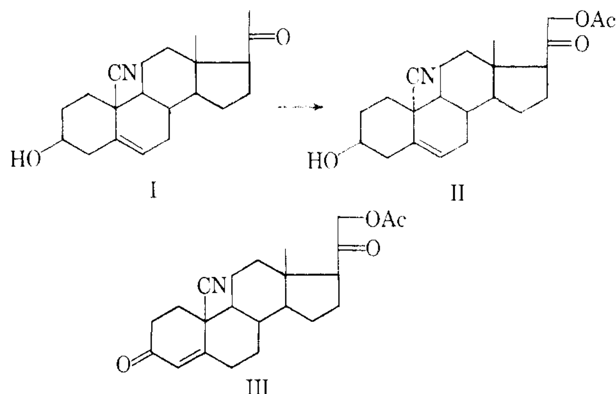

solution in the presence of azobisisobutyronitrile[4,5] gave the corresponding 21-iodo steroid. It was found that the use of redistilled tetrahydrofuran was essential for the success of the reaction, probably owing to the presence of chain-breaking stabilizers in the commercial product. The resulting crude iodo ketone was allowed to react with triethylammonium acetate in acetone solution to give the acetate II in 30% over-all yield. Oxidation of II by the Oppenauer method was unsuccessful, but the ketone III was obtained by oxidation with chromic acid in acetone followed by isomerization of the intermediate Δ^5-ketone on alumina.[2]

For the biological assay,[6] the test materials, in sesame oil as vehicle, were administered by subcutaneous injection in divided doses at 0 and +3 hr. The test animals were groups of seven male rats adrenalectomized approximately 48 hr. prior to the initial injection. The urethras were ligated at the end of 4 hr. and urine was collected from the fourth through the sixth hour. Urinary sodium and potassium determinations were made by means of flame photometry after suitable dilutions of the urine. The "t" test was used to evaluate the significance of the data at the 95% level of confidence.

The results of the testing are displayed in Table I. It can be seen that III is inactive in the sodium retention test and has only about 5% of the activity of DOCA as a potassium excretor. The concomitant administration of 20 parts of III and 1 part of DOCA produces an effect which is not significantly different from the effect of DOCA alone. It is concluded that III is not a DOCA antagonist.

Table I

Mineral Balance Tests

Compound (total dose/rat, γ)	Av. final body wt., g./rat	Sodium excreted, mg., mean ± S.D.	Potassium excreted, mg., mean ± S.D.
Control	146	2.03 ± 0.79	2.45 ± 0.60
DOCA (6)	145	0.94 ± 0.63	3.47 ± 1.18
III (120)	149	1.45 ± 0.93	3.84 ± 0.87
DOCA (6) and III (120)	150	0.81 ± 0.49	3.75 ± 0.59

	Sodium "t"	Inference[a]	Potassium "t"	Inference[a]
Control *vs.* DOCA	2.86	S	2.40	S
Control *vs.* III	1.26	NS	3.38	S
Control *vs.* III + DOCA	3.45	S	3.81	S
DOCA *vs.* III + DOCA	0.04	NS	0.50	NS

[a] S = significant, NS = not significant.

(4) H. J. Ringold and G. Stork, *J. Am. Chem. Soc.*, **80**, 250 (1958).

(5) E. S. Rothman, T. Perlstein, and M. E. Wall, *J. Org. Chem.*, **25**, 1966 (1960).

(6) The biological assays were performed at The Endocrine Laboratories, Madison, Wis.

Experimental[7]

3β,21-Dihydroxy-20-oxo-pregn-5-ene-19-nitrile 21-Acetate (II).—A solution of 1.00 g. (0.002 mole) of I[2,3] in 8 ml. of redistilled tetrahydrofuran and 8 ml. of methanol was treated with 1.7 g. of powdered calcium oxide and 57 mg. of recrystallized azobisisobutyronitrile. To the stirred mixture, immersed in a 25° water bath, there was added 1.2 g. (0.005 mole) of iodine dissolved in a mixture of 5 ml. of tetrahydrofuran and 3 ml. of methanol. The iodine solution was added dropwise but fast enough to slightly exceed the rapid decolorization rate. The mixture was stirred for 2-4 hr. until a pale yellowish color was observed. It was diluted with ether and filtered. The filter cake was washed with ether and was discarded. The ether filtrate was washed free of excess iodine with 15% aqueous sodium iodide solution, dried over sodium sulfate, and evaporated under reduced pressure. Without purification, the 21-iodo residue was dissolved in 30 ml. of acetone, and treated with a mixture of 5 ml. of acetic acid and 8 ml. of triethylamine. The resulting solution was heated under reflux for 1 hr., cooled, and diluted with water. The crude product was filtered and recrystallized from aqueous acetone to afford 0.30 g. (30%) of product, m.p. 197-199°. Further recrystallization gave an analytical sample, m.p. 200–201°, $[\alpha]^{25}D$ −89° (*c* 1%, $CHCl_3$).

