ing stilbazole. After several recrystallizations from alcohol, 1.1 g. (45%) of the stilbazole was obtained which melted at 133–133.5° dec. (lit.[11] 136°).

(11) K. Feist, *Ber.*, **34**, 466 (1901).

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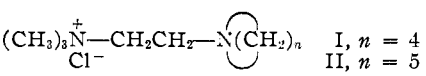
Nature of the Acetyl Cholinesterase Surface. II. The Ring Effect in Enzymatic Inhibitors of the Substituted Ethylenediamine Type[1]

BY S. L. FRIESS AND W. J. MCCARVILLE

RECEIVED JANUARY 13, 1954

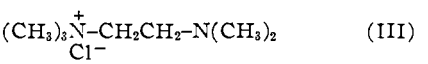
In a recent study[2] it was observed that compounds of the general type



I, $n = 4$
II, $n = 5$

are potent competitive inhibitors of the catalyzed hydrolysis of acetylcholine (AC) by the enzyme acetylcholinesterase (AChE). A general interpretation of the high order of activity of these inhibitors, comparable to or greater than that shown by the powerful anticholinesterase eserine, was based on the duality of catalytic enzymatic sites proposed by Nachmansohn and co-workers,[3] and the added working stipulation that the molecular region attracted to the so-called "esteratic" site on the enzyme is exclusively nucleophilic in its properties.[4]

The variation of enzyme–inhibitor dissociation constants with the size of the heterocyclic ring[2] in compounds I and II ($K_I = 2.3$ and 1.6×10^{-8}, respectively, at *p*H 7.4 and 25°) has now led to the study of the effectiveness of III as an AChE inhibitor.



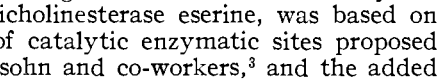
(III)

In compound III the constraint imposed on the valence angles about the ring N atom in I and II has been removed by cleavage of the ring, leaving a pair of methyl groups attached to this tertiary nitrogen center of high electron density.

The net result of this structural change is a marked diminution in the ability of III to compete with substrate for the catalytic sites on the enzyme, as compared with I and II. Under the standard conditions previously employed,[2] the value of the enzyme—inhibitor dissociation constant (K_I) deduced from the linear plot of the Wilson equation[5] was found to be $(8.3 \pm 0.6) \times 10^{-8}$. Using K_I values under comparable conditions as an index of the binding capacity of inhibitors for enzyme, this would imply that III is less effective than compounds I and II by factors of 3.5 and 5.2, respectively.

This decrease in inhibitory activity resulting from ring cleavage could arise from several structural features. First, the somewhat greater steric requirements of the $-\ddot{N}<$ function in III, as compared with I and II where the ring *o*-methylene groups are held back under greater restraint, might be approaching the limit of the effective dimensions of the cavitation available at the "esteratic" site. Secondly, it is possible that van der Waals interaction of the entire cyclic moiety of I and II with the site and its environs supplements the previously postulated electrostatic interaction of the localized high electron density function $-\ddot{N}<$ with the site to give increased strength of bonding over that shown by III. Both of these views assume direct interaction of the tertiary end of the inhibitor molecule with the surface of the site, and make no allowance for an intermediary spatial role of the Mg^{++} ion[2] at this point.

In the course of work on III, the chloro derivative of choline



(IV)

came to hand as a synthesis intermediate. Since its primary chloro function also fulfills the condition of serving as a localized region of high electron density, this compound too was tested for its inhibitory power on AChE. It proved to be a surprisingly effective competitive inhibitor, with a K_I value (at *p*H 7.0 and 25.12°) of 12×10^{-8}. This makes it less potent than the series I, II, III and eserine, but more effective than the prominent anticholinesterase prostigmine ($K_I = 16 \times 10^{-8}$).[3]

This relatively high level of activity of IV lends further support to the primary assumption that the non-quaternized end of the $Me_3\overset{+}{N}-CH_2CH_2$ chain need only possess a central locus of high electron density to complete the requirements for effective bonding at the esteratic site and produce a potent competitive inhibitor.

Experimental[6]

The known chloro derivative of choline chloride was prepared in standard fashion from crystalline choline chloride and Eastman thionyl chloride. The product was recrystallized repeatedly from methanol–ether mixture as the anhydrous salt, at the temperature of a Dry Ice–methanol-bath. *Anal.* Calcd. for $C_5H_{13}NCl_2$: N, 8.86. Found: N, 8.89.

