perchloric acid. After 3 days at 25° saturated sodium chloride was added and the mixture extracted with chloroform. Paper chromatography of the crude crystalline product showed it to consist of 2 substances both considerably more polar than Vb. The major and more polar component (isolated by fractional crystallization from acetone–ether) was 12α-hydroxycorticosterone (Xa), m.p. 208–212°, $[\alpha]_D$ +194°, λ_{max} 241 mμ (15,900); λ_{max}^{Nujol} 2.90, 3.02, 5.84, 6.08 and 6.18 μ.

Anal. Calcd. for $C_{21}H_{30}O_5$: C, 69.58; H, 8.34. Found: C, 69.97; H, 8.47.

The minor component, m.p. 175–177°, was assigned the structure 12α-hydroxycorticosterone 21-acetate (Xb) on the basis of its polarity.

12α-Hydroxycorticosterone 12α,21-Diacetate (Xc).—Acetylation of 120 mg. of the perchloric acid product (Xa and a minor amount of Xb) in 3 ml. of pyridine and 2 ml. of acetic anhydride for 16 hr. at 25° followed by chromatography on neutral alumina gave 95 mg. of the 12α,21-diacetate Xc, m.p. 221–223°; λ_{max}^{Nujol} 2.90, 5.70, 5.79, 5.98 and 6.16 μ as well as 16 mg. of Xb. Thus the 12α-hydroxyl group appears to be partly resistant to acetylation under these conditions.

12α-Hydroxy-11-dehydrocorticosterone 12α,21-Diacetate (XI).—To 90 mg. of the 11β-hydroxy-12α,21-diacetate Xc in 5 ml. of acetic acid was added 17 mg. of chromic oxide in 0.1 ml. of water and 0.5 ml. of acetic acid. After 16 hr. at 20°, 5% sodium sulfate solution was added and the mixture extracted with chloroform. The solid residue on crystallization from acetone–ether gave the 11-keto-12α,21-diacetate XI as prismatic plates (70 mg., 1st crop), m.p. 171–172°, $[\alpha]_D$ +241°; λ_{max} 238 mμ (16,000); λ_{max}^{Nujol} 5.71, 5.80, 5.97 and 6.12 μ.

Anal. Calcd. for $C_{25}H_{32}O_7$: C, 67.54; H, 7.26. Found: C, 67.29; H, 7.06.

RAHWAY, N. J.

[CONTRIBUTION FROM THE MERCK-SHARP & DOHME RESEARCH LABORATORIES]

The Transformation of Stigmasterol to 17α-Hydroxypregnenolone and 17α-Hydroxyprogesterone

BY E. M. CHAMBERLIN, E. TRISTRAM, T. UTNE AND J. M. CHEMERDA

RECEIVED JULY 25, 1956

The transformation of stigmasterol to 17α-hydroxypregnenolone and 17α-hydroxyprogesterone is described. Stigmasterol acetate is hydrochlorinated to 5α-chlorostigmasterol acetate which on ozonization gives 3β-acetoxy-5α-chlorobisnorcholan-22-al. This aldehyde on bromination and dehydrobromination at C-20 gives 3β-acetoxy-5α-chloro-17(20)-bisnorcholen-22-al. The α,β-unsaturated aldehyde on treatment with excess peracid gives 3β-acetoxy-5α-chloro-17,20-epoxypregnan-20-ol 20-formate which compound on alkaline hydrolysis gives 17α-hydroxypregnenolone. Acid hydrolysis of the epoxy formate followed by oxidation with chromic acid–pyridine complex and dehydrohalogenation with base gives 17α-hydoxyprogesterone.