Anal. Calcd. for $C_{23}H_{31}NO_4$: C, 71.66; H, 8.11. Found: C, 71.43; H, 7.96.

3,20-Dioxo-21-hydroxypregn-4-ene-19-nitrile 21-Acetate (III).—A solution of 0.10 g. (0.0002 mole) of II in 20 ml. of acetone was treated at 10–15° with 0.2 ml. of 8 *N* chromic acid reagent for 10 min. Excess oxidant was destroyed with 2-propanol, and the solvent was partially evaporated under reduced pressure. After dilution with water, the product was filtered and dried. The crude product was chromatographed on neutral alumina to effect isomerization of the double bond and gave 0.025 g. (25%) of product, m.p. 125–129°. Recrystallization from aqueous acetone gave an analytical sample, m.p. 129–130°, λ_{max}^{EtOH} 232 mμ (ε 16,500), $[\alpha]^{25}D$ +220 (*c* 1%, $CHCl_3$).

Anal. Calcd. for $C_{23}H_{29}NO_4$: C, 72.03; H, 7.62. Found: C, 72.01; H, 7.46.

(7) Melting points were determined with a Thomas–Hoover apparatus and are corrected. Ultraviolet spectra were obtained with a Cary Model 11 instrument. Microanalyses were performed by the Microanalytical Department, University of California, Berkeley, Calif. Optical rotations were obtained in a 0.5-dm. tube with a Rudolph photoelectric polarimeter.

Anticholinesterase Activity of Phenylalkyltrimethylammonium Compounds[1]

A. S. Hume and W. C. Holland

Department of Pharmacology, University of Mississippi Medical Center, Jackson, Mississippi

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Thomas and Marlow[2] have reported on the anti-acetylcholinesterase (red cell) activities of a series of phenylalkyltrimethylammonium compounds. They noted, as the homologous series was ascended, that their inhibitory effect decreased to the fourth member, then increased. The pattern of results obtained differs fundamentally from a "normal" series such as *n*-alkyltrimethylammonium. They interpreted their results in

(1) This investigation was supported in part by Public Health Grant HE-06072-03.

(2) J. Thomas and W. Marlow, *J. Med. Chem.*, **6**, 107 (1963).

TABLE I

PHENYLALKYLTRIMETHYLAMMONIUM COMPOUNDS

$C_6H_5(CH_2)_nN^+(CH_3)_3 \cdot X$

Compound	M.p., °C.	Yield, %	n	X	Formula		C (%)	H (%)	N (%)	X (%)
Phenyltrimethylammonium iodide	224–225	70.0	0	I	$C_9H_{14}IN$	Calcd.	41.1	5.4	5.3	48.2
						Found	41.0	5.3	5.4	48.2
Benzyltrimethylammonium chloride	241–243 dec.	60.4	1	Cl	$C_{10}H_{16}ClN$	Calcd.	64.7	8.7	7.5	19.1
						Found	64.9	8.7	7.5	19.0
Phenylethyltrimethylammonium iodide	229–230	56.9	2	I	$C_{11}H_{18}IN$	Calcd.	45.4	6.2	4.8	43.6
						Found	45.2	6.4	4.8	44.0
3-Phenylpropyltrimethylammonium bromide	151–152	45.0	3	Br	$C_{12}H_{20}BrN$	Calcd.	55.8	7.8	5.4	31.0
						Found	55.7	7.7	5.5	31.0

terms of charge delocalization on the cationic head and certain stereochemical factors.

Recently, we have examined the anticholinesterase activity of the first four members of this same series using a highly purified serum cholinesterase. The results obtained were just the reverse of those reported by Thomas and Marlow. In light of the two-point attachment theory of esterase activity,[3] we have interpreted both groups of data by assuming that, in addition to charge delocalization on the quaternary nitrogen atom, an additional factor, electronic activation of the benzene ring, is of equal importance in determining their inhibitory activity.

