[CONTRIBUTION FROM THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY]

A Steroidal Mustard of the Androstane Series[1]

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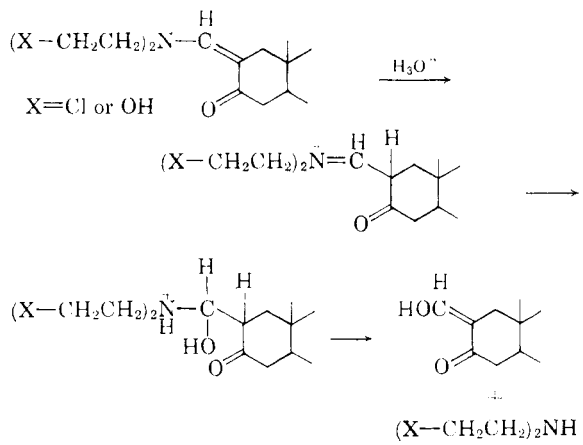
2-(Bis-β-chloroethylaminomethylene)-5α-androstan-17β-ol-3-one 17-acetate (III) was synthesized from 2-hydroxymethylene-5α-androstan-17β-ol-3-one diacetate (Ib) *via* the bis-β-hydroxyethylaminomethylene derivative IIb. Acid hydrolysis experiments are described.

Recent experiments[2] have indicated that the therapy of breast cancer with androgens and alkylating agents combined may be more effective than with either individual agent. It thus seemed highly desirable to combine chemically an alkylating agent and an androgenic type steroid with the hope that the steroid would act as a biologically acceptable platform capable of transporting the alkylating agent to the tumor site in a rather specific manner. Should transport be so effected, it would then be possible for the compound *per se*, or by hydrolysis to its two components, to act upon the tumor.

While the literature cites a number of examples[3] of nitrogen mustards chemically combined with steroids, none have involved androgens, and, with the exception[3c] of an alkylating agent bound to the 3-hydroxyl group of cholesterol, none have left the steroid molecule intact.



It has been demonstrated[4] that 2-hydroxymethylene-3-keto-5α-androstanes readily react with a number of secondary amines yielding 2-aminomethylene derivatives which are, in fact, enamines. 2 - Hydroxymethylene - 5α - androstan - 17β - ol - 3-one[5] (Ia) was therefore selected as our starting material since condensation with diethanolamine would lead to a derivative theoretically convertible to the desired mustard. Another consideration for our starting material was the antitumor[6] and anabolic[5] activity of the closely related 2-hydroxymethylene-17α-methyl-5α-androstan-17β-ol-3-one.

Compound Ia, in a model experiment, readily condensed with diethanolamine in boiling benzene to yield the expected amino derivative, IIa. Our efforts were then directed toward the enol acetate 17-acetate Ib, which was derived by treatment of Ia at room temperature with acetic anhydride–pyridine. The enol acetate grouping proved to be highly unstable and after a few days standing at room temperature in the solid state, Ib underwent partial hydrolysis to the 17-monoacetate Ic. Selective hydrolysis of the diacetate with dilute hydrochloric acid in acetone solution also produced the 17-monoacetate in high yield. It was reasoned however that selective hydrolysis previous to diethanolamine condensation would not be necessary due to the basicity of the reagent. Thus, the total crude diacetate Ib derived from the acetylation of Ia was condensed directly with diethanolamine either in hot benzene or preferably at room temperature in acetone solution, to yield 2-(bis-β-hydroxyethylaminomethylene) - 5α - androstan-17β-ol-3-one 17-acetate (IIb). The 17-ester was desired at this stage since an unesterified 17-hydroxyl group would not be stable to the subsequent reaction.

The dihydroxyethanol derivative IIb reacted smoothly with thionyl chloride at room temperature furnishing the desired bis-β-chloroethylamino derivative III. For maximum yield it appeared necessary for the thionyl chloride to be freshly distilled before use. A by-product which arose, in particular in larger scale experiments or with thionyl chloride which had not been freshly distilled was

(1) This work was aided by grant no. T-185 from the American Cancer Society.