The importance of bio-oxidative methods for the introduction of oxygen into ring C of steroids[1] has focused attention on the synthesis of substrates (*e.g.*, progesterone, Compound S) for this oxidation. The most abundant raw materials for this purpose are stigmasterol, ergosterol and diosgenin. Several simplified and improved methods of preparation of progesterone and pregnane-3,20-dione from the first two sterols have been reported.[2] A more valuable substrate than progesterone or pregnane-3,20-dione would result from a degradation procedure which resulted in the introduction of the 17α-hydroxy group in the final product without materially increasing the number of steps in the process. This paper describes such a procedure utilizing stigmasterol as a raw material. As a final product either 17α-hydroxypregnenolone or 17α-hydroxyprogesterone may be obtained. The reaction scheme is formulated in the flow sheet.

This scheme required the C-22 aldehyde from stigmasterol with the 5,6-double bond protected. In the usual ozonization of stigmasterol to obtain the C-22 aldehyde the 5,6-double bond is protected as the 5,6-dibromide. Since subsequent decomposition of the ozonide by zinc dust removes the bromine, this practice was not useful for our purpose. Although the 5,6-dichloro derivative of stigmasterol acetate offered a more attractive prospect, it proved impracticable to test since partial chlorination could not be achieved. It was found that the 5α-chloro compound I obtained by the addition of hydrogen chloride to stigmasterol acetate was stable to the zinc dust treatment for the decomposition of the ozonide and this compound I represents the key intermediate in the degradation.

5α-Chlorostigmasterol acetate (I) had been prepared previously by Ruzicka, Fischer and Meyer[3] by hydrochlorination in ether. The low solubility of stigmasterol acetate in ether is disadvantageous. Hydrochlorination proceeds slowly in chloroform because of the low solubility of hydrogen chloride. Finally a mixture of chloroform–ethanol saturated with hydrogen chloride at 5–10° was found to be an excellent medium for the hydrochlorination of stigmasterol acetate in good, reproducible yields. During the 70-hour period at room temperature, partial deacetylation is effected by ester interchange and a reacetylation is required. The steric orientation of the chlorine atom to the 5α (axial) position was assigned by analogy with Barton's[4] results on the stereochemistry of the ionic addition of chlorine to cholesterol acetate. Fur-