Experimental[4]

The phenyltrimethylammonium salts and their analytical data are listed in Table I.

Phenyltrimethylammonium and 3-phenylpropyltrimethylammonium salts were prepared according to Thomas and Marlow.[2] The syntheses of the remaining compounds are outlined below.

Benzyltrimethylammonium Chloride.—Benzyl chloride (12.7 g.) was treated overnight with trimethylamine (5.9 g., 23.6 ml. of 25% in methanol). The precipitate was collected and recrystallized from an ether–alcohol solution.

2-Phenylethyltrimethylammonium Iodide.—Phenethyldimethylamine (24.2 g.) was prepared by the method of Icke and Wisegarver[5] and then allowed to react with methyl iodide (27.9 g.) in ether. The product was recrystallized from an ether–ethanol solution.

Measurement of Enzyme Inhibition.—The inhibitory effects of the phenylalkyltrimethylammonium compounds on the hydrolysis of acetylcholine were determined manometrically by means of the standard Warburg technique. Each manometer vessel contained 0.4 ml. of enzyme solution (400 mg./100 ml.) and x ml. of a solution (concentration arbitrarily selected) of the inhibitor. The enzyme, purified serum cholinesterase (fraction IV-4, run 457), was obtained from Protein Foundation Laboratories, Jamaica Plain, Mass. The substrate, acetylcholine iodide (0.1 M) dissolved in 0.9% NaCl solution, was added to the side bulb. Sodium bicarbonate solution (1 ml., 0.154 M) and sufficient distilled water to adjust the final volume to 2.0 ml. were added. The manometers were gassed 15 min. with 5% CO_2–95% N_2 mixture and the temperature was maintained at 37°.

Results and Discussion

Table II and Fig. 1 are a summary of our data of the inhibitory effects of a series of phenylalkyltrimethylammonium compounds on serum cholinesterase and acetylcholinesterase. Included, for comparison, are the data of Thomas and Marlow and that of Bergmann and Shimoni.[6] It is clear from the data that there is a fundamental difference in effects of the inhibitor on the two enzymes. The pattern of results obtained with the serum enzyme (pseudocholinesterase) is similar to that obtained with the series of n-alkyltrimethylammonium compounds.

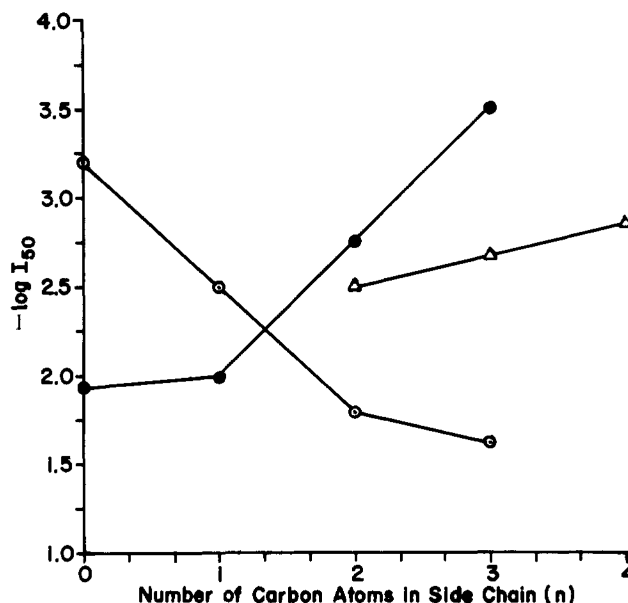

Fig. 1.—Inhibitory activities of phenyltrimethylammonium compounds on serum cholinesterase, –●–●–; on acetylcholinesterase, –O-O– (from Thomas and Marlow[2]); and of n-alkyltrimethylammonium compounds, –Δ–Δ– (from Bergmann and Shimoni[6]).

TABLE II

INHIBITION OF SERUM CHOLINESTERASE AND ACETYLCHOLINESTERASE BY PHENYLALKYLTRIMETHYLAMMONIUM COMPOUNDS

Compound	n	I_{50} (g./mole/l.) Serum cholinesterase	Acetylcholinesterase[a]
Phenyltrimethylammonium iodide	0	1.2×10^{-2}	5.9×10^{-4}
Benzyltrimethylammonium chloride	1	1.0×10^{-2}	3.1×10^{-3}
Phenylethyltrimethylammonium iodide	2	2.3×10^{-3}	1.6×10^{-2}
3-Phenylpropyltrimethylammonium bromide	3	3.1×10^{-4}	2.3×10^{-2}

[a] Values taken from ref. 2.

