from inadequate ranges of π, σ, and E_R values. One should be careful to avoid the use of only those substituents which lie on or near a straight line in Figures 1–3; *i.e.*, those which are highly correlated.

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Inhibitors and Stimulators of Cholesterolgenesis Enzymes. A Structure–Activity Study *in Vitro* of Amino and Selected N-Containing Analogs of 5α-Cholestane-3β,5α,6β-triol[1a-c]

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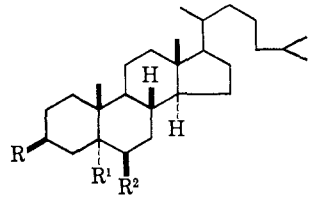
The stereoselective synthesis and biological evaluation *in vitro* of the 3β-, 3α-, 5α- and 6β-monoamino and 3β,6β-diamino analogs of 5α-cholestane-3β,5α,6β-triol and selected azido and oximino intermediates are discussed. Compounds were studied for their inhibitory action on acetate-2-^{14}C and mevalonate-2-^{14}C incorporation into nonsaponifiable products catalyzed by a rat liver homogenate preparation and for their inhibitory or stimulatory action on two semipurified liver enzymes, Δ^7-sterol Δ^5-dehydrogenase and $\Delta^{5,7}$-sterol Δ^7-reductase. Some of our preliminary studies designed to probe the mechanism of action of three inhibitors and one stimulator of the Δ^7 reductase enzyme are also described. The results suggest that the analogs exert their actions by direct effect on the microsomal enzyme and by altering the function of a sterol carrier protein (SCP) required for full activity of the enzyme.

Studies with oxo analogs and esters of 5α-cholestane-3β,5α,6β-triol (**1**) suggested the free 5α-OH function to be important for lowering serum cholesterol levels in the cholesterol-fed hypercholesterolemic rabbit.[2] Triol **1** also inhibits cholesterol biosynthesis *in vitro*, causing accumulation of a previously undetected 29–30 C atom intermediate.[1c,2] We anticipated therefore that replacement of the 5α-OH with a 5α-NH_2 would render the compound a more potent inhibitor of cholesterol biosynthesis; *i.e.*, the NH_2 function, either protonated or unprotonated, would bind strongly to a specific enzyme system. In this regard, examination of Dreiding molecular models[3] shows the topographical relationship between the 5α-NH_2 and 3β-OH functions of **2** to be similar to the relationship between the 4α-Me and 3β-OH groups of lanosterol, in which the A ring probably exists in a flattened chair conformation.[4] Further, it is known that removal of the 4α-Me represents the first step in the enzymatic conversion of lanosterol to cholesterol.[5]

For these stereochemical reasons we proposed[1a,1d] **2** would block the biosynthesis of cholesterol after or during squalene cyclization. Such a block may enable isolation of presently unidentified intermediates in cholesterol biosynthesis and elucidate mechanisms of specific cholesterolgenesis enzymes. In this communication the biological effects on various cholesterolgenesis enzymes *in vitro* of **1** and **2** are compared with results obtained for the 3β-, 3α-, and 6β-monoamino and 3β,6β-diamino analogs of **1**, as well as with some selected synthetic intermediates.

Results and Discussion

Synthesis.—LAH reduction of 5α-azido-5α-cholestane-3β,6β-diol (**3**) afforded the known 5α-amino-5α-cholestane-3β,6β-diol (**2**).[6a] The 5α-azido intermediate **3** was prepared from cholesterol β-epoxide[7] by a



1, R = R¹ = R² = OH
2, R = R² = OH; R¹ = NH_2
3, R = R² = OH; R¹ = N_3
4, R = OAc; R¹ = N_3; R² = OH
5, R = OAc; R¹ = N_3; R² = keto

(1) (a) D. T. Witiak, R. A. Parker, W. E. Connor, and D. M. Brahmankar, 155th National Meeting of the American Chemical Society, San Francisco, Calif., April 1968, p N70; (b) D. T. Witiak, W. E. Connor, D. M. Brahmankar, A. Wartman, and R. Parker, *J. Clin. Invest.*, **47**, 104 (1968); (c) M. E. Dempsey, M. C. Ritter, D. T. Witiak, and R. A. Parker, *Atherosclerosis: Proc. Int. Symp.*, **2**, Springer-Verlag, New York and Heidelberg, 1970, p 290; (d) abstracted in part from the dissertation presented by R. A. P., July 1969, to the Graduate School of the Ohio State University; (e) Dow-Pitman-Moore Graduate Fellow 1965–1966; (f) U. S. Public Health Service Predoctoral Fellow (5-F1-GM-29,392) 1966–1969; (g) U. S. Public Health Service Predoctoral Fellow(1-FO1-GM42265)1968–1970.

(2) D. T. Witiak, R. A. Parker, D. R. Brann, M. E. Dempsey, M. C. Ritter, W. E. Connor, and D. M. Brahmankar, *J. Med. Chem.*, **14**, 216 (1971).

(3) A. Dreiding, *Helv. Chim. Acta*, **42**, 1339 (1959).

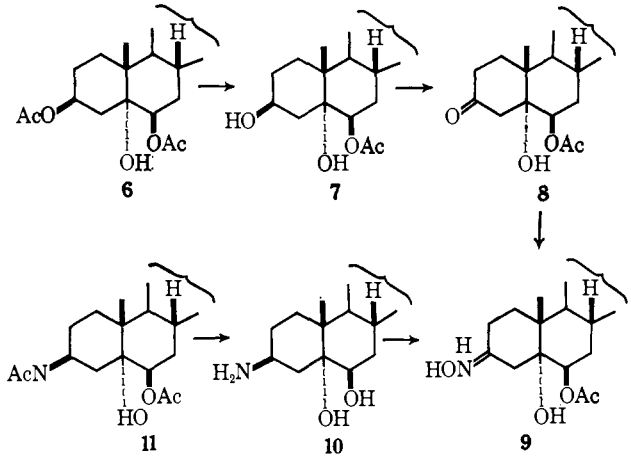
(4) (a) N. L. Allinger and M. A. DaRouge, *J. Amer. Chem. Soc.*, **84**, 4561 (1962); (b) J. Lehn, J. M. Levisalles, and G. Ourisson, *Tetrahedron Lett.*, 682 (1961).

(5) R. Rahman, K. B. Sharpless, T. A. Spencer, and R. B. Clayton, *J. Biol. Chem.*, **245**, 2667 (1970).

(6) (a) K. Ponsold, *Ber.*, **95**, 1727 (1962); (b) K. Ponsold, *ibid.*, **96**, 1411 (1963), (c) G. Snatzke and A. Veithen, *Justus Liebigs Ann. Chem.*, **703**, 159 (1967).

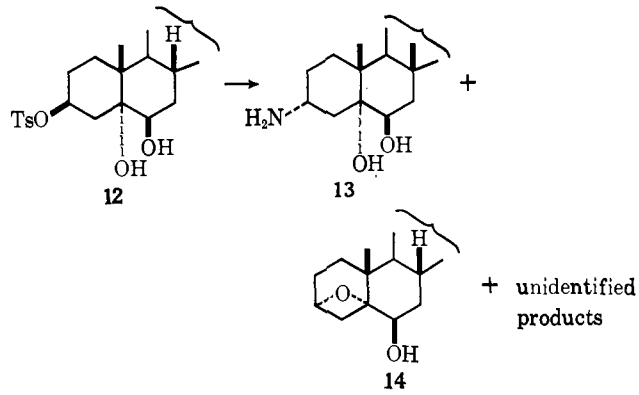
modified method of Ponsold;[6a] reaction of the β-epoxide in DMSO–H_2SO_4 with NaN_3 at 120° afforded **3** in higher yields than reported by Ponsold. 5α-Azido-5α-cholestane-3β,6β-diol 3-acetate (**4**) was obtained from the β-epoxide of 5α-cholestan-3β-yl acetate.[6a] Oxidation of acetate **4** with H_2CrO_4 yielded the 6-keto derivative **5**.[6b] Compound **5** was also synthesized by selective oxidation of the azidodiol **3** followed by acetylation.

3β-Amino-5α-cholestane-5α,6β-diol (**10**) was synthesized by LAH reduction of 3-oximino-5α-cholestane-5α,6β-diol 6-acetate (**9**). This oxime **9** was obtained in good yields from 5α-cholestane-5α,6β-diol-3-one 6-acetate (**8**). Keto analog **8** was prepared by selective hydrolysis of diacetate **6**, as described by Ellis and Petrow,[8] followed by H_2CrO_4 oxidation of the resulting monoacetate **7**. Assignment of the β configuration to the 3-NH_2 group of **10** was partly based on the work of Shoppee;[9] LAH reduction of **9** afforded the 3β equatorial isomer. Comparison with the 3α-NH_2 compound **13**, synthesized using several different routes, revealed the NH_2 group of **10** to have the β configuration. The diacetate derivative **11** was prepared and found to be different than the diacetate of the 3α-NH_2 isomer **13**.



The 3α-NH_2 analog **13** was obtained in poor yield from the 3-tosyl derivative **12**[10] of triol **1** by displacement with NH_3 at 100° under pressure. Under these reaction conditions a number of unidentified products were obtained. One compound isolated from the reaction mixture was 3α,5α-oxido-5α-cholestan-6β-ol (**14**). This was expected since Clayton and coworkers[11] reported formation of **14** from tosylate **12** on treatment with strong base. Since the 3α,5α-oxide is a possible intermediate when **12** undergoes reaction with NH_3, the 3β-NH_2 sterol **10** was also expected to be present in the reaction mixture. Thus far, we have only isolated the 3α-NH_2 compound **13**.

