[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PENNSYLVANIA]

Synthesis of Some Steroidal Nitrogen Mustards

GANTI V. RAO[1] AND CHARLES C. PRICE

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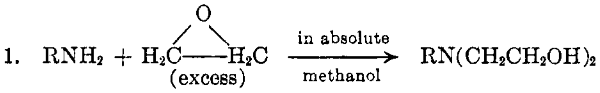
Synthetic schemes for the preparation of a number of steroidal nitrogen mustards have been developed. Some of the mustards produced contained hydroxyl, methoxyl, or carbonyl functional groups as well.

The syntheses of three nitrogen mustards of the general formula $RN(CH_2CH_2Cl)_2$, where R is a steroidal unit have been described by Hazens.[2] They are related to cholesterol, ergosterol, and stigmasterol. The nitrogen mustard unit $—N(CH_2CH_2Cl)_2$ was attached to the 3-position, replacing the hydroxyl group. The biological activity of these mustards has not been reported, and Hazens[3] has indicated that he was not aware of any screening experiments for carcinolytic activity. Vavasour *et al.*[4] have also reported the synthesis of a nitrogen mustard related to cholesterol without making any reference to its biological activity. Recently Gensler and Sherman[5] have reported the synthesis of a nitrogen mustard $R—CH_2N(CH_2CH_2Cl)_2$, where R is cholestene.

As all the four nitrogen mustards described so far in the literature have the $—N(CH_2CH_2Cl)_2$ grouping in the 3- position, we have investigated the possibilities of introducing the grouping in other positions, and of incorporating other functional groupings such as 3-oxygenated and 20-keto steroids.

The penultimate step in the synthesis of $RN(CH_2CH_2Cl)_2$, where R is a steroidal unit, is the formation of $RN(CH_2CH_2OH)_2$. Two methods were followed:



$$1.\quad RNH_2 + \underset{\text{(excess)}}{H_2C\overset{O}{—}H_2C} \xrightarrow[\text{methanol}]{\text{in absolute}} RN(CH_2CH_2OH)_2$$

$$2.\quad RX + \underset{\text{(large excess)}}{HN(CH_2CH_2OH)_2} \xrightarrow[\text{diglyme}]{\text{in refluxing}} RN(CH_2CH_2OH)_2$$

(X = halogen or *p*-toluenesulfonate)

The use of diglyme as a solvent in the second method is an improvement over earlier procedures,[2,4] as the lower reaction temperature and dilution with solvent minimize the resinification. The complete miscibility of diglyme with water is an added advantage, as the reaction product separates on dilution with water. The use of the solvent dispenses with the mechanical stirring of the thick syrupy diethanolamine and the reaction time is considerably shorter.

The chlorination of the diols $[RN(CH_2CH_2OH)_2]$ was carried out in anhydrous chloroform solution with thionyl chloride, and the nitrogen mustard was isolated as the hydrochloride in 40 to 60% yield, except in the case of 12-hydroxy-24-cholanyl diethanolamine. Table I, which follows, summarizes the results.

TABLE I

STEROID MUSTARDS PREPARED AND YIELD OF DIETHANOLAMINE INTERMEDIATES

System	Position of $—N(CH_2CH_2Cl)_2$	% Yield of $—N(C_2H_4OH)_2$
Cholestane	3β-	71[a]
Cholane	24-	58[b]
3β-Methoxycholane	24-	47[b]
12-Hydroxycholane	24-[e]	57[c]
5-Pregnen-20-one	3-	3.5[c]
3β-Methoxy-5-pregnene	20-	32, 21[a, d, f]
4-Androsten-17β-ol	3-	69[a]
1,3,5(10)-Estratrien-3-ol	17-	6.8[a, g]
3-Methoxy-1,3,5(10)-estratriene	17-	65[a]

[a] By RNH_2 and ethylene oxide. [b] By RCl and diethanolamine. [c] By ROTS and diethanolamine. [d] For this case, RCl + diethanolamine failed. [e] Chlorination by thionyl chloride failed to produce the bis(β-chloroethyl)amine in this case. [f] The reduction of the oxime group produced both α- and β-isomers, but their identity was not established. [g] In this case, the hydroxylethyl compound separated from solution in 50% yield, greatly reducing the yield of the desired bis(hydroxyethyl)amine.

The reaction of ethylene oxide with 24-aminocholane and 3β-methoxy-24-aminocholane in methanol solution proceeded beyond the diol stage, and yielded mainly the quaternary ammonium alcoholate, $R\overset{\oplus}{N}(CH_2CH_2OH)_3\ \overset{\ominus}{O}CH_3$. These products were insoluble in ether and benzene, slightly soluble in chloroform, and very soluble in methanol and ethanol.

The reaction of ethylene oxide with 17ξ-amino-1,3,5(10)-estratriene-3-ol results mainly in a product which is insoluble in the common solvents but is soluble in hot dimethylformamide. Analysis suggests it to be the mono-*N*-hydroxyethyl derivative, ($RNHCH_2CH_2OH$, R = estratriene unit). The prod-

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[1] Supported in part by U.S.P.H.S. Grants CY-2189 and CY-2714.

[2] G. G. Hazens, *Dissertation Abstracts*, **12**, 449 (1952).

[3] G. G. Hazens, personal communication.

[4] G. R. Vavasour, H. R. Bolker, and A. F. McKay, *Canad. J. Chem.*, **30**, 933 (1952).

[5] W. J. Gensler and G. W. Sherman, *J. Org. Chem.*, **23**, 1227 (1958).

uct dissolved in alkali and the alkaline solution showed the characteristic shift in the ultraviolet spectra as for similar phenolic compounds.