Compound III was prepared by heating the chloro derivative above with approximately a fivefold excess of anhydrous dimethylamine, in a glass bomb at 100°, for a period of 24 hours. The salt remaining after evaporation of the excess amine was recrystallized repeatedly from a 1:1 methanol-ether mixture containing several drops of concd. hydrochloric acid per liter. It proved to be relatively hygroscopic; dec. above 235°. *Anal.* Calcd. for $C_7H_{19}N_2Cl \cdot HCl$: N, 13.79. Found: N, 13.97.

Enzymatic Rate Determinations.—The stock enzyme preparation used was the highly-purified one previously prepared[2] from electric eel tissue. Dilutions of the stock were made just prior to use in the kinetic determinations.

(1) Presented in part before the Division of Biological Chemistry, Meeting of the American Chemical Society, Kansas City, Mo., March, 1954. The opinions in this paper are those of the authors and do not necessarily reflect the views of the Navy Department.

(2) S. L. Friess and W. J. McCarville, THIS JOURNAL, **76**, 1363 (1954).

(3) See for example the review by D. Nachmansohn and I. B. Wilson, *Advances in Enzymol.*, **12**, 259 (1951).

(4) This constitutes a departure from the conclusion of I. B. Wilson that a basic group in the esteratic site is responsible for the nucleophilic binding to a relatively *positive* center (such as a carbonyl carbon) on a substrate or inhibitor. See I. B. Wilson, *J. Biol. Chem.*, **197**, 215 (1952).

(5) P. W. Wilson, "Respiratory Enzymes," H. A. Lardy, ed., Burgess, Minneapolis, Minn., 1949, p. 24.

(6) Analyses by courtesy of Dr. W. C. Alford, Microanalytical Laboratory, National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.

These hydrolysis runs, with and without the presence of added inhibitor, were carried out in the phosphate buffer (*p*H 7.0) as previously described.[2] All inhibitor dilutions and acetylcholine solutions were freshly prepared before use in the standard sequence of kinetic determinations (at 25.12°) employing a series of inhibitor concentrations varying over the range of $1\text{–}10 \times 10^{-7}$ *M*.

The Wilson plot of v/v_I *vs.* concentration for each of the inhibitors III and IV was linear over this concentration range, with least squares fits of about ±6% paralleling the observed magnitudes of precision of the slopes of individual rate plots. The velocity values *v* were obtained as the slopes of these rate plots for the first six to seven minutes of reaction, corresponding to about 10% completion of AC hydrolysis.

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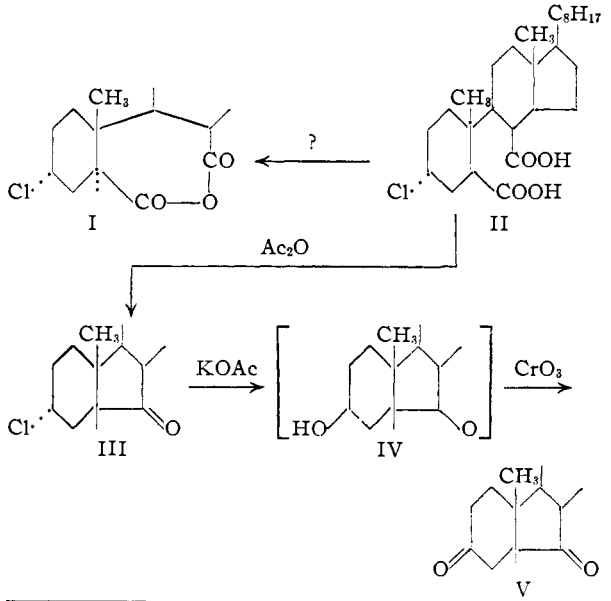
3α-Chloro-B-norcoprostane-6-one

BY MARCEL GUT

RECEIVED JANUARY 16, 1954

A few years ago we were interested in preparing 3α-chloro-6,7-secocholestane-6,7-dioic acid anhydride (I). Windaus and Stein[1] reported that 3α-chloro-6,7-secocholestane-6,7-dioic acid (II)[2] formed a crystalline anhydride melting at 187°. No further data on this compound were reported. A re-examination of this reaction produced a substance of the indicated melting point, the structure of which was, however, established to be 3α-chloro-B-norcoprostane-6-one (III). Therefore, this ring closure proceeded according to Blanc's rule.[3]

In a recent publication Fieser[4] reinterpreted Butenandt's oxidation and subsequent ring closure of 3,6-diketocholestene yielding B-norcoprostane-3,6-dione (V).[4,5] This prompted us to transform the chloro analog into the known diketone V. The chloro compound III obtained by the ring closure was easily transformed into V and its identity established by comparison with authentic material.[6] 3α-Chloro-B-norcoprostane-6-one (III) was converted under conditions employed by Marker[7] in similar cases to 3-hydroxy-B-norcoprostane-6-one (IV) (mainly the β-epimeride[8]) and the crude mixture was oxidized with chromic anhydride to the known B-norcoprostane-3,6-dione (V).