Displacement of the tosyl group of **12** with azide ion in DMF represents a second approach to the synthesis for **13**. The resulting 3α-azido-5α-cholestane-5α,6β-diol (**15**), which failed to crystallize, was characterized by conversion to the 6-acetate derivative **16**; this compound readily recrystallized from 1:1 MeOH–Me_2CO. The 3α-azido analog (**16**) was prepared in better yields by displacement of the mesyl group of **17** with azide ion. Compound **17** was prepared from **7** by reaction with MsCl in pyridine.



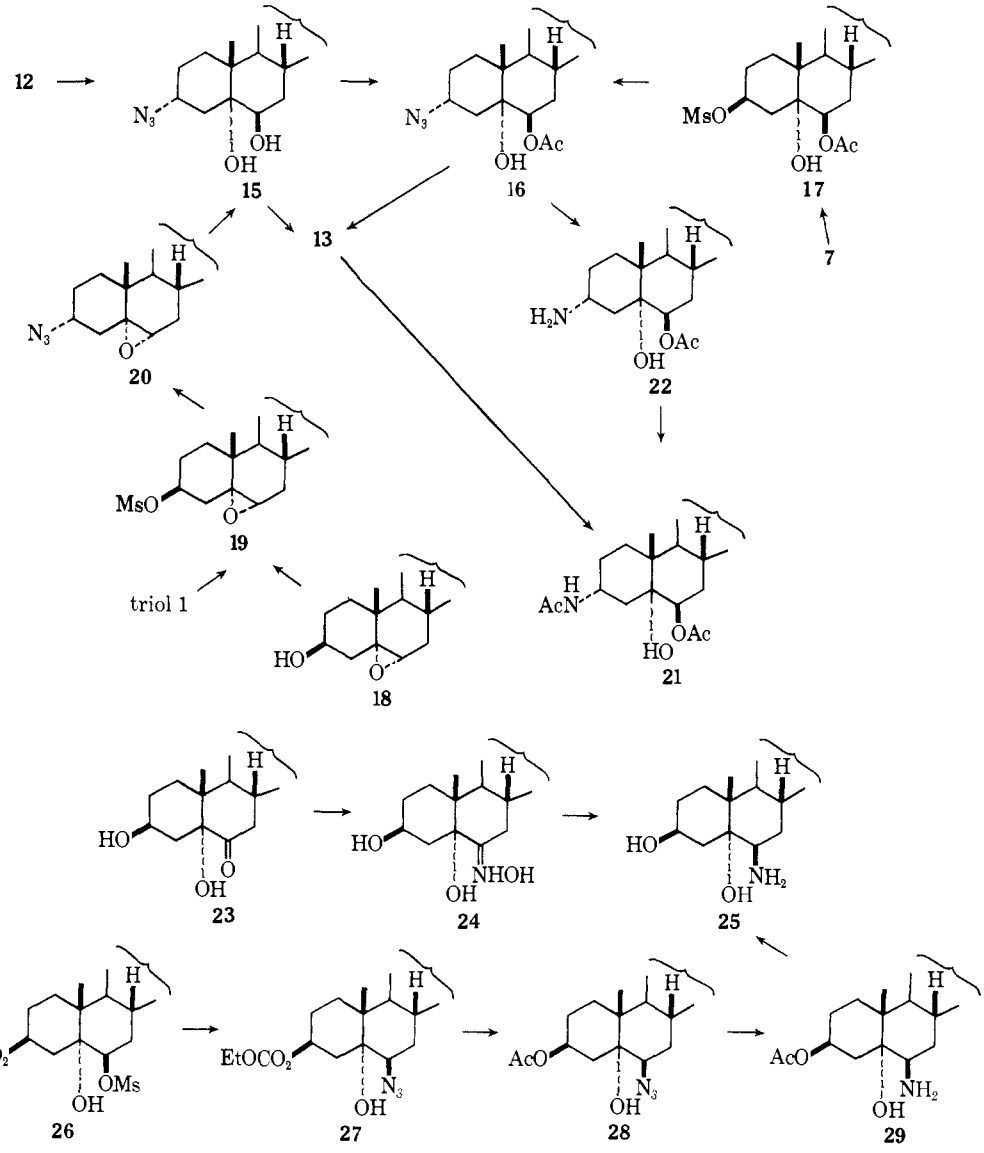
To establish the configuration of the 3-azido group of **16** the following reaction sequence was employed. Reaction of triol **1** with MsCl in pyridine followed by recrystallization from Me_2CO yielded 5α,6α-epoxy-5α-cholestan-3β-ol 3-methanesulfonate (**19**). Compound **19** was also prepared by treatment of the 5α,6α-epoxide **18** with MsCl in pyridine. Reaction of **19** with azide ion in DMF afforded 3α-azido-5α,6α-epoxy-5α-cholestane (**20**). In this reaction anchimeric assistance by a 5α-OH is not possible and the azide function should have the 3α configuration. Ponsold[6a] has shown epoxide opening with NaN_3 requires strongly acidic conditions; *i.e.*, generation of the 5,6-diol as an intermediate which could anchimerically assist is unlikely in this reaction. Treatment of **20** with HIO_4 in aq Me_2CO afforded 3α-azido-5α-cholestane-5α,6β-diol (**15**). Compound **15** was converted to its 6-acetate derivative **16** which is identical in all respects with the product obtained by azide displacement of the 3-tosylate **12** (followed by acetylation) or by azide displacement of the 3-mesylate of 5α-cholestane-3β,5α,-6β-triol 6-acetate (*i.e.*, **12** or **17**→**16**).

LAH reduction of azide **15** or acetate azide **16** afforded 3α-amino-5α-cholestane-5α,6β-diol (**13**). This was acetylated affording 3α-acetamido-5α-cholestane-5α,6β-diol 6-acetate (**21**), identical in all respects to the amide obtained from the 3α-amino compound **13** prepared by displacement of the 3β-tosylate of triol **1** with NH_3. These data establish the 3α configuration for azido and amino analogs **15**, **16**, and **13**, respectively, and substantiate the β assignment for the NH_2 group of compound **10** obtained by hydride reduction of oxime **9**.

The azido compound **16** was reduced to the amine more conveniently through use of hydrazine–Raney Ni.[6a] Reaction of the azido acetate **16** under these conditions afforded 3α-amino-5α-cholestane-5α,6β-diol 6-acetate (**22**); diacetyl derivative **21** was prepared from **22**. Utilizing the same method, azidodiol **15** afforded the corresponding 3α-NH_2 compound **13**.

Although crystalline samples of both 3β-amino-5α-cholestane-5α,6β-diol (**10**) and 3α-amino-5α-cholestane-5α,6β-diol (**13**) were obtained for elemental analysis and biological studies, both of these compounds were difficult to crystallize. For characterization purposes

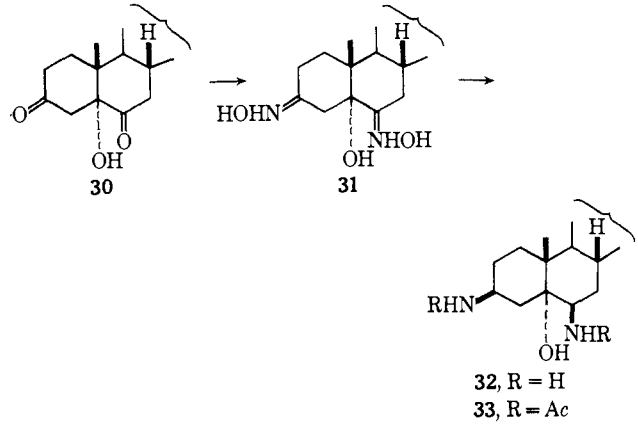
(7) L. F. Fieser and M. Fieser, "Steroids," Reinhold, New York, N. Y., 1959, p 198.
(8) B. Ellis, and V. Petrow, *J. Chem. Soc.*, 1078 (1939).
(9) (a) C. W. Shoppee, R. E. Lack, and P. Ram, *ibid.*, *C*, 1018 (1966); (b) C. W. Shoppee, P. Ram, and S. K. Roy, *ibid.*, 1018, 1023 (1966).
(10) R. Bourdon and S. Ranisteano, *Bull. Soc. Chim. Fr.*, 1977, 1982 (1960).
(11) R. B. Clayton, H. B. Henbest, and M. Smith, *J. Chem. Soc.*, 1982 (1957).



we converted the amines to their respective 3-acetamido 6-acetate derivatives, **11** and **21**, respectively. The 3β-acetamido isomer **11** melts at 205–207°; the 3α isomer **21** is hydroscopic and melts at 126–127°.

The known 6β-amino analog **25** of triol **1** was prepared by two different methods. One approach involved synthesis of the oxime **24** of 5α-cholestane-3β,-5α-diol-6-one (**23**) by methods similar to the ones reported by Shoppee[12] and Drefahl.[13] LAH reduction of the resulting 6-oximino compound **24** afforded 6β-amino-5α-cholestane-3β,5α,-diol (**25**). Alternatively, reaction of 5α-cholestane-3β,5α,6β-triol 3-ethylcarbonate 6-methanesulfonate (**26**) with NaN_3 in DMF gave excellent yields of the 6β-azidocholestane **27**. Proof for the 6β-azido configuration, resulting from 5α-OH participation in the reaction, was obtained by hydrolysis followed by acetylation. The known 6β-azido-5α-cholestane-3β,5α-diol 3-acetate (**28**) was formed; reduction with hydrazine–Raney Ni[6] afforded 6β-amino-5α-cholestane-3β,5α-diol 3-acetate (**29**). Base-catalyzed hydrolysis of acetate ester **29** yielded the aminodiol **25** identical in all respects with the compound prepared by reduction of the 6-oximino derivative **24**. Purification of the 6-NH_2 sterol **25** was difficult, but crystalline samples were obtained for biological evaluation.