The 3β-cholestanyl mustard was inactive in mice infected with Gardner ascites tumor at 100 mg./kg. doses. The two isomers of 3β-methoxy-5-pregnen-20-ol mustard were inactive at 10 mg./kg. doses. The androstene mustard at 10 mg./kg. and the 3-methoxyestratriene mustard at 15 mg./kg. gave about 100% extension of survival time.

EXPERIMENTAL

All melting points reported are uncorrected.

Diglyme is the commercial name for diethylene glycol dimethyl ether, $CH_3OCH_2CH_2OCH_2CH_2OCH_3$, b.p. 162° at atmospheric pressure. The optical rotations reported were measured at 20–21°, using a 1% solution in the solvents indicated.

3β-Bis(β-hydroxyethyl)aminocholestane. The reduction of cholestanone oxime was carried out according to the procedure of Shoppee *et al.*[6] A solution of 800 mg. of 3β-aminocholestane in the minimum amount of chloroform was added to a solution of 1 g. of ethylene oxide in 30 ml. of absolute methanol at 0–5°. The mixture was stirred for one hour and allowed to stand at room temperature for 18 hr. The mixture was then refluxed on a water bath at 40–50° using a Dry Ice–acetone cooling system to condense the ethylene oxide. After refluxing for 2 hr., the solvent was removed by evaporation and the residue recrystallized from methanol, m.p. 206–208°, yield 700 mg. (71%), $[\alpha]_D$ +24.5° (chloroform).

Anal. Calcd. for $C_{31}H_{57}NO_2$: C, 78.25; H, 12.08; N, 2.94. Found: C, 78.07; H, 12.30; N, 2.94.

3β-Bis(β-chloroethyl)aminocholestane hydrochloride. Thionyl chloride (1 ml.) was added to a solution of 300 mg. of 3β-bis(β-hydroxyethyl)aminocholestane in 50 ml. of chloroform. After refluxing for 2 hr., the solvent and excess thionyl chloride were removed by distillation under reduced pressure. The residue was dissolved in the minimum amount of warm absolute methanol, filtered, and 250 ml. of anhydrous ether saturated with hydrogen chloride was added to the filtrate. On standing the hydrochloride was obtained as a fine crystalline powder, m.p. 218–221°, yield 150 mg. (43%). An analytical sample was obtained by redissolving in absolute ethanol and reprecipitation by ether saturated with hydrogen chloride. The melting point was unchanged, $[\alpha]_D$ +19° (chloroform).

Anal. Calcd. for $C_{31}H_{56}NCl_3$: C, 67.80; H, 10.28; N, 2.55; Cl, 19.37. Found: C, 68.10; H, 10.43; N, 2.63; Cl, 18.50.

Ionic chlorine estimated conductimetrically by titrating with silver nitrate both before and after alkali treatment gave the following results with the above-mentioned mustard.

Before treatment with alkali, $C_{31}H_{55}Cl_2N\cdot HCl$ requires: Cl, 6.46. Found: 6.88. After alkali treatment, $C_{31}H_{55}Cl_2N\cdot HCl$ requires: Cl, 19.37. Found: Cl, 19.58.

24-Bis(β-hydroxyethyl)aminocholane hydrochloride. Cholanyl chloride was prepared according to the procedure of Wessely and Swoboda.[7] To a solution of 4 g. of cholanyl chloride in 200 ml. of freshly distilled diglyme, 15 ml. of diethanolamine was added and the mixture refluxed for 8 hr. After cooling, the dark-brown solution was poured into 2 l. of water, when a gelatinous precipitate was obtained in a short time. The material was collected by filtration, repeatedly washed with water, and dissolved in 300 ml. of ether. The ether solution was washed with water several times to remove all of the diethanolamine in solution and dried over anhydrous magnesium sulfate. The product was isolated by passing hydrogen chloride through the dried ethereal solution, and the hydrochloride was recrystallized from absolute ethanol twice, m.p. 235–237°, yield 3 g. (58%), $[\alpha]_D$ +24.5° (absolute ethanol).

Anal. Calcd. for $C_{28}H_{52}NO_2Cl$: C, 71.52; H, 11.15; N, 2.98: Cl, 7.54. Found: C, 71.46; H, 11.11; N, 2.89; Cl, 7.54.

24-Bis(β-chloroethyl)aminocholane hydrochloride. A mixture of 100 ml. of 5% sodium hydroxide and 3 g. of 24-bis(β-hydroxyethyl)aminocholane hydrochloride was warmed on a steam bath for 10 min., cooled and the liberated free amine was extracted with 300 ml. of ether. The ethereal solution was washed with water, dried over anhydrous magnesium sulfate, and on evaporation left a residue of the amine. The amine was dissolved in 100 ml. of anhydrous chloroform, 5 ml. of thionyl chloride was added, and the mixture refluxed for 2 hr. The solvent and excess thionyl chloride were removed by distillation under reduced pressure, when a pale brown residue was left behind. The product was dissolved in the minimum amount of warm absolute ethanol, the solution was filtered and, with the addition of 250 ml. of anhydrous ether saturated with hydrogen chloride, yielded the nitrogen mustard hydrochloride, m.p. 195–197°. Further purification by redissolving in absolute ethanol and reprecipitation as the hydrochloride raised the m.p. to 196–198°, yield 1.4 g. (43%), $[\alpha]_D$ +26.5° (absolute ethanol).

Anal. Calcd. for $C_{28}H_{50}NCl_3$: C, 66.32; H,9.94; N, 2.76; Cl, 20.98. Found: C, 66.22; H, 9.79; N, 2.95; Cl, 19.69.

Ionic chlorine, estimated conductimetrically by titration with silver nitrate both before and after treatment with alkali, gave the following results.

Before treatment with alkali, $C_{28}H_{49}Cl_2N\cdot HCl$ requires: Cl, 6.99. Found: Cl, 6.50. After treatment with alkali, $C_{28}H_{49}Cl_2N\cdot HCl$ requires: Cl, 20.98. Found: Cl, 21.36.