(2) *Cf.* G. Whyte Watson and R. L. Turner, *Brit. Med. J.*, 1315 (1959).

(3) (a) G. R. Vavasour, H. I. Bolker, and A. F. McKay, *Can. J. Chem.*, **30**, 933 (1952); (b) G. G. Hazen, *Dissertation Abstr.*, **12**, 449 (1952); (c) L. N. Owen, M. H. Benn, and A. M. Creighton, *British Empire Cancer Campaign Annual Report*, **34**, 417 (1954); (d) W. J. Gensler and G. M. Sherman, *J. Org. Chem.*, **23**, 1227 (1958); (e) G. V. Rao, *Dissertation Abstr.*, **20**, 1590 (1959); R. E. Havranek and N. J. Doorenbos, *J. Am. Pharm. Assoc. (Sci. Ed.)*, **49**, 328 (1960).

(4) J. A. Zderic, O. Halpern, H. Carpio, A. Ruiz, D. Chavez Limon, L. Magana, H. Jimenez, A. Bowers, and H. J. Ringold, *Chem. & Ind.*, 1625 (1960).

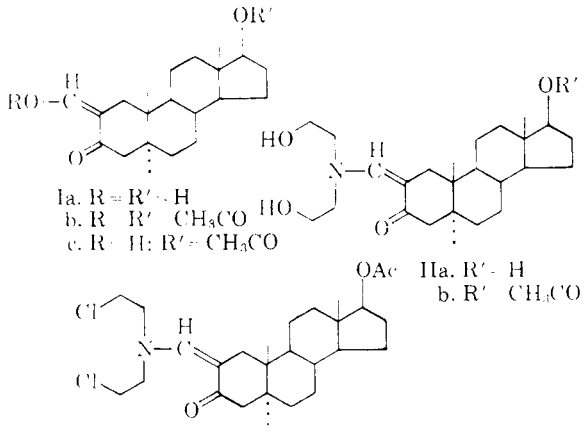
(5) H. J. Ringold, E. Batres, O. Halpern, and E. Necoechea, *J. Am. Chem. Soc.*, **81**, 427 (1959).

(6) G. Montano and J. R. Hinojosa, *Rev. med. hosp. gen. (Mex.)*, **22**, 465 (1959).

readily identified as 2-hydroxymethylene-5α-androstan-17β-ol-3-one 17-acetate (Ic).

The structure of the steroid mustard III rests upon elemental analysis, the similarity of its ultraviolet and carbonyl region infrared spectrum to that of the bis-β-hydroxyethylamino derivative IIb, as well as on hydrolysis experiments. The NMR[7] was consistent with the assigned structure and exhibited one olefinic hydrogen atom as expected. Further, III, in common with other alkylating agents, reacted[8] with 4-(p-nitrobenzyl)-pyridine yielding a purple solution after alkalinization.

As IIb and III are both enamines, it was expected that acid hydrolysis would regenerate the same 2-hydroxymethylene derivative which would serve as added evidence that the thionyl chloride reaction followed the anticipated reaction course. We also wished to establish the stability of the steroid mustard to acid conditions. The dihydroxyethanol derivative IIb readily dissolved in dilute aqueous hydrochloric acid and after a few minutes deposited a high yield of 2-hydroxymethylene-5α-androstan-17β-ol-3-one 17-acetate (Ic) while the dichloro compound III was insoluble in the same medium and was recovered unchanged after overnight treatment. However, solution in aqueous acetone containing a few drops of hydrochloric acid readily hydrolyzed the mustard to Ic, presumably liberating β,β′-dichloroethylamine (nor-nitrogen mustard), a potent cytotoxic[9] agent. The failure of the dichloro compound to dissolve in aqueous acid, in contrast to the dihydroxy compound, is probably due to decreased water solubility and to decreased basicity of the already weakly basic enamine.



