2-methyl-6-phenyl-2,3-dihydropyran-5-carboxylic acid (XXXI) and 6-phenyl-2,3-dihydropyran-5-carboxylic acid were prepared by the method of Perkin.[19] Similarly, 4′-hydroxybutyrophenone, which previously has been prepared by a different method,[20] was prepared from 5-phenyl-2,3-dihydrofuran-4-carboxylic acid by the Perkin method.

5-Phenyl-2,3-dihydrofuran-4-carboxylic acid (XXXII) was prepared from ethyl benzoylacetate and 1,2-dibromoethane by the same method[19] and recrystallized from benzene–hexane; m.p. 151–144° dec., yield 20%.

Anal. Calcd. for $C_{11}H_{10}O_3$: C, 69.5; H, 5.3. Found: C, 69.2; H, 5.5 Infrared: acid OH, 3.8; C=O, 5.98 μ.

Lactobacillus Assay.—Anti-MVA activity for the inhibition of growth of *Lactobacillus acidophilus* ATCC 4963 was measured according to the procedure of Wright,[7] except that the 24-hr. milk inoculum was diluted 1:500 in saline, and the assay tubes were incubated for 40 hr. at 37°. All compounds were tested in the presence of 0.05 μg./ml. of DL-mevalonic acid.[21] Those which inhibited growth were retested with higher concentrations of MVA in order to determine whether or not the inhibition was reversible and competitive.[6] In order to avoid dehydration during sterilization (which was found to occur with compounds such as XVII when autoclaved in the basal medium) neutralized solutions of all compounds were sterilized by filtration and added to sterile basal medium. Results are shown in Table III. The abbreviated data from a typical assay of 4-methyl-MVA (II) are shown in Table IV. In addition to compounds listed in Table III, all substances synthesized, with the exception of some of the acetoxy esters of Table I, were similarly bioassayed and found inactive. Inactive compounds showed no inhibition of growth at 5 mg./ml.

Yeast Assay.—*Saccharomyces cerevisiae* was grown on a synthetic medium similar to that of Woolley and White.[22] The cells were harvested, and the ergosterol was extracted and determined by both the Liebermann–Burchard[23,24] method and by direct ultraviolet absorption at 282 mμ. All compounds shown in Table III except V, XXIII, XXIV, XXV, XXVIII, XXIX, 3-methoxybutyric acid and 5′-hydroxyvalerophenone, were thus tested for ability to inhibit ergosterol synthesis. Because it is known that several organic acids fail to penetrate into yeast, whereas the corresponding esters do, all of the acetoxy esters shown in Table I were tested. The following additional compounds were also assayed: butyrophenone, isobutyrophenone, isovalerophenone, caprophenone, 5′-hydroxycaprophenone, 2-methyl-6-phenyl-2,3-dihydropyran-5-carboxylic acid, ethyl 6-phenyl-2,3-dihydropyran-5-carboxylate and 5-phenyl-2,3-dihydro-

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TABLE III

ANTI-MVA ACTIVITY OF ANALOGS FOR *L. Acidophilus*

Compound	Inhib. concn. mg./ml.[a]	Inhibition index[b]	Type of inhibition[c]
3-Methyl-5-valerolactones			
II (4-methyl-MVA)	0.03	1,200[d]	R, C
III (unsat. from II)	0.5	20,000	
V (2,4-dimethyl-MVA)	0.2	8,000	R, C
VI (unsat. from V)	4.0	160,000	
3-Methyl-4-valerolactones			
IX (iso-MVA)	2.5	100,000	Never complete
XI, XIII	Inactive		
3-Methyl-4-butyrolactones			
XVII	>2	>80,000	
XX	5		
XV, XVIII, XXI	Inactive		
Derivatives of 4-methyl-MVA (II)			
XXII Amide	0.15	60,000	R, C
XXIII Anilide	3.0	120,000	Never complete
XXIV *p*-Chloroanilide	0.2	8,000	R
XXV Hydrazide	0.3	12,000	R, C
Derivatives of 3,4-dihydroxy-3-methylbutyric acid (XV)			
XXVI Amide	3.0	120,000	R, C
XXVII, XXVIII, XXIX	Inactive		
Miscellaneous compounds			
2-Phenylbutyric acid	1.0		Partially R, NC
3-Methoxybutyric acid	5.0		IR
n-Valerophenone	0.3		IR
5′-Hydroxyvalerophenone	>3		IR
XXXI	0.2		IR
XXXII	1.5		IR

[a] The concentration of antimetabolite which gave half maximal inhibition of growth in the presence of 0.05 μg./ml. of MVA. [b] The amount of antimetabolite needed to neutralize a unit weight of MVA. These amounts determined from point of half maximal inhibition. [c] R, reversible; IR, irreversible; C, competitive; NC, non-competitive. [d] Lactone from high b.p. ester. The other lactone was only half as active.