Cholanamide was reduced according to the procedure of Wessely and Swoboda[7] to yield 24-aminocholane. The literature,[8] records the melting point of cholanamide to be 75°, which was found to be incorrect, since the melting point of the amide we obtained from the acid chloride of cholanic acid by treatment with ammonia in ether had a melting point of 185–187°. The acid chloride was prepared by the earlier workers using phosphorus trichloride, and the only analysis reported was for nitrogen, and no record of specific rotation are to be found in the literature for the amide. Wessely and Swoboda did not isolate the amide,[7] but reduced the amide directly without any purification. The amide we reduced was made as follows.

To a solution of 1.5 g. of cholanic acid in 40 ml. of anhydrous carbon tetrachloride 4 ml. of thionyl chloride was added and the mixture refluxed for one hour. The solvent was removed along with the excess thionyl chloride by reduced pressure distillation. The residue was dissolved in 100 ml. of anhydrous ether and the ethereal solution added to 250 ml. of ether saturated with ammonia. The mixture was stirred for one hour, allowed to stand overnight, and washed with water several times to remove ammonium salts and excess ammonia. The ether solution on evaporation left a residue, which crystallized from ethanol (95%), m.p. 185–187°, yield 1.1 g. (74%), $[\alpha]_D$ +22.5° (chloroform). The infrared spectrum showed the characteristic carbonyl absorption of an amide at 6.05 μ.

Anal. Calcd. for $C_{24}H_{41}NO$: C, 80.16; H, 11.49; N, 3.90. Found: C, 79.91; H, 11.47; N, 4.00.

24-Cholanyl-tris-(β-hydroxyethyl)ammonium methoxide. A mixture of 100 ml. of 10% sodium hydroxide solution and 2 g. of 24-aminocholane hydrochloride was warmed on the steam bath for 15 min., cooled, and the free amine extracted with 250 ml. of ether. The ether solution was washed with water to remove alkali, dried over anhydrous magnesium

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(6) C. W. Shoppee, D. E. Evans, H. C. Richards, and G. H. R. Summers, *J. Chem. Soc.*, 1649 (1956).

(7) F. Wessely and W. Swoboda, *Monatsh.*, **82**, 437 (1951).

(8) W. Borsche, *Ber.*, **52**, 1353 (1919).

sulfate, filtered, and on evaporation left an oily residue which on standing in a vacuum desiccator turned opaque. The amine solution in 250 ml. of absolute methanol was added to a solution of 2–3 g. of ethylene oxide in 100 ml. of methanol at 0–5°. The mixture was stirred for one hour, allowed to stand overnight at room temperature, and then warmed on a water bath at 40–50° for 2 hr., using a Dry Ice–acetone cooling system to condense the ethylene oxide. The solvent and ethylene oxide were removed by evaporation and the gummy residue solidified on the addition of anhydrous ether, m.p. 135–140° (frothing at the start). The material was insoluble in ether, benzene, or petroleum ether. It dissolved readily in ethanol, methanol, and somewhat more difficultly in chloroform. The material in methanol solution was precipitated by the addition of anhydrous ether. A sample precipitated three times from methanolic solution melted at 147–150° (frothing at the start) $[\alpha]_D$ +21° (absolute ethanol).

Anal. Calcd. for $C_{31}H_{59}NO_4$: C, 73.03; H, 11.67; N, 2.75. Found: C, 72.83; H, 11.41; N, 2.98.

The analysis indicates that this product was the quaternary methoxide resulting from reaction of three rather than two ethylene oxide molecules.

3β-Methoxycholanyl chloride. To a solution of 3 g. of 3β-methoxycholanyl alcohol[9] in 50 ml. of anhydrous chloroform, a few drops of pyridine and 5 ml. of thionyl chloride were added, and the mixture refluxed for one hour. The solvent and excess thionyl chloride were removed by distillation under reduced pressure. The residue was dissolved in 300 ml. of ether, and the ether solution washed with 100 ml. of 5% hydrochloric acid followed by 5% sodium hydroxide and finally with water. After drying over anhydrous magnesium sulfate, evaporation of the ethereal solution left an oily residue which presented considerable difficulty in crystalzation. It crystallized from ethyl acetate–acetone, m.p. 91–94°, yield of crystalline material, 0.05 g., yield of the oily material 2.8 g. (89%).

Anal. Calcd. for $C_{25}H_{43}OCl$: C, 76.00; H, 10.97; Cl, 8.98. Found: C, 76.24; H, 10.93; Cl, 8.99.

3β-Methoxy-24-bis(β-hydroxyethyl)aminocholane hydrochloride. Using five grams of 3β-methoxycholanyl chloride, the displacement with diethanolamine was carried out in the same way as the one described above with cholanyl chloride. The melting point of the hydrochloride was 219–221° (yield, 47%), $[\alpha]_D$ +25° (absolute ethanol).

Anal. Calcd. for $C_{29}H_{54}O_3NCl$: C, 69.63; H, 10.88; N, 2.88; Cl, 7.09. Found: C, 69.31; H, 10.88; N, 2.80; Cl, 7.65.

3β-Methoxy-24-bis(β-chloroethyl)aminocholane hydrochloride. Using 3 g. of the above diol hydrochloride, chlorination to the mustard was carried out as described in the case of the cholane system; m.p. 186–188° dec., yield 1.9 g. (59%), $[\alpha]_D$ +25.5° (absolute ethanol).

Anal. Calcd. for $C_{29}H_{52}ONCl_3$: C, 64.85; H, 9.76; N, 2.61; Cl, 19.80. Found: C, 64.67; H, 9.89; N, 2.34; Cl, 19.20.

Ionic chlorine estimate before and after treatment with alkali gave the following values: $C_{29}H_{51}OCl_2N \cdot HCl$ requires Cl, 6.60. Found: Cl, 7.34. After treatment with alkali requires: Cl, 19.80. Found: Cl, 20.62.