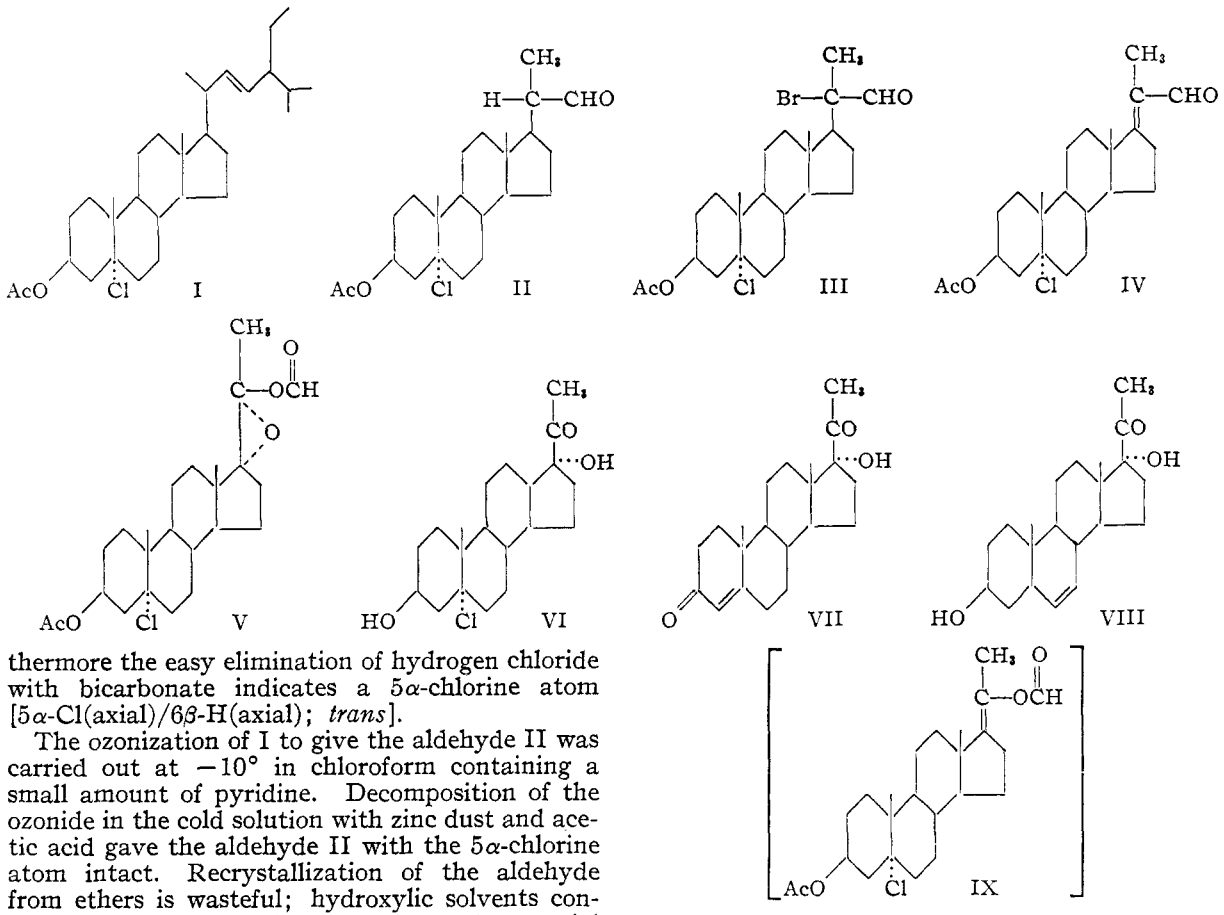
(1) D. R. Colingsworth, M. P. Brunner and W. J. Haines, THIS JOURNAL, **74**, 2381 (1952); D. H. Peterson and H. C. Murray, *ibid.*, **74**, 1871 (1952); D. H. Peterson, H. C. Murray, S. H. Eppstein, L. M. Reineke, A. Weintraub, P. D. Mister and H. M. Leigh, *ibid.*, **74**, 5933 (1952); O. Mancera, A. Zaffaroni, B. A. Rubin, F. Sondheimer, G. Rosenkranz and C. Djerassi, *ibid.*, **74**, 3711 (1952); J. Fried, R. W. Thoma, J. R. Gerke, J. E. Herz, M. N. Donin and D. Perlman, *ibid.*, **74**, 3962 (1952).

(2) F. Johnson, G. T. Newbold and F. S. Spring, *J. Chem. Soc.*, 1302 (1954); A. F. Daglish, J. Green and V. D. Poole, *Chemistry & Industry*, **45**, 1207 (1953); *J. Chem. Soc.*, 2627 (1954); D. A. Shepheard, R. A. Donin, J. Allan Campbell, B. A. Johnson, R. P. Holysz, G. Slomp, Jr., J. F. Stafford, R. L. Pederson and A. C. Ott, THIS JOURNAL, **77**, 1212 (1955); G. Slomp, Jr., Y. F. Shealy, J. L. Johnson, R. A. Donin, B. A. Johnson, R. P. Holysz, R. L. Pederson, A. O. Jensen and A. C. Ott, *ibid.*, **77**, 1216 (1955).

(3) L. Ruzicka, W. Fischer and Jul. Meyer, *Helv. Chim. Acta*, **18**, 1483 (1935).

(4) D. H. R. Barton and E. Miller, THIS JOURNAL, **72**, 370 (1950).

FLOW SHEET



thermore the easy elimination of hydrogen chloride with bicarbonate indicates a 5α-chlorine atom [5α-Cl(axial)/6β-H(axial); *trans*].