Replacement of both the 3- and 6-OH groups of triol **1** with NH_2 was accomplished as follows. 5α-Cholestane-3,6-dion-5α-ol (**30**) was treated with $HONH_2$ affording the dioximino analog **31**. LAH re-

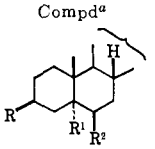
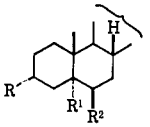


duction of the dioximino derivative **31** afforded the diamino compound **32**; this was difficult to purify

(12) C. W. Shoppee and S. K. Roy, *J. Chem. Soc.*, 3774 (1963).
(13) G. Drefahl and K. Ponsold, *Ber.*, **91**, 271 (1958).

TABLE I

COMPARATIVE EFFECTS *in Vitro* OF CHOLESTANE-3β,5α,6β-TRIOL (1) AND SELECTED AMINO AND OTHER NITROGEN-CONTAINING ANALOGS ON THE INCORPORATION OF ACETATE-2-^{14}C AND MEVALONATE-2-^{14}C INTO NONSAPONIFIABLE PRODUCTS AND CHOLESTEROL

Compd[a]	% Inhibition of ^{14}C incorp into cholesterol from Acetate[b]	% Inhibition of ^{14}C incorp into cholesterol from Mevalonate[b]	Nonsaponifiable products containing 50% or more of the radioactivity originally as acetate or mevalonate[c]
 1, R = R¹ = R² = OH	98.0[d]	97.2[e]	C-29-30
2, R = R² = OH; R¹ = NH_2		98.7[f]	C-28-30; C-27
4, R = OAc; R¹ = N_3; R² = OH	23.0[g]	59.5	C-28-30; C-27
10, R = NH_2; R¹ = R² = OH		58.1	C-27
24, R = R¹ = OH; R² = NHOH		97.0[d]	C-29-30
25, R = R¹ = OH; R² = NH_2		98.9[f]	C-28-30; C-27
31, R = R² = NHOH; R¹ = OH		18.0[d]	C-27
32, R = R² = NH_2; R¹ = OH		94.7[d]	Analog caused marked inhibition prior to nonsap products
 13, R = NH_2; R¹ = R² = OH		98.6[f]	C-28-30; C-27

[a] Analogs were dissolved in propylene glycol, $CHCl_3$, or dioxane. [b] Concns of the constituents of each incubation medium (total volume 1.35 ml) were 0.1 *M* phosphate buffer, pH 7.35, 2.3 × 10^5 dpm of NaOAc-2-^{14}C (2.0 mCi/m*M*) or 3.9 × 10^5 dpm of mevalonate-2-^{14}C (3.1 mCi/m*M*), 0.8 m*M* NADPH, NADP, and NAD, and 5 m*M* ATP, 5 m*M* $MgCl_2$, analog concn 60 μ*M*, and 22.5 mg of protein from the 500*g* supernatant fraction of a rat liver homogenate. Incubations were for 30 min at 37° under O_2. Conversions to nonsaponifiable compounds, sterols, and cholesterol were detd as described in detail elsewhere (M. E. Dempsey, *Methods Enzymol.*, **15**, 501 (1969); *Progr. Biochem. Pharmacol.*, **2**, 21 (1967); "Drugs Affecting Lipid Metabolism," W. Holmes, L. Carlson, and R. Paoletti, Ed., Plenum Press, New York, N. Y., 1969, p 511). Per cent inhibition data listed are the average value with a standard error no greater than ±0.4%. [c] The nonsaponifiable products which accumulate in the presence of the analog and which contain 50% or more of the radioactivity originally present as acetate or mevalonate are indicated (C-28-30 = squalene plus 28-30 C-atom sterols; C-27 = cholesterol and other 27 C atom sterols). [d] In the absence of the analog greater than 50% of the nonsaponifiable radioactivity was present as 27 C-atom sterols. [e] In the absence of analog greater than 50% of the nonsaponifiable radioactivity was present as 27-28 C-atom sterols. [f] Greater than 50% of the radioactivity present as 27 C-atom sterols was Δ⁷-cholestenol; greater than 50% of the radioactivity present as 28-30 C-atom sterols was an unidentified 29-30 C-atom sterol. [g] 35% of the nonsaponifiable radioactivity was present as 27 C atom sterols and the remaining radioactivity was distributed among 28-30 C-atom compds.

but a white crystalline sample was obtained for analytical and biological analysis. The easily crystallized 3β,6β-diacetamido-5α-cholestan-5α-ol (**33**) derivative was prepared by acetylation in pyridine. Theoretical and calcd elemental analyses were in excellent agreement; based on results obtained during LAH reduction of the 3- and 6-monoximino compounds **9** and **24**, we tentatively assigned the 3β,6β configuration to the NH_2 functions of **32**.

Biology.—The comparative effects of 5α-cholestane-3β-5α,6β-triol (**1**) and selected NH_2 and other N-containing analogs added *in vitro* on the incorporation of acetate-2-^{14}C and mevalonate-2-^{14}C into nonsaponifiable products are shown in Table I. At 5 μ*M* **1** inhibits 50% incorporation of acetate or mevalonate into cholesterol.[2] In addition an apparently new 29–30 C-atom sterol accumulated.[14] Similarly, the 5α-NH_2 analog **2** caused marked inhibition of incorporation of mevalonate into nonsaponifiable products (Table I). With this compound the unknown C-29–30 sterol was detected and also the radiolabel appeared in C-27 sterols, mainly Δ⁷-cholesten-3β-ol, with a small amount present in other C-28 sterols. Δ⁵,²⁴-Cholestadien-3β-ol did not accumulate, *i.e.*, there was no specific inhibition of the Δ²⁴-reductase.

The 6β-NH_2 diol **25** and 3α-NH_2 diol (**13**) (Table I) afforded results similar to those observed for the 5α-NH_2 analog **2** except that squalene also accumulated. However, the 3β-NH_2 compound **10** was less potent than any of the other NH_2 analogs on the incorporation of mevalonate into cholesterol. In addition, this inhibitor did not significantly block mevalonate incorporation into squalene and other 28–30 C-atom sterols. Instead, most of the radiolabel was present in 27 C-atom sterols. Therefore, replacement of the 5α and 6β OH groups of **1** with NH_2 in the same configuration or replacement of the 3β-OH with 3α-NH_2 afford potent inhibitors of sterolgenesis. These data indicate that the NH_2 derivatives function as inhibitors by a mechanism similar in most respects to that of **1**. It is striking and probably pertinent to complete understanding of the inhibition mechanism that the configuration of the NH_2 at C-3 markedly affects the degree of observed inhibition.

The activity observed for the 3β-Ac-5α-N_3-6β-ol analog **4** (Table I) of triol **1** is of particular interest. While this compound is less potent than the 5α-NH_2 diol **2** or triol **1**, its mechanism of action seems to be similar to the one observed for **1** and **2**. Possibly, the 5α-N_3 analog **4** serves as a precursor *in vitro* for the 5α-amino diol **2**,[15] but further work is necessary to substantiate this proposal. The relatively low inhibition seen with **4** probably reflects the importance, noted previously,[1c,2] of a free hydroxyl group at C-3 for maximum inhibitory activity by an analog.

The 3β,6β-di-NH_2 analog **32** (Table I) does not behave like any of the other compounds tested. Re-

(14) For a discussion of the properties of this unidentified sterol, see ref 2.

(15) One possibility is that the 3β-OAc function is hydrolyzed and the 5α-N_3 function reduced to NH_2 *in vitro*.

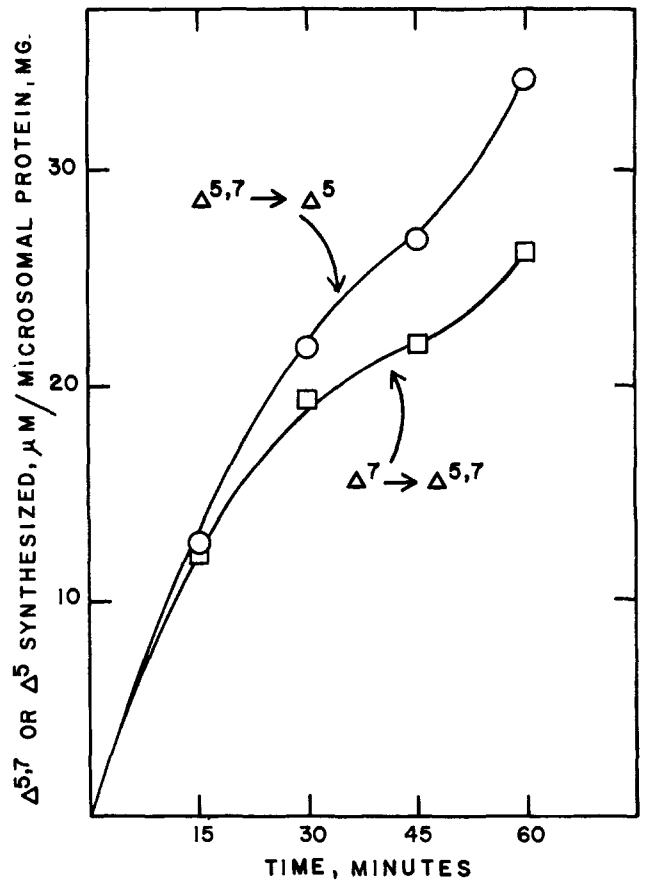


Figure 1.—Time courses of Δ^7-sterol Δ^5-dehydrogenase and $\Delta^{5,7}$-sterol Δ^7-reductase in the absence of cholestane analogs: □—□, Δ^5-dehydrogenase activity [conversion of Δ^7-cholestenol (Δ^7) to $\Delta^{5,7}$-cholestadienol ($\Delta^{5,7}$)]; ○—○, Δ^7-reductase activity, conversion of $\Delta^{5,7}$-cholestadienol-4-^{14}C ($\Delta^{5,7}$) to cholesterol-4-^{14}C (Δ^5). Incubation and assay conditions were as given with the footnotes for Table II, except that the substrate concentration for each time course was 64 μM.

placement of both of the 3β and 6β OH groups by NH_2 renders the compound a potent inhibitor prior to formation of nonsaponifiable products. This suggests that the diamino compound should be evaluated *in vivo* since accumulation of undesirable sterols may be less of a problem than with triol **1**. However, as discussed in a later section, the diamine does inhibit semipurified $\Delta^{5,7}$-sterol Δ^7-reductase as well as stimulate semipurified Δ^7-sterol Δ^5-dehydrogenase *in vitro*.[16,17] These two enzymes exert their catalytic action after squalene cyclization.