3β-Methoxycholanamide. To a solution of 3 g. of 3β-methoxycholanic acid[8] in 100 ml. of anhydrous benzene 7 ml. of thionyl chloride was added. After refluxing for one hour, the solvent and excess thionyl chloride were removed by distillation under reduced pressure. The residue of the acid chloride was dissolved in 100 ml. of anhydrous ether and added to 100 ml. of ether saturated with ammonia. The mixture was stirred for one hour and left to stand overnight at room temperature. The ether solution was washed with water several times to remove the ammonium salts, filtered, and, on evaporation, a residue was obtained which was recrystallized from aqueous methanol, m.p. 144–146°, yield 2 g. (67%), $[\alpha]_D$ +28° (chloroform).

Anal. Calcd. for $C_{25}H_{43}NO_2$: C, 77.07; H, 11.12; N, 3.60. Found: C, 77.38; H, 11.13; N, 3.69.

3β-Methoxy-24-aminocholane. In a one-liter three necked flask was placed 1.5 g. of 3β-methoxycholanamide, 500 ml. of anhydrous ether was added, and the mixture refluxed for 2 hr. to dissolve the amide. A solution of 1.2 g. of lithium aluminum hydride in 100 ml. ether was then added from a dropping funnel slowly and the mixture refluxed for 6 hr. The excess lithium aluminum hydride was decomposed by the slow addition of ethyl acetate, followed by 100 ml. of 15% sodium hydroxide solution. The contents were then transferred to a separatory funnel and the alkaline layer separated. The ethereal solution was washed with water several times, dried over anhydrous magnesium sulfate, and the amine was isolated as the hydrochloride by passing in hydrogen chloride for a short time. The hydrochloride crystallized from absolute ethanol-ether mixture, m.p. 268–271° dec., yield 1 g. (69%). The free amine was rather difficult to purify for purposes of analysis, as it was very soluble in most organic solvents, and the material crystallized from dilute methanol had a melting point of 85–91°, $[\alpha]_D$ +16° (chloroform).

Anal. Calcd. for $C_{25}H_{46}ONCl$: C, 72.86; H, 11.25; N, 3.40; Cl, 8.65. Found: C, 72.92; H, 11.17; N, 3.45; Cl, 8.75.

3β-Methoxy-24-cholanyl-tris(β-hydroxyethyl)ammonium methoxide. The reaction was carried out as under 24-aminocholane. The product was insoluble in ether, and was precipitated from an alcoholic solution by the addition of anhydrous ether. The precipitated material on treatment with hot benzene and cooling yielded a powdery mass, m.p. 145–147° (frothing at the start), $[\alpha]_D$ +20° (absolute ethanol).

Anal. Calcd. for $C_{32}H_{61}O_5N$: C, 71.19; H, 11.43; N, 2.60. Found: C, 70.90; H, 11.10; N, 2.57.

12-Hydroxycholanyl alcohol. To a solution of 5 g. of lithium aluminum hydride in 200 ml. of anhydrous ether under reflux, 7 g. of ethyl 12-hydroxycholanate[10] in 200 ml. of anhydrous ether was added from a dropping funnel (15 min.). After refluxing for another 3 hr., the excess lithium aluminum hydride was decomposed by the slow addition of ethyl acetate, followed by 5% sulfuric acid. The aqueous acid layer was separated, the ether solution washed twice with 200-ml. portions of water and dried over anhydrous magnesium sulfate. The solvents were removed by distillation and the residue recrystallized from petroleum ether (b.p. 60–110°), m.p. 109–111°. Two further recrystallizations raised the melting point to 113–115°, yield 4.5 g. (72%), $[\alpha]_D$ +42° (chloroform).

Anal. Calcd. for $C_{24}H_{42}O_2$: C, 79.50; H, 11.67. Found: C, 79.34; H, 11.33.

12-Hydroxycholanyl tosylate. To a solution of 12-hydroxycholanyl alcohol in 20 ml. of anhydrous pyridine 6 g. of *p*-toluenesulfonyl chloride was added and the mixture was gently swirled until the acid chloride went into solution. After standing at room temperature for 48 hr., the mixture was poured into ice-cold water and allowed to stand in the refrigerator for one day. The oily mass did not solidify, and hydrochloric acid was cautiously added until the solution was distinctly acidic. It was extracted with 500 ml. of ether, the ether solution was washed with 200 ml. of 10% hydrochloric acid, and then with water. After drying over anhydrous magnesium sulfate, filtering and evaporation, an oily mass was obtained which did not crystallize, yield 3 g. (53%).

12-Hydroxy-24-bis(β-hydroxyethyl)aminocholane. The above-obtained oily 12-hydroxycholanyl tosylate (3 g.) was dissolved in 200 ml. of diglyme, 25 ml. of diethanolamine was added, and the mixture refluxed for 12 hr. The dark-brown solution was cooled and poured into 2 l. of water when a gelatinous precipitate was obtained. The product was collected by filtration, washed repeatedly with water, and

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(9) J. C. Babcock and L. F. Fieser, *J. Am. Chem. Soc.*, **74**, 5472 (1952).

(10) J. Barnett and T. Reichstein, *Helv. Chim. Acta*, **21**, 926 (1938).

then dissolved in 300 ml. of ether. The ethereal solution was washed with water several times, dried over anhydrous magnesium sulfate, filtered, and the amine was isolated as the hydrochloride by treatment with hydrogen chloride for a short time. The hydrochloride was collected by filtration and the free base generated by treatment with dilute alkali and taken up in ether. Evaporation of the ether left a residue which crystallized from petroleum ether (b.p. 60–110°) m.p. 131–134°, yield 1.5 g., (57%), $[\alpha]_D$ +32° (chloroform).