(3) D. Nachmansohn and I. B. Wilson, *Advan. Enzymol.*, **12**, 259 (1951).

(4) All melting points are corrected. Microanalyses were performed by Microtech Labs, Skokie, Ill.

(5) R. N. Icke and B. B. Wisegarver in "Organic Synthesis," Coll. Vol. III, E. C. Horning, Ed., John Wiley and Sons, Inc., New York, N. Y., 1955 p. 723.

(6) F. Bergmann and A. Shimoni, *Biochim. Biophys. Acta*, **10**, 49 (1953).

Thomas and Marlow interpreted their data by assuming an alteration in the coulombic component of the total adsorptive forces, resulting from a progressive delocalization of the positive charge on the cationic head as one ascends the series. While charge delocalization and steric factors are surely involved in the interaction of these compounds with acetylcholinesterase, these factors alone will not explain their inhibitory effects on serum cholinesterase. An alternative explanation involving changes in charge distribution in the aromatic ring is also possible. Before this is considered it will be instructive to review our present day knowledge of the mechanism of cholinesterase action.

The hydrolysis of acetylcholine, as pictured by Wilson, *et al.*,[7] and Ormerod,[8] involves a two-point attachment of the substrate with the two enzymes. The cationic head of the substrate interacts with an anionic site and the acetoxy group with the esteratic site. With acetylcholinesterase, interattraction between the positively polarized carbon of the ester link and the basic group in the esteratic site was envisioned. In case of pseudocholinesterase (serum), interattraction between the negatively polarized carbonyl oxygen and a positively polarized site on the enzyme was suggested.

In the first two members of the series of phenylalkyltrimethylammonium compounds the aromatic ring is essentially positively polarized. In the remaining members, the ring is negatively polarized. This suggestion is supported by the studies of Ingold[9] on the rates of aromatic nitration of these compounds.

In light of this two-point attachment theory of enzyme action for the two different enzymes, it is not unreasonable to assume that the polarized aromatic ring interacts with the esteratic site, the interattraction being greater for the first two members with the esteratic site of acetylcholinesterase and diminishing thereafter. Just the reverse situation could be pictured for interattraction between the ring system and the esteratic site of pseudocholinesterase. Thus, by assuming that a change in charge distribution occurs in the aromatic ring as the series is ascended, one can offer an alternative explanation of the inhibitory effects of these compounds on the two different types of cholinesterases.

(7) D. Nachmansohn, I. B. Wilson, and F. Bergmann, *J. Biol. Chem.*, **186**, 603 (1950).

(8) W. E. Ormerod, *Biochem. J.*, **54**, 701 (1953).

(9) C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p. 232.

New Progestational Agents. Nonclassical 17-Alkylpregnene Structures

GEORGE R. ALLEN, JR., AND MARTIN J. WEISS

Organic Chemical Research Section, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York

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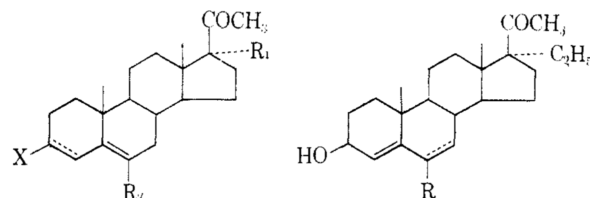
In the past decade the most important advances in the field of new progestational agents have been associated with structural modifications of 17-ethynyltestosterone and 17-acetoxyprogesterone.[1] Recently, however, several nonclassical steroids have been reported to be active progestins.[2] Among these compounds are certain 3-halopregn-5-enes,[2c] 3β-hydroxypregn-4-enes,[2a,b] and pregna-3,5-dienes.[2f] Another recent development in this field has been the observation that introduction of a 17-alkyl group confers oral activity on the progesterone molecule.[3] As a continuation of our interest in the 17-alkylprogesterone series, we now describe the inclusion of the above nonclassical structural features into certain 17-alkylpregnanes.