III

In short term acute toxicity studies, III was found not to be toxic to mice after six days administration, subcutaneous route, at a daily dosage of 500 mg./kg. Animal antitumor testing is now in progress.

EXPERIMENTAL[10]

2-Hydroxymethylene-5α-androstan-17β-ol-3-one enol acetate 17-acetate (Ib). A solution of 10 g. of 2-hydroxymethylene-5α-androstan-17β-ol-3-one[5,11] (Ia) in pyridine (20 ml.) and acetic anhydride (20 ml.) was allowed to stand overnight at room temperature and then poured into a stirred mixture of ice and water. Stirring was continued for an additional 30 min., the product was filtered, washed and dried *in vacuo*, yielding 11.2 g. of Ib, m.p. 156–163°. An analytical sample obtained by hexane recrystallization exhibited m.p. 162–164°, λ_{max} 256 mμ, log ε 4.03. Infrared[10] λ_{max} 5.66, 5.79, 5.93, 6.18, 8.03, and 8.31 μ.

Anal. Calcd. for $C_{24}H_{34}O_5$: C, 71.61; H, 8.51. Found: C, 71.64; H, 8.63.

2-Hydroxymethylene-5α-androstan-17β-ol-3-one 17-acetate (Ic). A 200-mg. sample of diacetate (Ib) in 5 ml. of acetone was treated with 1 ml. of water, 5 drops of 10% hydrochloric acid, and allowed to stand for 30 min. at 25°. Crystallization commenced after a few minutes. The mixture was chilled and the precipitate of Ic, 130 mg., m.p. 187°, λ_{max} 284 mμ, log ε 3.96, collected. The melting point was unchanged on further crystallization from aqueous acetone. Infrared λ_{max} 3.20, 3.70, 5.73, 6.35–6.45, 8.00, and 8.26 μ.

Anal. Calcd. for $C_{22}H_{32}O_4$; C, 73.30;H, 8.95. Found: C, 73.40; H, 8.96.

2-(Bis-β-hydroxyethylaminomethylene)-5α-androstan-17β-ol-3-one (IIa). A mixture of Ia (4.0 g.), benzene (100 ml.), and diethanolamine (8.0 g.) was heated under reflux for 2 hr. The solvent was removed *under vacuo* and water added. The resultant precipitate was filtered and recrystallized from acetone-ethanol yielding 4.06 g., m.p. 206–211°. Further crystallization from the same solvent pair raised the melting point to 215–217°, λ_{max} 333 mμ, log ε 4.22. Infrared λ_{max} 2.85, 3.07, 6.16, and 6.68 μ.

Anal. Calcd. for $C_{24}H_{39}NO_4 \cdot \frac{1}{2}\ C_3H_6O$: C, 70.39; H, 9.73; N, 3.21. Found: C, 70.39; H, 9.46; N, 3.61.

2-(Bis-β-hydroxyethylaminomethylene)-5α-androstan-17β-ol-3-one 17 acetate (IIb). (a) *Condensation in benzene.* The diacetate (Ib) (5.0 g.) was condensed with 10 g. of diethanolamine in 150 ml. of boiling benzene for 2.5 hr. The benzene was removed *in vacuo*, water was added, and the mixture extracted with ethyl acetate. The organic extract was washed several times with water, dried over sodium sulfate and concentrated to a small volume, yielding 2.1 g. of IIb, m.p. 194–197°, λ_{max} 333 mμ, log ε 4.30. Infrared λ_{max} 2.81, 5.75, 6.14, 6.60 and 8.00 (broad).

Anal. Calcd. for $C_{26}H_{41}NO_5$; C, 69.74; H, 9.23; N, 3.13. Found: C, 69.80; H, 9.25; N, 3.28.

(b) *Condensation in acetone.* A solution of 5.0 g. of Ib and 5.0 g. of diethanolamine in 75 ml. of acetone was allowed to stand at room temperature for 24 hr. whence crystallization occurred. The mixture was cooled for several hours at 0° and the product filtered yielding 2.0 g. of IIb, m.p. 194–196°. The mother liquors, after dilution with water, yielded a semisolid which was filtered and crystallized from ethyl acetate, furnishing an additional 0.51 g. of product, m.p. 187–191°.