Anal. Calcd. for $C_{28}H_{51}NO_3$: C, 74.78; H, 11.43; N, 3.12. Found: C, 74.74; H, 11.43; N, 3.24.

Attempted chlorination of 12-hydroxy-24-bis(β-hydroxyethyl)aminocholane. A solution of 0.9 g. of the amine in 50 ml. of anhydrous chloroform was mixed with 0.68 g. of thionyl chloride in 4 ml. of anhydrous benzene (2 equivalents) and refluxed for 2 hr. The solvents and unreacted thionyl chloride were removed by distillation under reduced pressure. The residue was dissolved in the minimum amount of warm absolute ethanol, filtered, and 200 ml. of anhydrous ether saturated with hydrogen chloride was added. A white crystalline deposit was obtained within a short time, which was collected by filtration, and washed with ether, m.p. 187–191°, yield 0.8 g.

Analysis indicates that this compound is the hydrochloride of the bis-(β-hydroxyethyl)amino compound.

Anal. Calcd. for $C_{28}H_{51}O_3N \cdot HCl$: C, 69.17; H, 10.78; N, 2.88; Cl, 7.29. Found: C, 68.55; H, 10.61; N, 2.66; Cl, 7.28.

An authentic sample of 12-hydroxy-24-bis(β-hydroxyethyl)aminocholane hydrochloride was prepared as follows: To a solution of 0.2 g. of the amine in 25 ml. of absolute ethanol 200 ml. of ether saturated with hydrogen chloride was added. After standing for one hour, the precipitated hydrochloride was collected by filtration and washed with ether, m.p. 191–193°. A mixture of this product with the one obtained above melted at 189–191°.

Chlorination of the amine using moderate excess or even large excess of thionyl chloride resulted only in tarry products.

3ξ-Bis(β-hydroxyethyl)amino-5-pregnen-20-one. To a solution of 2 g. of pregnenenolone tosylate[11] in 100 ml. of diglyme, 20 ml. of diethanolamine was added and the mixture refluxed for 72 hr. The cooled red-brown reaction mixture was poured with stirring into one liter of cold water. After one hour, a slimy precipitate was obtained which was filtered off, washed with water, and taken up in 300 ml. of ether. The ethereal solution was washed three times with water (200-ml. portions) and dried over anhydrous magnesium sulfate. The basic product in the ether solution was precipitated by passing in hydrogen chloride, yielding 70 mg. of crude hydrochloride. The free amine was regenerated by suspending the crude hydrochloride in alkali and extracting with ether. The ether solution was washed with water, and on evaporation left a crisp solid which crystallized from petroleum ether (b.p. 60–110°), m.p. 168–171°, yield 60 mg. (3.5%).

At times no crystallizable product resulted; a good amount of the originally precipitated material was ether soluble and found to be nonbasic. Change of reaction conditions, low temperature, substitution of solvents, or conducting the reaction in the absence of solvents did not materially improve the yield. If the material was impure—i.e., the melting point is lower than 160°—one recrystallization from acetone (small volume) yielded the product, m.p. 171–174°, $[\alpha]_D$ +26° (chloroform).

Anal. Calcd. for $C_{25}H_{41}NO_3$: C, 74.39; H, 10.24; N, 3.47. Found: C, 74.50; H, 10.17; N, 3.46.

3ξ-Bis(β-chloroethyl)amino-5-pregnen-20-one hydrochloride. To a solution of 0.25 g. of the above-mentioned diol in 25 ml. of anhydrous chloroform 1 ml. of thionyl chloride was added and the chlorination carried out as described in several of the previous chlorinations, melting point of the hydrochloride, 202–204°, dec., yield 0.125 g. (42%).

Anal. Calcd. for $C_{25}H_{39}OCl_2N \cdot HCl \cdot H_2O$: C, 60.66; H, 8.55; N, 2.83; Cl, 21.49. Found: C, 61.33; H, 8.48; N, 3.14; Cl, 22.30.

20ξ-Chloro-3β-methoxy-5-pregnene. To a solution of 2 g. of 3β-methoxy-5-pregnene-20β-ol[12] in 50 ml. of anhydrous chloroform, a few drops of pyridine and 5 ml. of thionyl chloride were added. The mixture was refluxed for one hour. After cooling an additional 100 ml. of chloroform was added, and the organic layer washed with 100 ml. of 5% sodium carbonate, followed by dilute hydrochloric acid and finally with water. After drying over anhydrous magnesium sulfate, the solvent was removed by distillation under reduced pressure, and the residue crystallized from acetone, m.p. 136–137°, yield 1.5 g. (71%), $[\alpha]_D$ −82° (chloroform).

Anal. Calcd. for $C_{22}H_{35}OCl$: C, 75.28; H, 10.05; Cl, 10.10. Found: C, 75.48; H, 10.05; Cl, 9.93.

Attempted displacement of the chlorine in 20 ξ-chloro-3β-methoxy-5-pregnene. To a solution of 2 g. of the chloro compound in 200 ml. of diglyme 25 ml. of diethanolamine was added and the mixture refluxed for 24 hr. The pale brown solution was cooled, poured into 2 l. of ice-cold water, and stirred for a short time, when a very fine crystalline deposit separated. The product was collected, washed with water several times, and dissolved in 250 ml. of ether. The ether solution was washed with water to remove the last traces of any diethanolamine, dried over anhydrous magnesium sulfate, filtered, and hydrogen chloride was passed in for some time. No precipitate was obtained even after 10 min., which indicated the absence of any basic material. The ether solution was then washed with water, dilute alkali, and finally with water. On evaporation of the ether, a crisp solid was left behind, which crystallized from acetone, m.p. 138°. The melting point of a mixture of this compound and the starting material was found to be undepressed; yield of the recovered material, 1.5 g. (75% recovery).