The ozonization of I to give the aldehyde II was carried out at −10° in chloroform containing a small amount of pyridine. Decomposition of the ozonide in the cold solution with zinc dust and acetic acid gave the aldehyde II with the 5α-chlorine atom intact. Recrystallization of the aldehyde from ethers is wasteful; hydroxylic solvents convert it to acetals or solvates. The crude material was found to be satisfactory for subsequent use without purification.

The 20-bromoaldehyde III was prepared easily by bromination of II in chloroform in the presence of powdered calcium carbonate.[5]

Selective dehydrobromination of the 5α-chloro-20-bromo compound III proved possible despite the relative ease of dehydrochlorination of the 5α-chloro compound, *e.g.*, treatment of the bromoaldehyde with lithium chloride in dimethylformamide at 85° removed both the 5α-chlorine and the 20-bromine.[6] A smooth dehydrobromination method was found during a study of the effect of solvents on the bromination of the aldehyde II. The aldehyde II reacted slowly with bromine in dimethylformamide at room temperature. After several days, the isolated product proved to be IV rather than the expected chlorobromo derivative III. Subsequent experiments with III showed that hydrogen bromide in dimethylformamide selectively and efficiently catalyzes the dehydrobromination of the bromoaldehyde in preference to the dehydrochlorination of the 5α-chloro substituent. The transformation of the aldehyde to the α,β-unsaturated aldehyde was best accomplished without isolation of the intermediate bromo-compound when the bromination was carried out in the presence of *p*-toluenesulfonic acid. The α,β-unsaturated aldehyde could then be isolated directly in high yield.

The introduction of the 17α-hydroxyl group and the 20-carbonyl group was accomplished by treatment of the α,β-unsaturated aldehyde IV with excess monoperphthalic acid followed by hydrolysis. The peracid afforded the epoxy formate V. Alkaline hydrolysis of this compound gave 17α-hydroxypregnenolone (VIII). When the epoxy compound V was subjected to hydrolysis with concentrated hydrochloric acid in methanol the product was 5α-chloropregnane-3β,17α-diol-20-one (VI).

We believe that the present case is the first report of the transformation of an α,β-unsaturated steroidal aldehyde to the epoxy formate. However, in the case of α,β-unsaturated carbonyl compounds in the aromatic series, von Wacek and von Bézard[7] obtained the monoformate ester of pyrocatechol from salicylaldehyde with peracetic acid. Furthermore Böeseken, *et al.*,[8] transformed benzalacetone to the enol acetate of phenylacetaldehyde with peracetic or perbenzoic acid. Reasoning by analogy from the example cited, one might expect that the peracid treatment of IV would have given the enol formate IX, a compound similar in structure to the enol acetate prepared in a different way

(5) I. Heilbron, E. R. H. Jones, R. W. Richardson and F. Sondheimer, *J. Chem. Soc.*, 737 (1949).

(6) R. P. Holysz, THIS JOURNAL, **75**, 4432 (1953).

(7) A. von Wacek and A. von Bézard, *Ber.*, **74**, 845 (1941).

(8) J. Böeseken and A. Kremer, *Rec. trav. chim.*, **50**, 827 (1931); J. Böeseken and A. L. Soesman, *ibid.*, **52**, 874 (1933); J. Böeseken and J. Jacobs, *ibid.*, **55**, 786 (1936).

by Kritchevsky and Gallagher[9] for the 17α-hydroxylation of cortical steroids. However the isolated product was in fact the 17,20-epoxy-20-formate (V). The data do not allow us to decide whether IX is an intermediate in the formation of V or whether the 17,20-epoxy aldehyde is produced first and then reacts further to give the epoxy formate.

The oxidation of the 3-hydroxyl group of VI to the 3-ketone proved difficult. Oxidation was eventually accomplished by the use of pyridine–chromic acid complex.[10] This difficult oxidation of C-3 is probably a reflection of the steric hindrance of hydrogen on C-3 to attack by the reagent due to shielding by the C-5 chlorine atom. Fudge, Shoppee and Sommers[11] have experienced a similar difficulty in the oxidation of a hydroxyl at C-3 when C-5 was substituted by hydroxyl or acetoxyl (*i.e.*, 5-hydroxycholestan-3β-ol and 5-acetoxycholestan-3β-ol).