The biological results obtained for the mono and diamino analogs (Table I) may be contrasted with the activity reported for certain amino compounds by Corey and coworkers.[18] While 2,3-iminosqualene blocks cyclization of 2,3-oxidosqualene to lanosterol, 3β-amino-8,25-lanostadiene and 3β-amino-8-lanostene show little inhibition. Counsell and coworkers[19] have also reported on 22,25-diazacholestan-3-ols which block cholesterol biosynthesis at a late reductive stage.[20] Azacholesterols have been shown to inhibit desmosterol reductase as well as sites prior to squalene cyclization. Most other A- and B-ring modified azacholesterols and cholesterol analogs with oza- and oxa-aza side chains[21] block the Δ^{24}-reductase step.[22]

The dioximinocholestane **31** (Table I), the synthetic precursor for diamine **32**, is only a very weak inhibitor of cholesterolgenesis. This result may be contrasted to finidings with the parent diketo analog, 5α-cholestane-3,6-dion-5α-ol. We previously reported[2] the latter analog to be a potent inhibitor of cholesterolgenesis *in vitro*, causing accumulation of squalene and other 28–30 C-atom sterols. In the presence of **31** the radiolabel originally present as mevalonate was found in cholesterol and/or other 27 C-atom sterols. On the other hand, the 6-oximino analog **24** is a very potent inhibitor of cholesterolgenesis and appears to be working by a mechanism similar to the one reported for triol **1**.[2] Again, formation of the unidentified 29–30 C-atom sterol was detected.[14] For cholestane derivatives to inhibit cholesterol biosynthesis by a mechanism similar to triol **1**, it seems a neutral compound having the 3β,5α-diol portion is most important; cholestane-3β,5α-diol, a compound having no substituent in the 6 position, also behaves like triol **1**, but is somewhat less effective.[2] For maximum inhibition of sterol synthesis from acetate or mevalonate required substitutions on cholestane are: a free OH or ketone at C-3 (or a 3α-NH_2); a free OH or NH_2 at C-5; and a free OH, NHOH, or NH_2 at C-6 (Table I and ref 1c and 2).

We previously showed triol **1** and selected oxo analogs work *in vivo* and *in vitro* by multiple mechanisms of action.[2] *In vitro*, **1** inhibited[2] liver Δ^7-sterol Δ^5-dehydrogenase and $\Delta^{5,7}$-sterol Δ^7-reductase enzymes.[16,17a] To probe further into structural requirements for activity on these enzymes we studied the N-containing analogs. The effects *in vitro* of triol **1** and selected analogs on these two semipurified liver enzymes are found in Table II. All compounds were evaluated at the concentrations indicated with incubation times of 45 min. Incubation times of this length were chosen since both the $\Delta^7 \rightarrow \Delta^{5,7}$-sterol and $\Delta^{5,7} \rightarrow \Delta^5$-sterol (cholesterol) conversions progress to a considerable extent during 45 minutes in the absence of inhibitor (Figure 1). The data of Figure 1 further indicate that after 45 min the rate of both enzyme reactions increases probably due to interaction of the substrates with the activator-carrier protein [squalene and sterol carrier protein (SCP)] required for maximum activity by both microsomal enzymes.[16,17] The rate of the Δ^5-dehydrogenase is measurably slower than that of the Δ^7-reductase, as reported previously.[16] The time

(16) M. E. Dempsey, *Ann. N. Y. Acad. Sci.*, **148**, 631 (1968).

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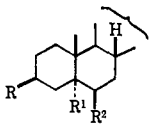
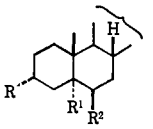
(22) (a) R. E. Ranney and D. L. Cook, *Arch. Int. Pharmacodyn.*, **154** 51 (1965); (b) H. S. Schutte and H. E. Neville, *Lab. Invest.*, **19**, 487 (1968); (c) R. Niemiro and R. Fumagilli, *Biochem. Biophys. Acta*, **98**, 624 (1965); (d) N. Winer, D. M. Klachko, R. D. Baer, P. D. Langley, and T. W. Burns, *Science*, **153**, 312 (1966); (e) B. A. Sachs and L. Wolfman, *Arch. Internal Med.*, **116**, 366 (1965).

TABLE II

COMPARATIVE EFFECTS *in Vitro* OF CHOLESTANE-3β,5α,6β-TRIOL (1) AND SELECTED AMINO AND OTHER NITROGEN-CONTAINING ANALOGS ON Δ⁷-STEROL Δ⁵-DEHYDROGENASE AND Δ⁵,⁷-STEROLS Δ⁷-REDUCTASE

Compd[a]	Substrate: Δ⁷-Cholestenol[b] % inhibition (−) or activation (+)/μM analog tested	Substrate: Δ⁵,⁷-Cholestadienol[c] % inhibition (−) or activation (+)/μM analog tested
		
1, R = R¹ = R² = OH	−11/52; −23/121	−25/67; −35/90
2, R = R² = OH; R¹ = NH_2	−7/53; −24/103	−24/32; −37/64
4, R = OAc; R¹ = N_3; R² = OH	0/55	+89/55
10, R = NH_2; R¹ = R² = OH	−21/56; −38/103	−40/31; −62/61
24, R = R¹ = OH; R² = NHOH	−15/96; −30/192	−10/32; −14/64
25, R = R¹ = OH; R² = NH_2	−3/51; −14/106	−24/31; −48/67
31, R = R² = NHOH; R¹ = OH	+3/51; +1/101	+24/31; +37/63
32, R = R² = NH_2; R¹ = OH	+22/53	−82/64
		
13, R = NH_2; R¹ = R² = OH	−5/53; −28/103	−60/31; −76/61

[a] Analogs were dissolved in propylene glycol, $CHCl_3$, or dioxane. [b] Conversion of Δ⁷-cholesterol to Δ⁵,⁷-cholestadienol was assayed by the uv absorption technique (M. E. Dempsey, *Progr. Biochem. Pharmacol.*, **2**, 21 (1967); "Drugs Affecting Lipid Metabolism," W. Holmes, L. Carlson, and R. Paoletti, Ed., Plenum Press, New York, N. Y., 1969, p 511). Concns of the constituents of each incubation medium (total vol, 1.3 ml) were 0.1 *M* phosphate buffer, pH 7.35, 1 m*M* NAD, 50 μ*M* Δ⁷-cholestenol, 0.1 n*M* AY-9944 (D. Dvornek, M. Kraml, and J. F. Bogli, *Biochemistry*, **5**, 1060 (1966)), analog as indicated, 2.5 mg of microsomal enzyme protein, and 15 mg of squalene and sterol carrier protein (SCP) (M. C. Ritter and M. E. Dempsey, *Biochem. Biophys. Res. Commun.*, **38**, 921 (1970); *Circulation*, **42**, *Suppl.*, **3**, 2 (1970); M. E. Dempsey, "Chemistry of Brain Development," R. Paoletti and R. Davison, Ed., Plenum Press, New York, N. Y., 1970). Incubations were for 45 min at 37° under O_2. [c] Conversion of Δ⁵,⁷-cholestadienol-4-^{14}C to cholesterol-4-^{14}C was assayed by the dibromide derivative technique (see first ref in footnote *b*). Concns of the constituents of each incubation medium (total vol, 2.2 ml) were 0.1 *M* phosphate buffer, pH 7.35, 1 m*M* NADPH, 20 μ*M* Δ⁵,⁷-cholestadienol-4-^{14}C, analog as indicated, and microsomal enzyme and activator proteins as for Δ⁷-cholestenol incubations (see ref in footnote *b*). Incubations were for 45 min at 37° under N_2.

courses for both enzyme reactions (Figure 1) are typical for the substrate concentration and ratio of SCP to microsomal enzyme protein employed (conditions, Figure 1).

The 5α-NH_2 diol **2** (Table II) is an inhibitor of the Δ⁵-dehydrogenase and Δ⁷-reductase behaving like triol **1**. The 3β-NH_2 (**10**), 6β-NH_2 (**25**), and 3α-NH_2 (**13**) diols also inhibit these two enzymes. Like triol **1** these compounds are consistently more potent inhibitors of the Δ⁷-reductase than the Δ⁵-dehydrogenase. Generally, replacement of OH by NH_2 increases the inhibitory activity on the reductase, but has little influence or decreases the inhibitory effect on the dehydrogenase enzyme. These data show that in addition to their inhibitory effect on mevalonate incorporation into nonsaponifiable products (Table I), they also influence later steps in cholesterolgenesis *in vitro*.