3β-Methoxy-5-pregnen-20-one oxime. A mixture of 3.5 g. of hydroxylamine hydrochloride and 8 g. of potassium acetate in 60 ml. of water was added to a solution of 3 g. of 3β-methoxy-5-pregnen-20-one[12] in 300 ml. of methanol and the mixture refluxed for four hours. The solution became turbid within a short time, because of the low solubility of the oxime formed, and additional volumes of methanol may be added to keep it in solution. The cooled mixture was poured into 500 ml. of water and the precipitated oxime was collected and dried, m.p. 223–224°, yield 3 g. (95%). A small amount was recrystallized from a fairly large volume of methanol, m.p. 224–225°, $[\alpha]_D$ −39° (chloroform).

Anal. Calcd. for $C_{22}H_{35}NO_2$: C, 76.47; H, 10.21; N, 4.05. Found: C, 76.27; H, 10.33; N, 3.98.

20ξ-Amino-3β-methoxy-5-pregnene. To a solution of 3 g. of the above oxime in 300 ml. of butanol at reflux temperature 30 g. of sodium metal was added in small lots during a period of one hour. Refluxing was continued until all the sodium dissolved. The mixture was cooled and 400 ml. of water was cautiously added with shaking. The alkaline aqueous layer was separated and the alcohol layer washed three times with water. The aqueous washings were combined and extracted with 500 ml. of ether and the ether washed with water. The ether extract and the butanol solution were combined and evaporated under reduced pressure, leaving an oily residue which was dissolved in 300 ml. of ether and dried over anhydrous magnesium sulfate. The amine was isolated as the hydrochloride by passing in hydrogen chloride for a short time. The free amine was regenerated by treating the hydrochloride with base and extracting with ether. The amine isolated by the procedure was an oily gum (1.8 g., 55%), which was found to be extremely soluble in the common organic solvents. When a

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(11) A. Butenandt and W. Grosse, *Ber.*, **70,** 1446 (1937).

(12) M. N. Huffman and J. W. Sadler, *J. Org. Chem.*, **18,** 919 (1953).

concentrated solution of the amine in petroleum ether (b.p. 60–110°) was chilled in the refrigerator for one day, crystals of the amine, m.p. 93–97° were obtained, yield 0.5 g., $[\alpha]_D$ −75° (chloroform).

Anal. Calcd. for $C_{22}H_{37}NO$: C, 79.70; H, 11.25; N, 4.23. Found: C, 79.25; H, 11.13; N, 3.94.

Condensation of ethylene oxide and 20ξ-amino-3β-methoxy-5-pregnene. A solution of 3 g. of the amine in 100 ml. of absolute methanol was added to approximately 3–4 g. of ethylene oxide in 100 ml. of absolute methanol at 0°. The mixture was then stirred for one hour and allowed to stand for 24 hr. at room temperature. The mixture was then warmed under reflux on a water bath at 40–50° for 2 hr., using a Dry Ice–acetone cooling system to condense the ethylene oxide. The solvent and ethylene oxide were removed by evaporation leaving an oily residue. Addition of 300 ml. of petroleum ether (b.p. 60–110°) and scratching with a glass rod resulted in sudden solidification of the mass. The mixture was warmed on the steam bath to dissolve the product in hot petroleum ether and filtered hot. The residue was treated with another 100 ml. of the same solvent, again heated to the boiling point and filtered hot. The combined filtrates on standing for fifteen minutes deposited a crystalline material (A) melting at 127–130°. The residue melted at 173–175° (B).

(*A*). Repeated recrystallizations of the petroleum ether soluble material (A), m.p. 127–130°, from the same solvent did not improve the melting point. However, one recrystallization from the smallest volume of acetone raised the melting point to 145–147°. On one further recrystallization from petroleum ether, it melted at 148–149°. Further recrystallizations either from acetone or petroleum ether did not affect the melting point, $[\alpha]_D$ −3.5° (chloroform).

Anal. Calcd. for $C_{26}H_{45}NO_3$: C, 74.41; H, 10.81; N, 3.34. Found: C, 74.39; H, 10.55; N, 3.42.

(*B*). The initial residue which did not dissolve in the described volume of petroleum ether was suspended in 400 ml. of the same solvent, brought to boil, and filtered hot; this operation was carried out twice, and from the filtrates a crystalline product separated out within a very short time. The product was collected and recrystallized from a larger volume of petroleum ether, m.p. 178–180°, $[\alpha]_D$ −83° (chloroform).

Anal. Calcd. for $C_{26}H_{44}NO_3$: C, 74.41; H, 10.81; N, 3.34. Found: C, 74.33; H, 10.73; N, 3.30.

The melting point of a mixture of the two products isolated above was 133–135°. The two products had virtually identical infrared absorption, with very minor differences in the 8–11 μ region.

Yield of (A) 1.2 g. (32%), (B) 0.80 g. (21%).

20ξ-Bis(β-chloroethyl)amino-3β-methoxy-5-pregnene hydrochloride. To a solution of 1 g. of the product (*A*) described above in 50 ml. of anhydrous chloroform 5 ml. of thionyl chloride was added and the mixture refluxed for one hour. The solvent and excess thionyl chloride were removed by distillation under reduced pressure and the residue dissolved in the minimum amount of warm absolute ethanol, filtered, and ether saturated with hydrogen chloride was added. Silky needles separated on standing for one hour. The product was redissolved in the minimum amount of absolute ethanol and reprecipitated by the addition of anhydrous ether saturated with hydrogen chloride. The chlorinated product crystallized on standing, m.p. 197–200°, yield 0.65 g. (55%), $[\alpha]_D$ −5° (absolute ethanol).

Anal. Calcd. for $C_{26}H_{44}ONCl_3$: C, 63.34; H, 8.99; N, 2.84; Cl, 21.58. Found: C, 63.00; H, 8.74; N, 2.94; Cl, 21.60.