Treatment of the oxidation product of VI with alkali without isolation gave 17α-hydroxyprogesterone identical in all respects with an authentic sample.

Acknowledgments.—The authors are indebted to Messrs. R. Walker and N. Trenner for the infrared spectra and Mr. R. N. Boos and staff for the analytical data.

Experimental

Melting points are uncorrected. Rotations were taken at 25 ± 1°.

5α-Chlorostigmasterol Acetate (I).—Thirty-two hundred milliliters of reagent chloroform was diluted with 800 ml. of absolute ethanol, cooled to 10° and saturated with hydrogen chloride (~2.7 molar). Stigmasterol acetate (100 g., 0.298 mole) was dissolved in this solution and refrigerated at 8° for 67 hours. At the end of this period the reaction mixture was washed with ice-water and saturated sodium bicarbonate. The dried solution ($MgSO_4$) was concentrated *in vacuo* and the residue dissolved in 750 ml. of dry pyridine. Acetic anhydride (100 ml.) was added and the acetylation mixture was allowed to stand overnight at room temperature. The product which separated was filtered off and washed with methanol to give 76.5 g. (71% of theory) of 5α-chlorostigmasterol acetate, m.p. 180–184; recrystallized from ethyl acetate, m.p. 183–185 (lit.[3] m.p. 183–183.5°), $[\alpha]^{24}D$ −8.1° ($CHCl_3$).

Anal. Calcd. for $C_{31}H_{51}O_2Cl$: C, 75.78; H, 10.47; Cl, 7.22. Found: C, 76.02; H, 10.46; Cl, 7.49.

3β-Acetoxy-5α-chlorobisnorcholan-22-al (II).—Twenty-six grams of 5α-chlorostigmasterol acetate (0.053 mole) was dissolved in 1400 ml. of chloroform and 200 ml. was distilled off to dry the solution. Dry pyridine (25 ml.) was added and the solution cooled to −10 ± 5°. Ozone was passed through the solution at the rate of 1.4 millimoles/minute for 46 minutes (20% excess). At the end of this time 26 g. of zinc dust was added and 400 ml. of glacial acetic acid. The cooling bath was removed and the suspension stirred for 1 hour (starch–iodide test negative). The reaction mixture was washed with water, saturated bicarbonate, and dried over magnesium sulfate. The solvent was removed *in vacuo* to a small volume and taken up in 1 l. of ether. The ether solution was shaken with a suspension of 50 g. of anhydrous sodium carbonate and 50 ml. of water, decanted and shaken with a suspension of 25 g. of anhydrous sodium carbonate and 25 ml. of water, again decanted and dried over magnesium sulfate. Removal of the ether *in vacuo* afforded 17 g. of crude aldehyde. A sample recrystallized from ether–ligroin showed a m.p. 172–176, $[\alpha]^{24}D$ −2.6 ($CHCl_3$); infrared 5.80 μ (OAc), 8.05 μ (Ac), 3.78 μ (–CHO).

Anal. Calcd. for $C_{24}H_{37}O_3Cl$: C, 70.47; H, 9.12; Cl, 8.67. Found: C, 70.36; H, 8.63; Cl, 8.56.

3β-Acetoxy-5α-chloro-20-bromobisnorcholan-22-al (III).—3β-Acetoxy-5α-chlorobisnorcholan-22-al (16 g., 0.0039 mole) was dissolved in 200 ml. of chloroform[12] and 5 g. of calcium carbonate was suspended in the solution. To the well-stirred suspension was added 42.5 ml. of a bromine solution (prepared by dissolving 6 g. of bromine in 50 ml. of chloroform) over a period of 1 hour. The reaction mixture was stirred 2–3 hours after bromine addition was complete, filtered, and the chloroform solution washed with saturated sodium bicarbonate. The dried solution (magnesium sulfate) was concentrated *in vacuo* under nitrogen. Addition of 95% ethanol caused the residue to crystallize. Filtered and washed with 95% ethanol, the yield of crude bromoaldehyde, m.p. 162–163° dec., was 11.8 g. (62% of theory). A sample recrystallized from 95% ethanol melted at 165.5–166.5° dec., $[\alpha]^{24}D$ +13.4° ($CHCl_3$); infrared spectrum 5.79 μ (OAc); –OH, –CHO not detected.