Interestingly, the 5α-N_3 analog **4**, a weaker inhibitor of cholesterolgenesis from acetate and mevalonate than the 5α-NH_2 diol (**2**) (Table I), is a selective potentiator of the reductase enzyme; azide **4** exerts no effect *in vitro* on the dehydrogenase system. It is less likely that **4** is enzymatically coverted to **2** in the semipurified enzyme preparations; these results are most probably a reflection of the activity of compound **4**. Since the triacetate, 6-keto-3β,5α-diol and 3-keto-5α,6β-diol 6-acetate analogs[2] of triol **1** as well as the 3,6-dioximino compound **31** (Table II), also stimulate the reductase enzyme, structural requirements for this kind of activity are difficult to classify at this time. For example, whereas the 6-keto-3β,5α-diol analog stimulated the reductase system,[2] the 6-oximino-3β,5α-diol **24** (Table II) had only a weak inhibitory effect on this enzyme. Its inhibitory activity on the dehydrogenase enzyme is also less than for the corresponding 6-keto analog. The probable explanation for the stimulatory or inhibitory effects of these analogs (Table II) is indicated in a later paragraph (*cf.* Figure 3).

Again, the 3β,6β-diamino compound **32** (Table II) exhibited anomalous behavior. While this analog is a potent inhibitor of the reductase system and, therefore, behaves like the monoamino compounds, it stimulates the dehydrogenase enzyme *in vitro*. The only other cholestane analog known to stimulate this enzyme *in vitro* is 5α-cholestane-3β,5α,6β-triol triacetate, which also stimulates the reductase system.[2] This great differentiation of action on the two enzyme systems by **32** may be a reflection of its dibasic nature. This is a structural characteristic not present in other analogs assayed. In this regard we showed previously that other Δ⁷-reductase inhibitors contain one or more NH_2 groups as part of their structure.[23]

To further investigate the mechanism of action by triol **1** and the 3α- and 5α-NH_2 diols (**13** and **2**, respectively) we studied the effect of varying concentrations of Δ⁵,⁷-cholestadien-3β-ol on the inhibition of the Δ⁷-reductase.[16,17] The results shown in Figure 2 illustrate the noncompetitive antagonism exhibited by these 3 compounds. In all cases, the inhibition of the reductase was not overcome by increasing the Δ⁵,⁷-cholestadienol level. These findings also demon-

(23) (a) M. E. Dempsey, *Progr. Biochem. Pharmacol.*, **2**, 21 (1967); (b) M. E. Dempsey, "Drugs Affecting Lipid Metabolism," W. Holmes, L. Carlson, and R. Paoletti, Ed., Plenum Press, New York, N. Y., 1969, p 511.

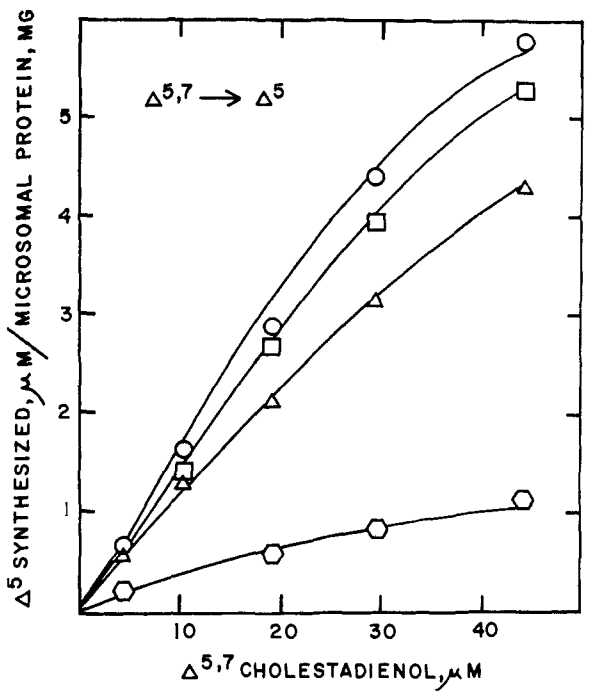


Figure 2.—Effect of varying concentrations of $\Delta^{5,7}$-cholestadienol-4-^{14}C on the inhibition of $\Delta^{5,7}$-sterol Δ^7-reductase by cholestane-3β,5α,6β-triol (1) and some related amino analogs: O—O, Δ^7-reductase activity (no inhibitor); □—□, effect of cholestane-3β,5α,6β-triol (1); Δ—Δ, effect of 3β,6β-dihydroxy-5α-aminocholestane (2); ○—○, effect of 3α-amino-5α,6β-dihydroxycholestane (13). Concentrations of each incubation medium (total volume, 2.65 ml) were 0.1 *M* phosphate buffer, pH 7.35, 1 m*M* NADPH, with or without cholestane derivative (27 μ*M*) as indicated, with $\Delta^{5,7}$-cholestadienol-4-^{14}C ($\Delta^{5,7}$) as indicated, and 5.2 mg of microsomal enzyme protein and 30.6 mg of squalene and sterol carrier protein (SCP).[11] Incubations were for 45 min under N_2. Cholesterol-4-^{14}C (Δ^5) synthesis was measured as described elsewhere.[28]

strate that the 5α-NH_2 diol (**2**) and the 3α-NH_2 diol (**13**) analogs are more powerful inhibitors of the reductase enzyme. Among these 3 compounds studied, the 3α-NH_2 analog is clearly the most potent and approaches the inhibitory effect of the diamine **32** (Table II).

As mentioned above, an activator–carrier protein (SCP) is required for maximum activity by the microsomal enzymes studied here.[16,17] The effects of varying the ratio of SCP to microsomal enzyme protein in the presence of selected inhibitors (5α- and 3α-NH_2 diols, **2** and **13**) and one stimulator (3,6-dioximino analog **31**) of the Δ^7-reductase at 2 substrate levels are shown in Figure 3. These data provide evidence that one mechanism by which the cholestane analogs cause their inhibition or activation is by direct action on the microsomal enzyme; *i.e.*, at concentrations of both 7.9 and 15.5 μ*M* substrate and in the absence of SCP the dioximino compound causes significant stimulation. Stimulatory effects by **31** indicate a direct activation or protection from deactivation during incubations of the microsomal enzyme. Similarly, in the absence of SCP the 5α-NH_2 diol (**2**) inhibits the reductase enzyme. The greater potency of the 3α-NH_2 diol **13**, in the absence of SCP is also shown at the 15.5 μ*M* substrate concentration (graph 2, Figure 3).

In addition to their direct activity on the reductase enzyme, these compounds also interfere with the activation by SCP of the reductase; the potent inhibitory effect of the 3α-NH_2 diol **13** is not reversed (graph 2, Figure 3) to any appreciable extent by increasing concentrations of SCP. The 5α-NH_2 diol **2** (graphs 1 and 2, Figure 3) also exerts its blocking effect by affecting the function of SCP. The differences in the slopes of the curves, with and without inhibitor and at different substrate concentrations are reflections of the changing rate of the enzymatic conversion of $\Delta^{5,7}$-cholestadienol to cholesterol and imply that the inhibitor exerts its influence by multiple modes of action.

The 3,6-dioximino analog **31** has an even greater stimulating effect as the ratio of SCP to microsomal enzyme is increased (graphs 1 and 2, Figure 3). This increase in potentiation by **31** reaches a maximum when the ratio of SCP to microsomal protein is 8 (graph 1, Figure 3). In the absence of the dioximino analog, an SCP to microsomal enzyme protein ratio of 20 is required to reach the same activation level. Similar to the experiments with inhibitor **2**, stimulator **31** influenced the nature of SCP's activation of the reductase enzyme both qualitatively and quantitatively at the two substrate concentrations. Apparently, the blockers and stimulators interfere with the binding of the substrate to SCP and/or formation of the active conformation of the sterol–SCP complex.[17c] The agents probably also affect the interaction of the sterol–SCP complex with the active and/or allosteric sites[24] of the enzyme. At different concentration levels of substrate or analog and because of their variations in affinity for different sites on the enzyme and SCP different rates of product formation are obsreved.

Experimental Section[25]

5α-Amino-5α-cholestane-5α,3β,6β-diol (2).—The synthesis of **2** from either the azidodiol **3** or the 3-acetate **4** was carried out as described by Ponsold.[6] Purification of the amine was accomplished by formation of the amine·HCl in Et_2O, filtration, and neutralization of the HCl in MeOH, followed by Et_2O–H_2O extn. The amine·HCl was again prepd and sepd from the Et_2O layer. The HCl was dissolved in hot MeOH and the soln was neutralized with 10% aq NaOH. H_2O was added to the point of turbidity, and the soln was allowed to cool. Compd **2** crystd as white needles: mp 241–243°; lit.[6a] mp 242–243°.

5α-Azido-5α-cholestane-3β,6β-diol (3) was synthesized by the method of Ponsold.[6] The following modified procedure afforded the best yield of the azidosteroid. 5β,6β-Epoxy-5β-cholestan-3β-ol (2.0 g, 5.0×10^{-3} mole) and NaN_3 (6.0 g, 9.2×10^{-2} mole) were suspended in 150 ml of dry DMSO, and the reaction mixt was stirred and heated to 120° on an oil bath. H_2SO_4 (2 g, 100%) in 10 ml of dry DMSO was added dropwise and the mixt was heated with stirring for an additional 36 hr. It was allowed to cool and poured into salt–H_2O and extd (Et_2O). The Et_2O layer was washed several times (H_2O), dried (Na_2SO_4), filtered, and removed under reduced pressure affording after 3 recrystns from MeOH, 0.80 g (36%) of **3**: mp 170–172°; lit.[6a] mp 171–172°.

5α-Azido-5α-cholestane-3β,6β-diol 3-acetate (4) was synthesized starting with 5β,6β-epoxy-5β-cholestan-3β-ol 3-acetate using the same reaction condns as described above affording 0.95 g (43%) of **4**: mp 184–188°; lit.[6] mp 188°; $[\alpha]D$ −22° (c, 2.0, $HCCl_3$); lit.[6a] $[\alpha]D$ −25°.