TABLE IV

GROWTH OF *L. Acidophilus* IN PRESENCE OF VARIOUS CONCENTRATIONS OF MVA AND 4-METHYL-MVA (II)[a]

MVA, μg./ml.	4-Methyl-MVA, μg./ml.	Transmission,[b] %
0.0	0	100
.05	0	69
.05	10	74
.05	20	82
.05	50	88
.05	100	94
.05	500	99
.5	0	55
.5	100	57
.5	500	79

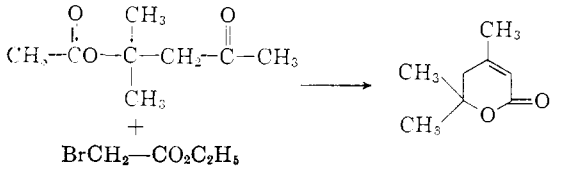
[a] In order to conserve space, all of the values are not shown. [b] Growth expressed as per cent. of incident light transmitted by the culture in comparison with uninoculated basal medium.

furan-4-carboxylic acid. Each compound was tried at several levels up to 2 mg./ml. No compound produced significant inhibition of ergosterol synthesis.

Mouse Assay.—Weanling Swiss mice were fed a fat- and sterol-free, but otherwise adequate, synthetic diet. The compounds to be tested were added to the diet, and in separate experiments were injected daily. When the mice had approximately doubled their original weight, the entire carcass was assayed for sterol by the Liebermann–Burchard method.[23] Four compounds, 2-phenylbutyric acid and compounds II, XV and XXX were thus examined up to dose levels which gave signs of toxicity. No inhibition of sterol formation was found.

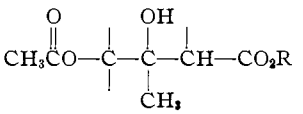
Discussion

Synthesis.—Most of the Reformatsky condensations of methyl ketones with ethyl bromoacetate proceeded normally to give the expected 3-hydroxy esters. However, the condensations of 2-acetoxy-2-methyl-3-butanone and 2-acetoxy-2-phenyl-3-butanone gave products which were partially dehydrated, and the condensation of 2-acetoxy-2-methyl-4-pentanone was especially remarkable in that the product was not only dehydrated, but it had also lost the elements of ethyl acetate to yield directly the lactone of 5-hydroxy-3,5-dimethyl-2-hexenoic acid (VI). This lactone was hydrolyzed



only slowly by aqueous-alcoholic sodium hydroxide at 60° (procedure A of the experimental), and while the free acid VII could be isolated, it lactonized slowly at room temperature. The remarkable stability to alkali of several of the lactones was noteworthy. Some of the butyrolactones formed even in alkaline solution.

The infrared spectra were of great help for indicating whether or not dehydration had occurred, either during the Reformatsky reaction or during saponification of the esters. All of the 4-acetoxy-3-hydroxy-3-methyl esters having the structure



had two carbonyl bands in their infrared spectra. The normal ester band was present at 5.72–5.78 μ, while an additional band was located at 5.6 μ. All of the 5-acetoxy-3-hydroxy-3-methyl esters showed only the normal carbonyl absorption. Other known 1,2-glycol monoacetates, *e.g.*, the pregnane- and allopregnane-triol diacetates,[25] do not show a displacement of the carbonyl absorption, and the reason for the shift in the present case is not known.

The stereoisomerism of the products obtained was not investigated. Although most of the compounds contain two or more asymmetric carbon atoms, in only one case, that of ethyl 5-acetoxy-3-hydroxy-3,4-dimethylvalerate (I), was there evidence for the separation of two diastereomers. In that case, two esters were obtained differing in boiling point and in biological activity of the derived lactones. These two esters (and their lactones) had identical infrared spectra.

Bioassay.—Among the compounds tested, the greatest anti-MVA activity was shown by those hydroxy acids having structures nearest to that of MVA. The most active, 3,5-dihydroxy-3,4-dimethylvaleric acid (4-methyl-MVA, II), differed from MVA only by addition of a methyl group at position 4. When another methyl group was added at position 2 (3,5-dihydroxy-2,3,4-trimethylvaleric acid (V)), the activity was lowered sevenfold. It is of interest to compare these results with those of Tamura, *et al.*,[8] who prepared and tested several substituted mevalonic acids. In his assay with *L. heterohiochii*, only 2-methyl- and 2-ethyl-MVA were found to have anti-MVA activity, whereas 4-methyl-MVA was inactive.