Anal. Calcd. for $C_{24}H_{37}O_3ClBr$: C, 58.95; H, 7.63. Found: C, 58.79; H, 7.43.

3β-Acetoxy-5α-chloro-17(20)-bisnorcholen-22-al (IV).

(a) **From III.**—Thirty-three grams (0.068 mole) of 3β-acetoxy-5α-chloro-20-bromobisnorcholan-22-al in 1320 ml. of dimethylformamide containing 5.5 g. (0.068 mole) of hydrogen bromide was allowed to stand at room temperature for 4 days. The solution was then stirred and cooled in an ice-bath while 600 ml. of water was added. After stirring for 3 hours the product was filtered and washed with much water and dried *in vacuo* to give 26.8 g. (96%) of α,β-unsaturated aldehyde, m.p. 194–195; λ_{max}^{MeOH} 2540 Å., $E_{1cm}^{1\%}$ 376, $[\alpha]^{24}D$ 0.0° ($CHCl_3$); infrared spectrum 5.79 μ (OAc), 6.0 μ, 6.15 μ, α,β-unsaturated carbonyl. Recrystallization from methanol did not change the physical constants.

Anal. Calcd. for $C_{24}H_{35}O_3Cl$: C, 70.82; H, 8.67; Cl, 8.71. Found: C, 70.51; H, 8.51; Cl, 8.58.

(b) **From II without Isolation of III.**—A solution containing 2.0 g. (0.005 mole) of 3β-acetoxy-5α-chlorobisnorcholan-22-al and 0.04 g. of *p*-toluenesulfonic acid monohydrate in 80 ml. of dimethylformamide was stirred at room temperature and treated over the course of three hours with 6.67 ml. of bromine solution (3.0 g. of bromine diluted to 25 ml. with dimethylformamide). After standing for 24 hours the light yellow reaction mixture was cooled in an ice-bath and quenched by the dropwise addition of 80 ml. of water. The crude product was aged for an hour in the refrigerator, filtered, washed with water and dried *in vacuo;* weight 1.86 g., m.p. 158–163° dec.; ultraviolet spectrum, λ_{max}^{MeOH} 2540 Å., $E_{1cm}^{1\%}$ 200. Several recrystallizations from acetone–water and then from ethanol raised the melting point to 191–193°; ultraviolet spectrum, λ_{max}^{MeOH} 2560 Å., $E_{1cm}^{1\%}$ 379.

3β-Acetoxy-5α-chloro-17,20-epoxypregnane-20-ol Formate (V).—A solution containing 15.7 g. (0.0386 mole) of 3β-acetoxy-5α-chloro-17(20)-bisnorcholen-22-al in 150 ml. of dry benzene was treated with 56 g. (0.309 mole) of monoperphthalic acid in 435 ml. of ethyl acetate at 10°. After two days at room temperature the insoluble phthalic acid was filtered off and the solution washed with 10% sodium bicarbonate solution. After drying (sodium sulfate) the solvent was removed *in vacuo* to give pure 3β-acetoxy-5α-chloro-17,20-epoxypregnane-20-ol formate 15.1 g. (89%), m.p. 139–142°, $[\alpha]^{24}D$ −5.2 ($CHCl_3$); infrared spectrum 5.79 μ (carbonyl), 8.0 μ (OAc); 8.5 μ (—OC(=O)—H).