(24) J. Monod, J. Wyman, and J.-P. Changeux, *J. Mol. Biol.*, **12**, 88 (1965).

(25) Melting points are corrected and were taken using a Thomas-Hoover melting point apparatus. Rotations at the Na D-line were taken with a Ziess polarimeter. Ir spectra were determined in $HCCl_3$ on a Perkin-Elmer (Model 257) spectrophotometer. Elemental analyses were performed by Clark Microanalytical Laboratory, Urbana, Ill.

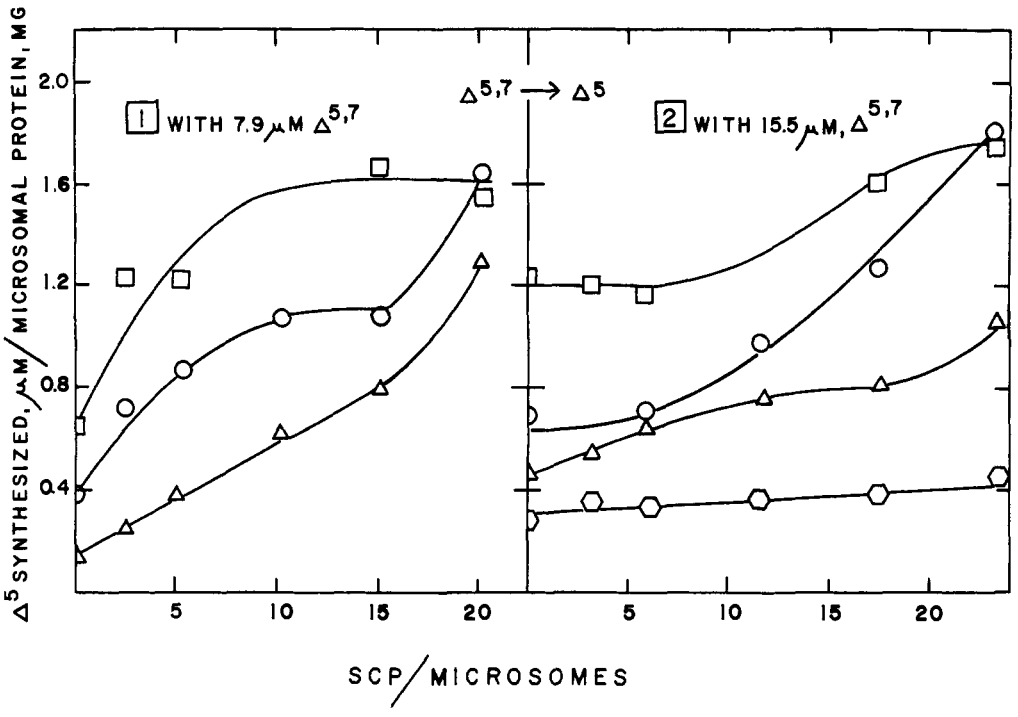


Figure 3.—Effects of varying the ratio of squalene and sterol carrier protein (SCP) to microsomal enzyme protein on the inhibition or activation of $\Delta^{5,7}$-sterol Δ^{7}-reductase at two substrate levels by cholestane derivatives: (graph 1) ○—○, Δ^{7}-reductase activity [conversion of $\Delta^{5,7}$-cholestadienol ($\Delta^{5,7}$) to cholesterol (Δ^{5}),; no inhibitor or activator]; □—□, effect of 3,6-dioximino-5α-hydroxycholestane (**31**); △—△, effect of 3β,6β-dihydroxy-5α-aminocholestane (**2**). Assay conditions were as given with Table II and Figure 2 except that the ratio of SCP to microsomal enzyme protein was varied as indicated and the concentration of cholestane derivatives was 27 μ*M*; (graph 2) ○—○, □—□, △—△, and other conditions are as defined for graph 1; ⬡—⬡, effect of 3α-amino-5α,6β-dihydroxycholestane (**13**).

5α-Azido-3β-acetoxy-5α-cholestan-6-one (5).—CrO_3 (0.176 g, 5.7×10^{-3} mole) in HOAc (10 ml, 97.5%) was slowly added to **4** (1.0 g, 2.1×10^{-3} mole) in 100 ml of HOAc at room temp. After the addn was complete the reaction mixt was dild with excess H_2O and extd (Et_2O). The Et_2O was washed (H_2O), filtered, and evapd under reduced pressure affording 0.94 g of white solid. Recrystn from MeOH afforded 0.70 g (70%) of **5**: mp 186–188°; lit.[6b] mp 188.5–189°; $[\alpha]_D$ −45° (*c* 1.0, $HCCl_3$); lit.[26] $[\alpha]_D$ −47.2°. *Anal.* ($C_{29}H_{47}N_3O_3$) C, H, N.

5α-Azido-3β-acetoxy-5α-cholestan-6-one (5) from 5α-Azido-5α-cholestane-3β,6β-diol (3).—CrO_3 (0.340 g, 1.1×10^{-2} mole) in HOAc (30 ml, 95%) was added dropwise (1 drop every 15 sec) with stirring to the azidodiol **3** (2.0 g, 4.2×10^{-3} mole) in HOAc (150 ml, 95%) at room temp. After the addition was complete the reaction mixt was poured into ice H_2O and extd (Et_2O). The Et_2O layer was washed several times (H_2O), dried (Na_2SO_4), filtered, and removed under reduced pressure affording 1.9 g of white residue. The product was acetylated without further purification. Ac_2O (5 ml) in 5 ml of pyridine was added to the oxidation product in 50 ml of pyridine. The reaction mixt was allowed to stand at room temp overnight, poured into ice H_2O, and extd (Et_2O). The Et_2O layer was washed with H_2O, dried (Na_2SO_4), and evapd under reduced pressure affording 2.0 g of white cryst residue. Recrystn (twice from MeOH) afforded 1.5 g (68%) of **5**, mp 187–189°. The product was identical in all respects with the product obtained by oxidation of 5α-azido-5α-cholestane-3β,6β-diol 3-acetate (**4**).

3-Oximino-5α-cholestane-5α,6β-diol 6-Acetate (9).—Cholestane-5α,6β-diol-3-one 6-acetate[8] (**8**, 2.4 g, 5.2×10^{-3} mole), $HONH_2 \cdot HCl$ (3.0 g, 4.3×10^{-2} mole) and $NaOAc \cdot 3H_2O$ (4.5 g, 3.5×10^{-2} mole) were stirred with 100 ml of abs EtOH. The reaction mixt was refluxed for 8 hr, poured into ice H_2O, and extd (Et_2O). The Et_2O layer was washed (H_2O), dried (Na_2SO_4), filtered, and removed under reduced pressure affording 2.5 g of white product. Recrystn 3 times from Et_2O–MeCN afforded 1.4 g (56%) of **9**, softening point 115°, 200° dec. *Anal.* ($C_{29}H_{49}NO_4$) C, H, N.

3β-Amino-5α-cholestane-5α,6β-diol (10).—The 3-oxime **9** (2.4 g, 5.2×10^{-3} mole) in 50 ml of anhyd Et_2O was added dropwise with stirring to LAH (2.5 g, 6.6×10^{-2} mole) in 50 ml of Et_2O at room temp. The reaction mixt was stirred at room temp for an additional 48 hr. Excess LAH was decompd by dropwise addn of 2.5 ml of 10% NaOH and 10.0 ml of H_2O. The filtrate was collected, and the ppt was extd twice with THF. The combined filtrate was evapd under reduced pressure affording 2.0 g (86%) of white residue. Crystals formed from MeOH, mp 175–178°. *Anal.* ($C_{27}H_{49}NO_2$) C, H, N.

3β-Acetamido-5α-cholestane-5α,6β-diol 6-Acetate (11).—Ac_2O (10 ml) in dry pyridine was added to the 3β-NH_2 **10** (2.0 g, 4.8×10^{-3} mole) in 50 ml of dry pyridine. The reaction mixt was allowed to stand at room temp overnight, poured into ice H_2O, and extd (Et_2O). The Et_2O layer was washed several times (H_2O), dried (Na_2SO_4), and evapd under reduced pressure yielding 2.20 g of white residue. Recrystn 3 times from Me_2CO afforded 1.6 g (67%) of white crystals, mp 205–207°. *Anal.* ($C_{31}H_{53}NO_4$) C, H, N.

5α-Cholestane-3β,5α,6β-triol 3-*p*-Toluenesulfonate (12).—Triol 1[2] (10.0 g, 2.4×10^{-2} mole) dissolved in 200 ml of dry pyridine was added dropwise with stirring to the reaction mixt at room temp. The mixt was allowed to stand at room temp overnight, poured into ice H_2O, and extd (Et_2O). The Et_2O layer was washed with 10% aq $NaHCO_3$ and H_2O, dried (Na_2SO_4), and removed under reduced pressure affording 13.0 g of cryst product. The crude tosylate was recrystd twice from Me_2CO affording 8.5 g (62%) of **12**: mp 148–150° dec; lit.[10] mp 166° dec.

Reaction of 5α-cholestane-3β,5α,6β-triol 3-*p*-Toluenesulfonate (12) with NH_3.—Several attempts had been made to displace the 3β-Ts of **12** with NH_3. All attempts failed with excess NH_3 in a citrate bottle at room temp. Only starting material was recovered.