Ionic chlorine estimated conductimetrically by titrating with silver nitrate after treatment with alkali requires: Cl, 21.58. Found: 20.68.

Chlorination of (*B*), similar to the one described above, gave the mustard hydrochloride, m.p. 186–188°, $[\alpha]_D$ −49° (absolute ethanol).

Anal. Calcd. for $C_{26}H_{44}ONCl_3$: C, 63.34; H, 8.99; N, 2.84; Cl, 21.58. Found: C, 63.25; H, 8.88; N, 2.76; Cl, 21.86.

Ionic chlorine estimated conductimetrically gave the following result: Total chloride found 21.86, requires 21.58.

3ξ-Amino-4-androsten-17β-ol. A solution of 2 g. of testosterone oxime[13] in 200 ml. of freshly distilled butanol was brought to gentle boil under reflux and 20 g. of sodium metal was added during a period of one hour in small lots. The mixture was refluxed until all the sodium dissolved, cooled and diluted with 500 ml. of water, the aqueous layer removed and the butanol layer washed three times with 100-ml. portions of water. The aqueous layers were combined and extracted with ether. The ether extract and butanol solution were combined and the solvents removed by distillation under reduced pressure. The residual oily material was dissolved in 300 ml. of ether, filtered from the insoluble material, and hydrogen chloride was passed in for a short time to precipitate the amine hydrochloride, yield 1.1 g. (50%). The crude hydrochloride was suspended in 200 ml. of 10% sodium hydroxide solution and warmed on the steam bath for a short time, cooled, and the liberated amine extracted with 200 ml. of ether. The ether solution was washed with water, and on evaporation yielded a gummy mass, 0.9 g. (47%). The material was very soluble in the common organic solvents, with the exception of light petroleum ether (b.p. 30–60°), and addition of 50 ml. of the solvent resulted in solidification of the oil. Repeated recrystallizations from benzene (smallest volume) resulted in a product, m.p. 191–194°, 0.15 g. (8% crystalline product), $[\alpha]_D$ +120° (chloroform).

Anal. Calcd. for $C_{19}H_{31}NO$: C, 78.83; H, 10.80; N, 4.84. Found: C, 79.05; H, 11.16; N, 4.81.

3ξ-Bis(β-hydroxyethyl)amino-4-androsten-17β-ol. The condensation with ethylene oxide using the oily gummy amine (2.0 g.) was carried out in the usual manner as described in several of the earlier condensations. The diol (1.8 g., 69%) is sparingly soluble, but was recrystallized from methanol, m.p. 215–217°. Since the material did not dissolve to an appreciable extent in any of the solvents, the optical rotation was not determined.

Anal. Calcd. for $C_{23}H_{39}NO_3$: C, 73.16; H, 10.41; N, 3.71. Found: C, 73.33; H, 10.79; N, 3.41.

Chlorination of 3ξ-bis(β-hydroxyethyl)amino-4-androsten-17β-ol. When the chlorination was carried out with the requisite amount of thionyl chloride (2 equivalents) either in benzene or chloroform as the solvent, the product isolated was the hydrochloride of the diol. An authentic sample of the hydrochloride of the diol was made, and it melted at 224°, and the melting point of a mixture of the product of controlled chlorination melted at 220–221°.

Chlorination with a reasonable excess of thionyl chloride in chloroform solution (as described in several earlier chlorinations) for a short time yielded the mustard hydrochloride, m.p. 194–197° dec., yield (33%), $[\alpha]_D$ +16° (absolute ethanol).

Anal. Calcd. for $C_{23}H_{38}ONCl_3$: C, 61.26; H, 8.49; N, 3.11; Cl, 23.59. Found: C, 61.64; H, 8.87; N, 3.04; Cl, 21.64.

Total chloride estimated conductimetrically was found to be 21.30; required 23.59.

17ξ-Amino-1,3,5(10)-estratrien-3-ol. The crude material was generously supplied by Eli Lilly Research Laboratories, Indianapolis, Ind. The crude amine melted at 206–209° dec., which increased to 216–218° dec. when crystallized twice from benzene. Further recrystallizations did not alter the melting point appreciably since after five recrystallizations it melted at 217–218°, dec., λ_{max} 280 mμ, (ϵ 3500) (neutral alcohol solution), λ_{max} 300 mμ (ϵ 2700) (alkaline solution), $[\alpha]_D$ +60° (chloroform).

Anal. Calcd. for $C_{18}H_{25}NO$: C, 79.66; H, 9.29; N, 5.16. Found: C, 79.95; H, 9.33; N, 5.26.

Condensation of ethylene oxide and 17ξ-amino-1,3,5(10)-estratrien-3-ol. A solution of 2 g. of the amine in 150 ml. of methanol was added to approximately 1–2 g. of ethylene oxide in

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[13] L. Ruzicka and A. Wettstein, *Helv. Chim. Acta*, **18**, 1264 (1935).

100 ml. of methanol at 0°. The mixture was stirred for one hour and allowed to stand at room temperature for one day. A thick deposit of pale yellow crystalline material formed around the walls of the flask. The mixture was then warmed on a water bath at 40–50° for 2 hr., using a Dry Ice–acetone cooling system to condense the ethylene oxide. The ethylene oxide was later removed by evaporation and the methanol-insoluble material was filtered hot and dried, m.p. 246–250°, dec., yield 0.8 g. The methanol filtrate was evaporated to dryness and the residue treated with 100 ml. of ethyl acetate, brought to boil on a steam bath, and filtered hot, leaving a residue which melted at 246–250°, dec., yield 0.2 g. The filtrate of the ethyl acetate on standing for an hour deposited 0.3 g. of material, melting at 202–204°, dec. On two further recrystallizations from ethyl acetate this material melted at 214–216°, the mixture melting point of this product with the starting material was 215°.