Anal. Calcd. for $C_{24}H_{35}O_5Cl$: C, 65.66; H, 8.04; Cl, 8.08. Found: C, 65.64; H, 7.97; Cl, 8.12.

17α-Hydroxypregnenolone (VIII).—One gram (0.0024 mole) of 3β-acetoxy-5α-chloro-17,20-epoxypregnane-20-ol formate was dissolved in 150 ml. of 95% ethanol at 30° and treated with 30 ml. of 2.5 *N* sodium hydroxide solution. After 90 minutes at room temperature 6 ml. of glacial acetic

(9) T. H. Kritchevsky and T. F. Gallagher, THIS JOURNAL, **73**, 184 (1951).

(10) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, *ibid.*, **75**, 422 (1953).

(11) A. J. Fudge, C. W. Shoppee and G. H. R. Summers, *J. Chem. Soc.*, 958 (1954).

(12) There is an unevaluated factor in the chloroform used. It was found that some bottles of chloroform reacted with bromine very rapidly to give hydrogen bromide—such chloroform gives poor results in this reaction.

acid was added and the solution was partially concentrated under vacuum and diluted with 75 ml. of water. After aging overnight in the ice-box the product was filtered, washed with water and dried *in vacuo* to give 0.55 g. of crude 17α-hydroxypregnenolone, m.p. 200–215°. A sample recrystallized from ethanol showed a m.p. 205–210° and did not depress the melting point of an authentic sample of 17a-hydroxypregnenolone.

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.85; H, 9.70. Found: C, 75.41; H, 9.66.

5α-Chloropregnan-3β,17α-diol-20-one (VI).—A slurry of 2.5 g. (0.0057 mole) of 3β-acetoxy-5α-chloro-17,20-epoxypregnane-20-ol formate in 125 ml. of methanol was treated at room temperature with 12.5 ml. of concentrated hydrochloric acid. A greenish-blue color developed and within 1 hour solution was practically complete. After standing overnight the reaction mixture was cooled 1 hour in the refrigerator. The heavy precipitate of product was filtered, washed with methanol and dried *in vacuo;* yield 1 g., m.p. 200–205°. Slurrying with boiling ethyl acetate raised the melting point to 205–210°, $[\alpha]^{24}D$ +1 (pyridine); infrared spectrum 2.82 μ, 2.91 μ (OH), 5.90 μ (carbonyl).

Anal. Calcd. for $C_{21}H_{33}O_3Cl$: C, 68.36; H, 9.02; Cl, 9.61. Found: C, 68.33; H, 9.03; Cl, 9.51.

17α-Hydroxyprogesterone (VII).—Chromic anhydride–pyridine complex was formed by adding 1.3 g. (0.013 mole) of chromic anhydride in small portions with stirring to 13 ml. of pyridine while keeping the temperature below 25°. To this stirred slurry was added 1.3 g. (0.0035 mole) of 5α-chloropregnane-3β,17α-diol-20-one in 26 ml. of pyridine. After stirring overnight the brown solution was poured into 500 ml. of dilute sodium hydroxide solution. The precipitated product was aged 1 hour, filtered and washed with water. The product was dissolved in 25 ml. of pyridine and 50 ml. of methanol, filtered to remove insoluble material and treated with 10 ml. of 2.5 *N* sodium hydroxide solution at room temperature for 1 hour. The reaction mixture was then diluted with 100 ml. of water and refrigerated for 1 hour. The filtered product was washed with water and dried *in vacuo;* yield 0.6 g. (51%) of 17α-hydroxyprogesterone, m.p. 200–212°; ultraviolet spectrum λ_{max}^{MeOH} 2420 Å., $E_{1cm}^{1\%}$ 488. A sample recrystallized from ethanol melted at 213–218°, $[\alpha]^{CHCl_3}D$ +91°. Identity of the sample with authentic material was established by mixed melting point and identity of the infrared absorption curves. The authentic sample had the following characteristics, m.p. 212–215°, $[\alpha]^{CHCl_3}D$ +93.8°, λ_{max}^{MeOH} 2420 Å., $E_{1cm}^{1\%}$ 498, phase solubility 99.6%, (lit.[13] m.p. 212–215°, $[\alpha]^{CHCl_3}D$ +102°).