Compd **12** (10 g, 1.74×10^{-2} mole) was placed in a glass-lined stainless steel bomb. The reaction bomb was cooled in a Dry Ice–Me_2CO bath for 5 min, and excess liquid NH_3 was added (approx 100 ml). The bomb was sealed and heated in an oven at 100° for 48 hr. The excess NH_3 was allowed to escape at room temp. The residue was extd with Et_2O and H_2O, and the Et_2O layer was dried (Na_2SO_4) and evapd under reduced pressure affording 7.3 g of residue. Attempts to cryst this product failed. The residue was dissolved in Et_2O, gaseous HCl was added, and

(26) H. R. Roscoe, R. Goldstein, and M. J. Fahrenbach, *Biochem. Pharmacol.*, **17**, 1189 (1968).

the ppt was collected. The Et_2O layer was evapd under reduced pressure affording a white residue which crystd from EtOH affording 2.1 g (30%) of white crystals of 3α,5α-oxido-5α-cholestan-5β-ol (**14**), mp 130–131°. *Anal.* ($C_{27}H_{46}O_2$) C, H.

The amine·HCl soln contg **13** was neutralized and extd (Et_2O). The dried (Na_2SO_4) Et_2O layer was removed under reduced pressure affording 4.0 g of yellow residue. All attempts to cryst this product failed. Acetylation was carried out with Ac_2O (10 ml) in 50 ml of dry pyridine affording 4.1 g (47%) of a white product. Chromatography on 100 g of silicic acid with $HCCl_3$ and $HCCl_3$–MeOH (93:5) afforded **3α-acetamido-5α-cholestane-5α,6β-diol 6-acetate (21)**. Recrystn from Me_2CO yielded white needles, mp 126–127° (foams). *Anal.* ($C_{31}H_{53}NO_4$) C, H, N.

3α-Amino-5α-cholestane-5α,6β-diol (13) from LAH Reduction of 3α-Azido-5α-cholestane-5α,6β-diol (15).—Azidodiol **15** (0.25 g, 5.6×10^{-4} mole) in 20 ml of Et_2O was added dropwise with stirring to LAH (0.25 g, 6.6×10^{-3} mole) in 50 ml of Et_2O at 0°. The reaction mixt was stirred at room temp overnight. The excess LAH was decompd by slow addn of 0.25 ml of aq NaOH (10%) and 1.0 ml of H_2O. The filtrate was collected, and the ppt was extd twice with THF. The combined filtrates were distd under reduced pressure affording 0.050 g (21%) of yellow cryst **13**, mp 120–132°. *Anal.* ($C_{27}H_{49}NO_2$) C, H, N. The crude amine was converted to amide **21** by acetylation procedures previously described. Amide **21** (mp 125–127°) was identical in all respects with the amide obtained by acetylation of the displacement product of tosylate **12** with NH_3.

3α-Amino-5α-cholestane-5α,6β-diol (13) from LAH Reduction of 3α-Azido-5α-cholestane-5α,6β-diol 6-Acetate (16).—Aminodiol 13 was synthesized from acetate **16** *via* LAH reduction as described above in 45% yield. The amine was characterized as the amide **21**.

3α-Amino-5α-cholestane-5α,6β-diol (13) from Hydrazine–Raney Ni Reduction of 3α-Azido-5α-cholestane-5α,6β-diol (15).—Azidodiol **15** (0.30 g, 6.7×10^{-4} mole), hydrazine hydrate (1 ml), and a small amount of W-2 Raney Ni were refluxed in 50 ml of EtOH for 1 hr. The reaction mixt was allowed to cool and 100 ml of Et_2O was added. The mixt was set aside until gas evoln ceased (overnight). The mixt was filtered, and the Et_2O layer was washed (H_2O), dried (Na_2SO_4), and distd under reduced pressure affording 0.275 g (97%) of white residue. This product was acetylated yielding the corresponding amide **21** for characterization purposes. The amide obtd was identical in all respects with amides prepd by the other methods.

3α-Azido-5α-cholestane-5α,6β-diol 6-Acetate (16) from 5α-Cholestane-3β,5α,6β-triol 3-Tosylate (12).—Compd **12** (0.50 g, 8.7×10^{-4} mole) and 2.0 g (3.1×10^{-2} mole) of NaN_3 were suspended in 50 ml of DMF. The mixt was heated and stirred at 100° for 10 hr, poured into salt–ice H_2O, extd (Et_2O), and washed several times (H_2O). The dried (Na_2SO_4) Et_2O layer was concd under reduced pressure affording 0.375 g (96%) of clear oil. Chromatography on 40 g of silica gel with $HCCl_3$ as the eluent afforded 0.350 g of azidosteroid **15** which failed to cryst. It was acetylated by treatment with 2 ml of Ac_2O in 10 ml of dry pyridine at room temp overnight. The reaction mixt was poured into ice–H_2O and extd (Et_2O). The Et_2O layer was washed several times (H_2O), dried (Na_2SO_4), and filtered, and Et_2O was removed under reduced pressure affording 0.350 g of white product. Recrystn from Me_2CO–MeOH (1:1) afforded 0.250 g (17%) of **16**, mp 77–78°. *Anal.* ($C_{29}H_{49}N_3O_3$) C, H, N.

3α-Azido-5α-cholestane-5α,6β-diol 6-Acetate (16) from 5α-Cholestane-3β,5α,6β-triol 3-Methanesulfonate 6-Acetate (17).—Mesylate **17** (0.550 g, 1.02×10^{-3} mole) and 1.0 g (1.5×10^{-2} mole) of NaN_3 were stirred with 30 ml of DMF. The mixt was heated to 90° for 20 hr, poured into salt–ice H_2O, and extd (Et_2O). The Et_2O was washed 3 times (H_2O), dried (Na_2SO_4), filtered, and removed under reduced pressure affording 0.450 g of cryst product. Recrystn from Me_2CO–MeOH (1:1) yielded 0.310 g (63%) of **16**, mp 78–79°. This product was identical in all respects with the azide obtd by displacement of the 3-tosylate followed by acetylation.

3α-Azido-5α-cholestane-5α,6β-diol 6-Acetate (16) from 3α-Azido-5α,6α-epoxy-5α-cholestane (20).—Azidoepoxide **20** (0.400 g, 9.4×10^{-4} mole) was refluxed in 30 ml of Me_2CO. $HIO_4 \cdot 2H_2O$ (0.245 g, 9.4×10^{-4} mole) in 5 ml of H_2O was added. The reaction mixt was refluxed for an addnl 30 min, poured into ice H_2O, and extd (Et_2O). The Et_2O layer was washed (H_2O), dried (Na_2SO_4), and evapd under reduced pressure affording 0.395 g (95%) of white cryst residue. Acetylation of the residue was carried out with 5 ml of Ac_2O in 30 ml of pyridine at room temp overnight. The reaction mixt was poured into ice H_2O and extd (Et_2O). The Et_2O layer was washed 3 times (H_2O), dried (Na_2SO_4), and removed under reduced pressure affording 0.390 g of white residue. Recrystn from Me_2CO–MeOH (1:1) yielded 0.270 g (59%) of **16**, mp 79–80°. This product was identical in all respects with **16** obtd by the other methods described.

5α-Cholestane-3β,5α,6β-triol 3-Methanesulfonate 6-Acetate (17).—5α-Cholestane-3β,5α-6β-triol 6-acetate[8] (**7**) (0.832 g, 1.8×10^{-3} mole) in 50 ml of dry pyridine was stirred and cooled in an ice bath. MsCl (2 ml) in 5 ml of dry pyridine was added dropwise. The reaction mixt was allowed to stand at 5° overnight, poured into ice H_2O, and extd (Et_2O). The Et_2O layer was washed (H_2O), dried (Na_2SO_4), and filtered, and Et_2O was removed under reduced pressure affording 0.814 g of white solid. Recrystn from Me_2CO afforded 0.670 g (69%) of **17**, mp 155–156° dec. *Anal.* ($C_{30}H_{52}O_6S$) H, S; C: calcd, 66.38; found, 67.17.

5α,6α-Epoxy-5α-cholestan-3β-ol (18).—5α-Cholestane-3β,5α,-6β-triol 3-ethylcarbonate 6-methanesulfonate[2] (20 g, 3.5×10^{-2} mole, **26**) was dissolved in 250 ml of hot EtOH. Alcoholic KOH (200 ml, 10%) was added, and the reaction mixt was refluxed for 2 hr and cooled to room temp. It was neutralized with glacial HOAc and extd (Et_2O). The Et_2O layer was washed 3 times (H_2O) and dried (Na_2SO_4), and Et_2O was removed under reduced pressure affording 13.0 g of white residue. Recrystn twice from MeOH yielded 9.5 g (64%) of **18**: mp 141–142°; lit.[27] mp 142.5°.

5α,6α-Epoxy-5α-cholestan-3β-ol Methanesulfonate (19).—α-Epoxide **18** (0.50 g, 1.2×10^{-3} mole) in 25 ml of dry pyridine was cooled on a salt–ice H_2O bath. MsCl (0.5 ml) in 5 ml of dry pyridine was added dropwise with stirring. The reaction mixt was allowed to stand at −5° overnight, poured into ice H_2O, and extd (Et_2O). The Et_2O layer was washed 3 times with H_2O, dried (Na_2SO_4), and filtered, and Et_2O was removed under reduced pressure affording 0.550 g of white product. Recrystn twice from Me_2CO yielded 0.20 g (34%) of **19**, mp 144–148° (dec 165°). This product was identical in all respects with the methanesulfonate **19** obtd from triol 1.

5α,6α-Epoxy-5α-cholestan-3β-ol Methanesulfonate (19) from 5α-Cholestane-3β,5α,6β-triol (1).—Compd 1 (5.0 g, 1.2×10^{-2} mole) in 100 ml of dry pyridine was cooled in an ice bath. MsCl (10 ml) in 20 ml of dry pyridine was added dropwise with stirring. The mixt was allowed to stand at room temp overnight, poured into ice H_2O, and extd (Et_2O). The Et_2O layer was washed (H_2O), filtered, and evapd under reduced pressure affording 5.0 g of white residue. Recrystn twice from Me_2CO afforded 3.5 g (51%) of white crystals, mp 146–150° (dec 167°). *Anal.* ($C_{28}H_{48}O_4S$) H, S; C: calcd, 70.00; found, 69.27.