After removing the starting material from the ethyl acetate solution and concentrating, 0.3 g. of product melting at 158–162° was obtained, which on subsequent recrystallization from ethyl acetate several times yielded a product melting at 163–164°, 0.15 g. (6.8%), $[\alpha]_D$ +76° (chloroform).

Anal. Calcd. for $C_{22}H_{33}NO_3$: C, 73.50; H, 9.25; N, 3.89. Found: C, 73.95; H, 9.60; N, 4.15.

The above analysis indicates that the material is the 17-bis(β-hydroxyethyl)amino derivative. It is soluble in alcoholic alkali and the ultraviolet absorption is shifted, showing the free phenolic group: λ_{max} 280 mμ (ε 3600) (neutral alcoholic solution); alcoholic alkaline solution λ_{max} 300 mμ, (ε 3000).

The major reaction product (1.0 g., 50%) was found to be insoluble in the common organic solvents and had a crude melting point of 246–250° dec. It dissolved in boiling dimethylformamide, and recrystallization from this solvent yielded a product melting at 252-254° dec. Analytical data and ultraviolet absorption in alkaline medium suggest this to be the mono-*N*-hydroxyethyl derivative.

Anal. Calcd. for $C_{20}H_{29}NO_2$: C, 76.15; H, 9.27; N, 4.44. Found: C, 75.57; H, 9.43; N, 4.48.

As the material did not dissolve in alcohol, the ultraviolet absorption in neutral medium was not determined. In alkaline alcoholic medium λ_{max} 300 mμ (ε 2800).

17ξ-Amino-3-methoxy-1,3,5(10)estratriene. Estrone methyl ether (2 g.) was converted to the oxime[14] and reduced with sodium and butanol, as described in several of the earlier reductions of oximes. The hydrochloride (1.2 g.) melted at 276–278°, dec., and the free amine was rather difficult to crystallize. It was obtained as a flaky resin, $[\alpha]_D$ +70.5° (absolute ethanol).

Anal. Calcd. for $C_{19}H_{28}ONCl \cdot H_2O$: C, 67.14; H, 8.90; Cl, 10.43, N, 4.12. Found: C, 67.84; H, 8.25; Cl, 10.55; N, 4.33.

Condensation of the reduction product of the oxime and ethylene oxide. The condensation was carried out with the free amine (1 g.) in methanol and ethylene oxide solution in methanol as described in the earlier condensations. The reaction product was recrystallized from petroleum ether (b.p. 60–110°), 0.75 g., m.p. 136–139°. An analytical sample was obtained by recrystallization from the minimum amount of acetone, m.p. 138–140°, $[\alpha]_D$ +73.5° (chloroform).

Anal. Calcd. for $C_{23}H_{35}NO_3$: C, 73.95; H, 9.45; N, 3.75. Found: C, 74.25; H, 9.68; N, 3.94.

Chlorination of 17ξ-Bis(β-hydroxyethyl)amino-3-methoxy-1,3,5(10)estratriene. It was found that chlorination proceeded to completion in the absence of any solvent, i.e., thionyl chloride acted as the solvent. A mixture of the diol (0.5 g.) and thionyl chloride (10 ml.) was boiled under reflux for one hour. The thionyl chloride was removed by distillation under reduced pressure. The dark brown residue was dissolved in 20 ml. of hot absolute ethanol, filtered, and to the filtrate 300 ml. of ether saturated with hydrogen chloride was added. A brown gum separated in 15 min. This gum was removed by filtration and to the resulting filtrate more ether was added. After standing for one day in the refrigerator, a pale yellow crystalline mass separated, m.p. 198–202° dec.

Anal. Calcd. for $C_{23}H_{34}ONCl_3$: C, 61.81; H, 7.67; N, 3.14; Cl, 23.80. Found: C, 61.61; H, 7.82; N, 3.36; Cl, 23.91.

PHILADELPHIA 4, PA.

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[14] B. M. Regan and F. N. Hayes, *J. Am. Chem. Soc.*, 78, 639 (1956).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PENNSYLVANIA]

Sulfur Analogs of δ-Aminolevulinic Acid. I. Phthalimide Derivatives[1]

CHARLES C. PRICE AND MAE L. BECK[2]

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The chloromethyl sulfide of methyl β-mercaptopropionate has been prepared. A phthalimide derivative of this ester has been made and hydrolyzed to the phthalimido acid and the (*o*-carboxy)benzamido acid. From these products of hydrolysis phthalimidomethyl β-carboxyethyl sulfone and (*o*-carboxy)benzamidomethyl β-carboxyethyl sulfoxide have been prepared. β,β′-Dithiodipropionyl chloride and methylene bis(β-thiopropionyl chloride) have been made and converted to chloromethyl ketones using diazomethane and hydrogen chloride. Treatment of the chloromethyl ketones with potassium phthalimide effected hydrogen elimination and cleavage of the sulfur-carbon bond.

As the compound δ-aminolevulinic acid has recently[3] been assigned a significant role as an intermediate in the metabolism of porphyrins, the synthesis of sulfur analogs has been undertaken which might possibly show interesting biological activity *e.g.*, as anticancer agents.

A. $H_2NCH_2SCH_2CH_2COOH$ (also, the sulfoxide and the sulfone)
B. $H_2NCH_2COCH_2CH_2SO_2NH_2$

EXPERIMENTAL

Chloromethyl β-carbomethoxyethyl sulfide (II). A solution of 0.15 mole (4.9 g.) of formaldehyde in water and 0.15 mole

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[2] Abstracted from the Ph.D. dissertation of Mae L. Beck, University of Pennsylvania, 1960.

[3] D. Shemin, *The Succinate-Glycine Cycle*, in Ciba Foundation *Symposium on Porphyrin Biosynthesis and Metabolism*, Little Brown and Co., Boston, Mass., 1955.