In our work, movement of the terminal hydroxyl from position 5 to 4 gave an antimetabolite IX, but with only 1/80 of the activity of 4-methyl-MVA. Other 4-hydroxy acids (the series of 3,4-dihydroxy-3-methylbutyric acids) were less active than the 5-hydroxy acids. While the parent compound of this series (XV) was totally inactive, addition of a 2-methyl group gave a compound XVII that was somewhat active. Replacement of this methyl by a benzyl radical gave a less active compound. All of the results suggested that the molecular size of the analog in comparison with that of MVA was important for activity.

In all of the acids tested, the hydroxyl group in position 3 was necessary for highest anti-MVA activity. This was best seen in 4-methyl-MVA, where dehydration to III lowered the activity by a factor of 17, and in 3,4-dihydroxy-2,3-dimethylbutyric acid (XVII), where dehydration led to complete loss of activity. Cornforth, *et al.*,[26] found that the metabolite activity of MVA for sterol synthesis was destroyed by dehydration.

Conversion of lactones to the corresponding amides occasionally led to enhanced activity. Thus, the inactive XV was made active by conversion to the amide XXVI. Substitution on the amide nitrogen seemed to depress, rather than to enhance, potency (*cf.* compounds XXIII, XXIV, XXV, XXVII, XXVIII and XXIX).

It is doubtful that the growth inhibitory activity of the phenyl ketones was due to interference with MVA. The inhibition was not overcome by MVA and furthermore the ketones did not interfere with ergosterol synthesis in yeast even at toxic concentrations.

The results with 2-phenylbutyric acid may be of interest, since this compound has been shown to inhibit cholesterol synthesis from MVA in liver homogenates[27] and to reduce serum cholesterol in hypercholesteremic patients.[28] Although it was a weak growth inhibitor for *L. acidophilus*, as

(25) G. Roberts, B. S. Gallagher and R. N. Jones, "Infrared Absorption Spectra of the Steroids," Vol. II, Interscience Publishers, Inc., New York, N. Y., 1958.

(26) J. W. Cornforth, R. H. Cornforth, G. Popjac and I. Y. Gore, *Biochem. J.*, **69**, 146 (1958).

(27) P. A. Tavormina and M. Gibbs, THIS JOURNAL, **79**, 758 (1957).

(28) J. Cottet, A. Mathivat and J. Redel, *Presse. med.*, **62**, 939 (1954).

reported by Wright,[7] its toxicity was antagonized by MVA only partially. Furthermore, it did not inhibit ergosterol synthesis in yeast, or cholesterol synthesis in growing mice, even at toxic concentrations.

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[CONTRIBUTION FROM THE LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID CO.]

16-Hydroxylated Steroids. X.[1a] The Synthesis of 21-Deoxytriamcinolone

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9α-Fluoro-11β,16α,17α-trihydroxy-4-pregnene-3,20-dione (XIId) was obtained by multi-stage syntheses starting from 4,9(11),16-pregnatriene-3,20-dione (VIa) and also from 16α,17α-epoxy-4,9(11)-pregnadiene-3,20-dione (X). Microbiological dehydrogenation of compound XIId gave 21-deoxytriamcinolone (Ib). Reaction of ethyl oxalate with 9α-fluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-4-pregnene-3,20-dione (XVIb) gave a product which was converted into 9α-fluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-1,4-pregnadiene-3,20-dione (XXI) and 9α-fluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-4,6-pregnadiene-3,20-dione (XXII).

It has been shown[2a] that triamcinolone, 9α-fluoro-11β,16α,17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione (Ia),[3] has high glucocorticoid activity and exhibits no sodium-retaining properties. Because of this, it was decided to prepare 21-deoxytriamcinolone, 9α-fluoro-11β,16α,17α-trihydroxy-1,4-pregnadiene-3,20-dione (Ib), in order to investigate its possible biological activity.