3α-Azido-5α,6α-epoxy-5α-cholestane (20).—Mesylate **19** (2.00 g, 4.2×10^{-3} mole) and NaN_3 (6.00 g, 9.1×10^{-2} mole) in 50 ml of DMF were heated and stirred at 90° for 24 hr. The reaction mixt was poured into salt–ice H_2O and extd (Et_2O). The Et_2O layer was washed (H_2O), dried (Na_2SO_4), filtered, and evapd under reduced pressure affording 1.62 g of cryst residue. Recrystn from Me_2CO yielded 1.35 g (75%) of needles of **20**, mp 134–135°. *Anal.* ($C_{27}H_{45}N_3O$) C, H, N.

3α-Amino-5α-cholestane-5α,6β-diol 6-Acetate (22).—Azide **16** (0.40 g, 8.2×10^{-4} mole) was converted to the amine **22** by the hydrazine–Raney Ni reduction previously discussed, except that 1.5 ml of hydrazine hydrate was used. The reaction product (0.370 g, 90%) would not cryst. *Anal.* (crude product) ($C_{29}H_{51}NO_3$) C, H, N.

Amino acetate **22** was treated with Ac_2O in pyridine under the usual reaction conditions affording amide **21** which was identical in all respects with the amide prepd by other methods.

6-Oximino-5α-cholestane-3β,5α-diol (24) was synthesized from ketone **23** by the method of Shoppee,[12] mp 188–189°, lit.[12] mp 170/190–192° (double mp), lit.[13] 248–250°.

6β-Amino-5α-cholestane-3β,5α-diol (25) from 6-Oximino-5α-cholestane-3β,5α-diol (24).—Amine **25** was prepared by LAH reduction as described by Shoppee.[12] The amine prepd by this method was very difficult to cryst: mp 196–199°; lit.[12] mp 199–200°.

6β-Amino-5α-cholestane-3β,5α-diol (25) from 6β-Amino-5α-cholestane-3β,5α-diol 3-Acetate (29).—Amine **25** was prepd by hydrolysis of acetate **29** as described by Ponsold:[6] mp 198–199°; lit.[6] mp 199–200°.

6β-Azido-5α-cholestane-3β,5α-diol 3-Ethylcarboxylate (27).—

(27) A. Fürst and F. Koller, *Helv. Chim. Acta*, **30**, 1454 (1947).

Mesylate **26** (0.50 g, 8.75×10^{-4} mole) and NaN_3 (0.550 g, 7.7×10^{-3} mole) in 50 ml of DMF were heated with stirring to 100° overnight. The reaction mixt was poured into salt–ice H_2O and extd (Et_2O). The Et_2O layer was washed (H_2O), dried (Na_2SO_4), filtered, and evapd under reduced pressure affording 0.360 g of white crystals. Recrystn twice from MeOH afforded 0.290 g (62%) of **27**, mp 128–130°. *Anal.* ($C_{30}H_{51}N_3O_4$) H, N, C: calcd, 69.59; found, 70.48.

6β-Azido-5α-cholestane-3β,5α-diol 3-Acetate (28).—Cathylate **27** (2.80 g, 5.4×10^{-3} mole) was dissolved in 100 ml of hot EtOH. Aq NaOH (10 ml 10%) was added, and the reaction mixt was refluxed for 2 hr cooled, neutralized with aq HCl (10%), and extd (Et_2O). The Et_2O layer was washed (H_2O), dried (Na_2SO_4), filtered, and removed under reduced pressure affording 2.40 g of white residue. The reaction product was acetylated under the usual condns of Ac_2O in pyridine yielding 2.45 g of **28**. Recrystn twice from MeOH afforded 2.00 g (76%) of **28**: mp 154–155°; lit.[6] mp 154°.

6β-Amino-5α-cholestane-3β,5α-diol 3-Acetate (29) from 6β-Azido-5α-cholestane-3β,5α-diol 3-Acetate (28).—The method of Ponsold[6] was used to prep **29**: mp 190–191°; lit. mp 190–191°.

3,6-Dioximino-5α-cholestan-5α-ol (31).—5α-Cholestane-3,6-dion-5α-ol[2] (4.5 g, 1.1×10^{-2} mole, **30**) and 10.0 g (0.144 mole) of $HONH_2 \cdot HCl$ were suspended in 50 ml of abs EtOH and 20 ml of dry pyridine. The reaction mixt was refluxed for 7 hr, poured into ice H_2O, and extd (Et_2O). The Et_2O layer was washed (H_2O), dried (Na_2SO_4), filtered, and evapd under reduced pressure affording 4.7 g of a white residue. Recrystn twice from MeOH afforded 3.6 g (74%) of **31**, mp 188–190° dec. *Anal.* ($C_{27}H_{46}N_2O_3$) C, H, N.

3β-6β-Diamino-5α-cholestan-5α-ol (32).—Compd **31** (1.00 g, 2.2×10^{-3} mole) in 75 ml of Et_2O was added dropwise with stirring to LAH (1.00 g, 2.9×10^{-2} mole) in 50 ml of Et_2O at 0°. The reaction mixt was stirred at room temp for 24 hr. Excess LAH was decompd by dropwise addn of 1 ml of NaOH (10%) and 4 ml of H_2O. The filtrate was collected and the ppt was extd twice with THF. The combined filtrate was distd under reduced pressure affording 0.50 g (47%) of white product which crystd with difficulty from MeOH–H_2O, affording the diamine **32**, mp 145–148°. *Anal.* ($C_{27}H_{50}N_2O$) C, H, N.

3β,6β-Diacetamido-5α-cholestan-5α-ol (33).—Ac_2O (5.0 ml) in 5.0 ml of dry pyridine was added to the diamine **32** (0.01 g, 1.5×10^{-3} mole) dissolved in 2[illegible] ml of dry pyridine. The reaction mixt was allowed to stand at room temp overnight, poured into ice H_2O, and extd (Et_2O). The Et_2O layer was washed (H_2O), dried (Na_2SO_4), filtered, and removed under reduced pressure affording 0.56 g of cryst residue. Recrystn from Et_2O afforded 0.31 g (42%) of diamide **33**, mp 119–120°. *Anal.* ($C_{31}H_{54}N_2O_3$) C, H; N: calcd, 6.69; found, 6.10.

Biological studies *in vitro* were carried out according to methods previously reported by Dempsey and coworkers.[16,17,23,28]

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(28) M. E. Dempsey, *Methods Enzymol.*, **15**, 501 (1969).

Potential Folic Acid Antagonists. 5. Synthesis and Biologic Evaluation of N^{10}-Deazapteroic Acid and N^{10}-Deazafolic Acid and Their 9,10-Dehydro Derivatives[1,2]

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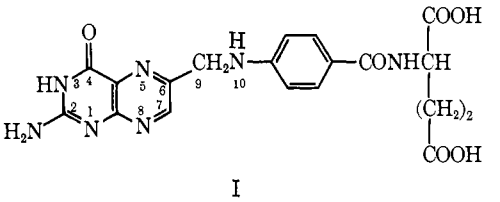
9,10-Dehydro-N^{10}-deazapteroic acid (V) was synthesized from 2-acetamido-6-formylpteridin-4(3*H*)-one (II) and the ylide from *p*-carbomethoxybenzyltriphenylphosphonium bromide by means of a Wittig reaction. Reduction of the acetylated methyl ester of V (IV) gave, after hydrolysis, N^{10}-deazapteroic acid (VI). Coupling of the acetylated derivatives of V and VI with diethyl glutamate by use of dicyclohexylcarbodiimide gave 9,10-dehydro-N^{10}-deazafolic acid (IX) and N^{10}-deazafolic acid (XII), respectively, after removal of acetyl and ester blocking groups. N^{10}-Deazafolic acid (XII), after reduction to the dihydro form, was shown to serve as a substrate for dihydrofolate reductase, whereas the unreduced acid XII was mildly inhibitory of the enzyme but did not serve as a substrate. The antagonistic effect of the analog XII was weak, as demonstrated by facile reversal of growth inhibition of *Streptococcus faecalis* by folic acid. No significant *in vitro* activity against human epidermoid cells or *in vivo* activity against leukemia L1210 was observed for the folic and pteroic acid analogs. Evaluation of the pteroic acid analogs in *Plasmodium berghei* revealed no antimalarial activity.

Chemistry.—Analogs of folic acid (I) are of interest for the determination of the structural features necessary for binding and inhibiting the enzymes involved in folic acid metabolism.[3] Replacement of the N in position 10 of folic acid (I) with CH_2 would alter the nucleophilicity of this portion of the molecule without significantly changing its steric properties. In addition, lacking the N^5,N^{10}-grouping, the compound would be incapable of being formylated and, therefore, functioning as a 1-C transfer intermediate.[4]



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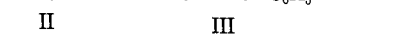

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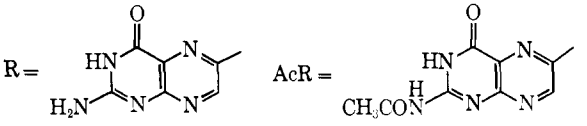

A potentially facile synthesis of N^{10}-deaza analogs of pteroic acid involved reaction of 2-acetamido-2-for-

(1) This investigation was supported by Contract NIH-71-2021 with Chemotherapy, National Cancer Institute, National Institutes of Health.

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