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.59; H, 9.17.

(13) J. J. Pfiffner and H. B. North, *J. Biol. Chem.*, **132**, 459 (1940); **139**, 855 (1941).

RAHWAY, NEW JERSEY

[CONTRIBUTION FROM THE U. S. NAVAL MEDICAL RESEARCH INSTITUTE AND THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH]

The Acetylcholinesterase Surface. VII. Interference with Surface Binding as Reflected by Enzymatic Response to Turicine, Betonicine and Related Heterocycles[1]

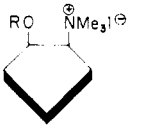
BY S. L. FRIESS, A. A. PATCHETT AND B. WITKOP

RECEIVED AUGUST 6, 1956

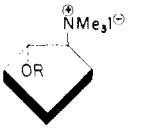
The response of the enzyme acetylcholinesterase to inhibition by the betaine amino alcohols D- and L-turicine (III, IV) and L-betonicine (V) is remarkably sensitive to the relative spatial orientations of the $-COO^-$ and –OH groups. When *trans* to one another as in V inhibition of the low order of strength shown by choline results, while in the *cis* derivatives III and IV no inhibition is observed even at concentrations two orders of magnitude higher. The anilide of V essentially repeats this picture, indicating that the effect of the 2-substituent on the pyrrolidine ring is steric and not electrostatic in character. This difference is discussed in terms of two-point attachment to the catalytic surface *via* the –OH and $-{}^+N\equiv$ functions, and steric interference with this binding when the 2-substituent is necessarily oriented toward the protein surface as compared with no hindrance to binding when the group is directed away from the surface. Additional information on the nature of the binding in these heterocycles comes from comparison of the relative inhibitory strengths of III, IV and V with those of the betaine of hydroxypipecolic acid (VIII), stachydrine (IX) and the methiodide of N-methylpyrrolidine (X). Measurements with compound IX in particular indicate that removal of the –OH function leads to a drop in inhibitory power as compared to betonicine. The acetates of betonicine and turicine appear to be accepted as substrates for the enzyme, but the enzymatic hydrolytic rates are not appreciably higher than those for the "uncatalyzed" water reaction of the betaine esters.

Introduction

Previous studies[2] have centered around a subtle variation in response of the enzyme acetylcholinesterase (AChE) to relatively weak reversible inhibitors of the choline type in the cyclohexane and cyclopentane series, as well as to the corresponding acetylated substrates. For example, the enzyme derived from electric eel tissue is inhibited somewhat more strongly by Ia than by IIa, and of the pair of substrates Ib and IIb the former evokes higher catalytic rates from the enzyme. These results were interpreted in terms of two-point binding to the enzymatic surface *via* the polar quaternized nitrogen function at one site and the –OH or –OAc function at the second site[3] of the catalytic unit.



Ia, R = H
Ib, R = Ac



IIa, R = H
IIb, R = Ac

On this assumption, it was of considerable interest to study the effect of steric influences in properly constituted inhibitors or substrates that might tend to impede this two-point adsorption process and lead to lower indices of enzymatic activity. One series of compounds ideally suited

(1) The opinions in this paper are those of the authors and do not necessarily reflect the views of the Navy Department.

(2) H. D. Baldridge, W. J. McCarville and S. L. Friess, THIS JOURNAL, **77**, 739 (1955); H. D. Baldridge and S. L. Friess, *ibid.*, **78**, 2482 (1956).

(3) For a summary of work leading to the postulation of a bifunctional catalytic unit on the enzymatic surface see D. Nachmansohn and I. B. Wilson, *Advances in Enzymol.*, **12**, 259 (1951).