The 17-alkyl-3β-halopregn-5-enes were readily prepared by known procedures. Thus, treatment of 17-methylpregnenolone,[3b,4,5] 17-ethylpregnenolone,[6] and 17-ethyl-6-methylpregnenolone[3d] with N-(2-chloro-1,1,2-trifluoroethyl)diethylamine[7] gave the corresponding 3β-fluorides (I–III). Moreover, reaction of 17-ethylpregnenolone with thionyl chloride afforded the 3β-chloride IV. For this work the requisite 17-ethylpregnenolone was prepared by preferential reduction of 3-acetoxy-17-ethylpregna-3,5-dien-20-one[3b] with methanolic sodium borohydride.



I, X = F; R_1 = CH_3; R_2 = H
II, X = F; R_1 = C_2H_5; R_2 = H
III, X = F; R_1 = C_2H_5; R_2 = CH_3
IV, X = Cl; R_1 = C_2H_5; R_2 = H
V, X = H; R_1 = C_2H_5; R_2 = H, Δ^3

VI, R = H
VII, R = H, Δ^6
VIII, R = Cl, Δ^6

For the preparation of a 17-alkyl-3β-hydroxypregn-4-ene, reduction of 17-ethylprogesterone[3d,5] with sodium borohydride readily furnished the 17-ethyl-Δ^4-3β-ol (VI), which was also characterized as its acetate. The β-configuration is assigned to this 3-ol, since the reduction of Δ^4-3-ketones with sodium borohydride is known to yield predominantly this isomer.[8] The preferential reduction of the 3-ketone, as well as that of the corresponding 3-enol acetate, further illustrates the steric effect exerted by the 17-ethyl group on the 20-keto function.[3c,5,9]

(1) For brief reviews see: (a) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p. 563; (b) H. J. Ringold in "Mechanism of Action of Steroid Hormones," Pergamon Press, New York, N. Y., 1961, p. 218 ff.

(2) (a) M. Gut, *J. Org. Chem.*, **21**, 1327 (1956); (b) F. Sondheimer and Y. Klibansky, *Tetrahedron*, **5**, 15 (1959); (c) M. S. deWinter, C. M. Siegmann, and S. A. Szpilfogel, *Chem. Ind.* (London), 905 (1959); (d) J. A. Edwards and A. Bowers, *ibid.*, 1962 (1961); (e) O. Halpern, J. A. Edwards, and J. A. Zderic, *ibid.*, 1571 (1962); (f) O. Halpern and J. A. Zderic, *ibid.*, 1540 (1962); (g) F. B. Colton and P. Klimstra, Abstracts of Papers Presented at International Congress on Hormonal Steroids, Milan, Italy, May 14–19, 1962, p. 57.

(3) (a) R. Deghengli and R. Gaudry, *J. Am. Chem. Soc.*, **83**, 4668 (1961); (b) M. J. Weiss, R. E. Schaub, J. F. Poletto, G. R. Allen, Jr., and C. J. Coscia, *Chem. Ind.* (London), 118 (1963); (c) R. Deghenghi, Y. Lefebvre, P. Mitchell, P. F. Morand, and R. Gaudry, *Tetrahedron*, **19**, 289 (1963); (d) R. Deghenghi, C. Revesz, and R. Gaudry, *J. Med. Chem.*, **6**, 301 (1963); (e) M. J. Weiss, R. E. Schaub, J. F. Poletto, G. R. Allen, Jr., and C. Pidacks, *Steroids*, **1**, 608 (1963).

(4) P. A. Plattner, H. Heusser, and P. T. Herzig, *Helv. Chim. Acta*, **32**, 270 (1949).

(5) M. J. Weiss, R. E. Schaub, G. R. Allen, Jr., J. F. Poletto, C. Pidacks, R. B. Conrow, and C. J. Coscia, *Tetrahedron*, **20**, 357 (1964).

(6) R. Deghenghi and R. Gaudry, *Tetrahedron Letters*, **No. 11**, 489 (1962).

(7) D. E. Ayer, *ibid.*, **No. 23**, 1065 (1962); L. H. Knox, E. Velarde, S. Berger, D. Cuadriello, and A. D. Cross, *ibid.*, **No. 26**, 1249 (1962).

(8) *Cf.* W. G. Dauben, R. A. Micheli, and J. F. Eastman, *J. Am. Chem. Soc.*, **74**, 3852 (1952); W. W. Zorbach, *ibid.*, **75**, 6344 (1953).

(9) It is noteworthy that in the 17-hydrogen series (progesterone), the corresponding preferential reduction is known to proceed at C-20 [J. K. Norymberski and G. F. Woods, *J. Chem. Soc.*, 3426 (1955)].