(1) A. Windaus and G. Stein, *Ber.*, **37**, 3699 (1904).

(2) At that time designated as 3β-chlorocholestane-6,7-dicarboxylic acid.

(3) H. G. Blanc, *Compt. rend.*, **144**, 1356 (1907).

(4) L. F. Fieser, THIS JOURNAL, **75**, 4386 (1953).

(5) A. Butenandt and E. Hausmann, *Ber.*, **70**, 1154 (1937).

Experimental

A solution of 500 mg. of 3α-chloro-6,7-secocholestane-6,7-dioic acid (II) in 15 ml. of acetic anhydride was heated on a steam-bath for 2 hours. Then the mixture was poured into ice, let stand for 2 hours, the solids filtered off, and finally recrystallized from methanol and dried; yield 215 mg., m.p. 180–183°. After sublimation at 140° (0.01 mm.) and recrystallization from acetone the melting point rose to 184–186°, α_D +10.3° Chf (*c* 0.58).

Anal. Calcd. for $C_{26}H_{43}OCl$: C, 76.71; H, 10.65; Cl, 8.71. Found: C, 76.72; H, 10.54; Cl, 8.60.

Treatment of 100 mg. of the above ketone and 450 mg. of potassium acetate in 3 ml. of valeric acid under the same conditions as used by Marker, *et al.*,[7] gave crude 3-hydroxy-B-norcoprostane-6-one (IV), which was oxidized with chromic anhydride in acetic acid. The resulting mixture gave after chromatographing 11 mg. of B-norcoprostane-3,6-dione (V),[4,5] m.p. 114–116°. The diketone could not be isomerized with alkali and the melting point was unchanged after admixture of authentic material.[6] The infrared spectrum shows two carbonyl bands at 5.76 and 5.81 μ.

Acknowledgment.—Thanks are due to the Damon Runyon Fund, U. S. Public Health Service (Grant No. C321) and to the American Cancer Society for an institutional grant which made this research possible.

(6) Obtained through the courtesy of Dr. L. F. Fieser.

(7) R. E. Marker, F. C. Whitmore and O. Kamm, THIS JOURNAL, **57**, 2358 (1935).

(8) C. W. Shoppee, *J. Chem. Soc.*, 1032 (1948).

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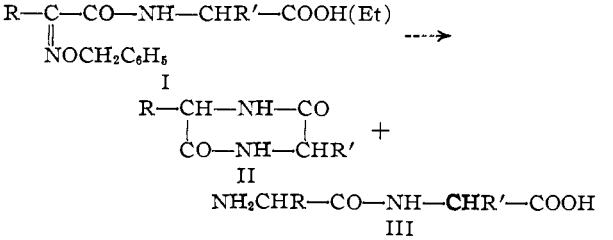
Synthesis of Peptides *via* α-Benzyloximino Acids[1]

BY WALTER H. HARTUNG,[2] DAVID N. KRAMER AND GEORGE P. HAGER

RECEIVED NOVEMBER 14, 1953

Weaver and Hartung[3] found that catalytic hydrogenation of N-benzyloximinoacylamino acids or their esters, I, in acidic media produced predominantly diketopiperazines, II. Nevertheless, the formation of small amounts of the dipeptide, III, indicated that under other conditions compounds of structure I may prove useful in the synthesis of peptides.



In the earlier conversion of oximes into primary amines acidic media were always employed.[4]

(1) No. 14 in amino acid series; for no. 13 see J. H. R. Beaujon and W. H. Hartung, THIS JOURNAL, **75**, 2499 (1953).

(2) University of North Carolina, Chapel Hill, North Carolina.

(3) W. E. Weaver and W. H. Hartung, *J. Org. Chem.*, **15**, 741 (1950).

(4) W. H. Hartung, THIS JOURNAL, **53**, 2248 (1931).