The most obvious route paralleled that used in the preparation[2a,b,4] of compound Ia and involved the preparation of 4,9(11),16-pregnatriene-3,20-dione (VIa), a compound reported previously by Szpilfogel and Gerris.[5] The desired intermediate VIa was synthesized by four methods. In the first, cortisone 21-mesylate (IIa) was converted into 21-deoxycortisone (IIb)[6] by treatment with sodium iodide in acetic acid, and the bis-ethylene ketal III then was reduced with sodium borohydride in ethanolic sodium hydroxide[7] to give 3,20-bis-ethylenedioxy-5-pregnene-11β,17α-diol (IV). Reaction of the bis-ethylene ketal IV with thionyl chloride–pyridine,[4] followed by acid hydrolysis without characterization of the intermediate V, gave the triene VIa.

In the second, pregnane-3,11,20-trione was converted into 4,16-pregnadiene-3,11,20-trione (VII) by the method of Magerlein, Lyttle and Levin.[8] The triene VIa was obtained readily from the compound VII by a process similar to that outlined above. Thus, reduction of 3,20-bis-ethylenedioxy-5,16-pregnadien-11-one (VIII) gave 3,20-bis-ethylenedioxy-5,16-pregnadien-11β-ol (IX) which, on treatment with thionyl chloride followed by acid hydrolysis, gave the compound VIa. The intermediate 3,20-bis-ethylenedioxy-5,9(11),-16-pregnatriene (V) was not characterized.

In the third, the 21-mesyl derivative VIb of 21-hydroxy-4,9(11),16-pregnatriene-3,20-dione[4] was transformed into the triene VIa using sodium iodide in acetic acid.

In the last method, 16α,17α-epoxy-4,9(11)-pregnadiene-3,20-dione (X), prepared[9,10] from 16α,-17α-epoxy-11α-hydroxy-4-pregnene-3,20-dione[11] by formation of the 11α-mesyl derivative and then elimination of the elements of methanesulfonic acid using sodium acetate in acetic acid, was treated with chromous chloride[12] to give the triene VIa.

Oxidation of the triene VIa with osmium tetroxide in benzene and pyridine gave 16α,17α-dihydroxy-4,9(11)-pregnadiene-3,20-dione (XIa) in excellent yield. The acetate XIb formed 16α-acetoxy-9α-bromo-11β,17α-dihydroxy-4-pregnene-3,20-dione (XIIa) on treatment[13] with N-bromosuccinimide in aqueous dioxane containing perchloric acid. 16α-Acetoxy-9α-chloro-11β,17α-dihydroxy-4-pregnene-3,20-dione (XIIb) was obtained using 1,3-dichloro-5,5-dimethylhydantoin[14] under the same conditions. Attempts to convert the bromohydrin XIIa into 16α-acetoxy-9β,11β-epoxy-17α-hydroxy-4-pregnene-3,20-dione (XIII) using anhydrous potassium acetate in ethanol under reflux[2a,13] failed, probably due to the sensitivity of the D-ring side-chain toward base.[15–18]

(1) (a) Paper IX, S. Bernstein and R. Littell, *J. Org. Chem.*, **24**, in press (1959); (b) Organic Chemical Research Section; (c) Biochemical Research Section.

(2) (a) S. Bernstein, R. H. Lenhard, W. S. Allen, M. Heller, R. Littell, S. M. Stolar, L. I. Feldman and R. H. Blank, THIS JOURNAL, **78**, 5693 (1956); *idem*, *ibid.*, **81**, 1689 (1959); S. Bernstein, *Rec. Prog. Hormone Res.*, **14**, 1 (1958); (b) R. W. Thoma, J. Fried, S. Bonnano and P. Grabowich, THIS JOURNAL, **79**, 4818 (1957), synthesized triamcinolone by a microbiological procedure starting from 9α-fluorohydrocortisone and 9α-fluoroprednisolone.

(3) The American Cyanamid Co.'s trade name for this compound is Aristocort.

(4) W. S. Allen and S. Bernstein, THIS JOURNAL, **77**, 1028 (1955).

(5) S. A. Szpilfogel and V. Gerris, *Rec. trav. chim.*, **74**, 1462 (1955).

(6) After completion of this work, A. Bowers and H. J. Ringold, THIS JOURNAL, **80**, 3091 (1958), described a preparation of 21-deoxycortisone from cortisone.

(7) The conditions (sodium borohydride in tetrahydrofuran containing aqueous sodium hydroxide) used by W. S. Allen, S. Bernstein and R. Littell, *ibid.*, **76**, 6116 (1954), to reduce the bis-ethylene ketal of cortisone to that of hydrocortisone failed with compound III.

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(17) G. Cooley, B. Ellis, F. Hartley and V. Petrow, *J. Chem. Soc.*, 4377 (1955).

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