2-(Bis-β-chloroethylaminomethylene)-5α-androstan-17β-ol-3-one 17-acetate (III). (a) The bis-β-hydroxyethylamino compound IIb, 300 mg., was dissolved in 5 ml. of cold freshly distilled thionyl chloride and the solution then

(7) Determined in deuterochloroform with tetramethylsilane as reference. We are grateful to Mr. T. A. Wittstruck for this determination and interpretation.

(8) J. Epstein, R. W. Rosenthal, and R. J. Ess, *Anal. Chem.*, **27**, 1435 (1955).

(9) L. H. Schmidt, *Ann. N. Y. Acad. Sci.*, **68**, 657 (1958).

(10) Melting points are uncorrected. Ultraviolet spectra were measured in 95% ethanol solution and infrared spectra in potassium bromide pellets. We are grateful to Mr. Frederick S. Skelton for technical assistance.

(11) This material, a gift from Syntex, S. A., exhibited m.p. 148–150°, λ_{max} 283 mμ, log ε 3.96.

allowed to come to room temperature and stand for 18 hr. under anhydrous conditions. After removal of thionyl chloride *in vacuo* without heating, the residue was diluted with methylene dichloride and the solution cautiously washed with dilute ice cold sodium bicarbonate solution followed by water washes to neutrality. Drying of the solution over sodium sulfate and evaporation under reduced pressure left a light yellow oil which crystallized from methylene chloride–ether, yielding 0.17 g. of the dichloro compound III, m.p. 166–169°dec. For the analytical specimen, a 100-mg. sample of III was chromatographed on 5 g. of silica gel. Methylene dichloride-hexane (1:1) and methylene chloride eluted traces of oil while the desired product was eluted with methylene dichloride–ether (4:1). Crystallization from methylene dichloride-ether gave material of m.p.[12] 172–174° dec., λ_{max} 326 mμ, log ε 4.20. Infrared λ_{max} 5.78, 6.08, 6.55, and 8.03 (broad) μ.

Anal. Calcd. for $C_{26}H_{39}Cl_2NO_3$: C, 64.45; H, 8.11; Cl, 14.64; N, 2.89. Found[13]: C, 63.98; H, 8.15, Cl, 15.26; N, 3.09.

(b) A solution of 11.0 g. of IIb in 100 ml. of thionyl chloride which had been distilled several days previous to the reaction was allowed to stand overnight at room temperature. The mixture was worked up as described for (a) and the residue remaining after removal of methylene dichloride was treated with ether yielding 8.31 g. of tan precipitate, m.p. 163–166° dec. This precipitate was taken up in 75 ml. of methylene dichloride and absorbed onto a column of 150 g. of silica gel. Elution with the same solvent gave small amounts of brown gummy material which was discarded while elution with methylene dichloride–ether (9:1) gave 0.9 g. of 2-hydroxymethylene - 5α - androstan - 17β - ol - 3 - one 17-acetate (Ic), m.p. 182–184°, identified by ultraviolet, infrared and mixed melting point determination. The methylene chloride-ether (4:1) eluate was crystallized as in (a) yielding 4.75 g. of III, m.p. 172–174° dec. and a second crop of 0.46 g., m.p. 168–172°.

Acid hydrolysis of 2(bis-β-hydroxyethylaminomethylene)-5α-androstan-17β-ol-3-one 17-acetate. When 0.15 g. of IIb was added to 6 ml. of 2% hydrochloric acid, solution occurred immediately followed by crystallization within a few minutes. Filtration yielded 97 mg. of 2-hydroxymethylene-5α-androstan-17β-ol-3-one 17-acetate (Ic), m.p. 183–185°.

Acid hydrolysis of 2-(bis-β-chloroethylaminomethylene)-5α-androstan-17β-ol-3-one acetate. (a) *Aqueous.* Treatment of 0.1 g. of dichloro compound III with 5 ml. of 2% hydrochloric acid (overnight stirring at room temperature) gave 90 mg. of recovered dichloro compound.

(b) *Acetone.* A solution of 100 mg. of III in 2.5 ml. of acetone, 0.5 ml. of water, and 10 drops of 5% hydrochloric acid was stirred for 1 hr. at room temperature whence precipitation occurred. Cooling and filtration yielded 85 mg. of Ic, m.p. 185–187°.

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(12) The melting point varied considerably with the rate of heating. Rapid determination for this sample gave 175–177° dec.

(13) Considerable difficulty was experienced in obtaining accurate and consistent analytical figures, apparently due in part to acetone of solvation. Chlorine values were usually high while direct oxygen determination was unsatisfactory.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

Steroidal Hormone Analogs. IX. Bisdehydrodoisynolic Acid Analogs Possessing the 1,2,3,4-Tetrahydrobenz[f]isoquinoline Nucleus[1]

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6-Methoxy-1-naphthylacetic acid was converted to 6-methoxy-1-naphthylacetonitrile. Reduction of the nitrile yielded the amine which, on acylation with propionic anhydride, gave *N*-(6-methoxy-1-naphthyl-β-ethyl)propionamide (IIa). Cyclodehydration of the amide and hydrogenation of the product gave 4-ethyl-8-methoxy-1,2,3,4-tetrahydrobenz[f]isoquinoline (VIa) which was converted to *N*-carbethoxy-4-ethyl-8-methoxy-1,2,3,4-tetrahydrobenz[f]isoquinoline (VIb). Acylation of 2-(6-methoxy-1-naphthyl)ethylamine with diethyl malonate gave the malonamate IIb which on cyclization and reduction afforded 4-(2-hydroxyethyl)-8-methoxy-1,2,3,4-tetrahydrobenz[f]isoquinoline (VId). The latter substance was converted to *N*-carbethoxy-4-(2-hydroxyethyl)-8-methoxy-1,2,3,4-tetrahydrobenz[f]isoquinoline (VIe) and *N*-carbomethoxymethyl-4-(2-hydroxyethyl)-8-methoxy-1,2,3,4-tetrahydrobenz[f]isoquinoline (VIf).

In continuing our work on the preparation of azasteroids we have undertaken the synthesis of some derivatives of 1,2,3,4-tetrahydrobenz[f]isoquinoline which bear a resemblance to the potent estrogen, bisdehydrodoisynolic acid.[3]

The starting material in our work, 6-methoxy-1-naphthylacetic acid (Ia), was prepared from 2-methoxynaphthalene by methods described in the literature.[4] Treatment of 6-methoxy-1-naphthylacetic acid with phosphorus pentachloride gave a crude acid chloride which was treated with ammonium hydroxide to afford 6-methoxy-1-naphthylacetamide(Ib) in 79% yield. Attempts to reduce the amide to the corresponding amine Ic by means of lithium aluminum hydride in ether or tetrahydrofuran solution were unsuccessful, presumably because of the insolubility of the amide (or salt of the amide) in the solvents used. To circumvent this obstacle, we chose to dehydrate the amide and reduce the resulting nitrile to the desired amine. 6-

(1) This investigation was supported in part by a research grant, CY-2999 (C3), from the National Cancer Institute, Public Health Service.

(2) Department of Chemistry, The Upjohn Company, Kalamazoo, Mich.

(3) For a review of the doisynolic acids, see L. F. Fieser and M. Fieser, *Steroids*, Reinhold Publishing Corp., New York, N. Y., 1959, and references contained therein.

(4) G. Stork, *J. Am. Chem. Soc.*, 69, 576 (1947); G. Haberland, *Ber.*, 69, 1380 (1936); E. Buchta, M. Klisch, S. Maier, and H. Bayer, *Ann.*, 576, 7 (1952).