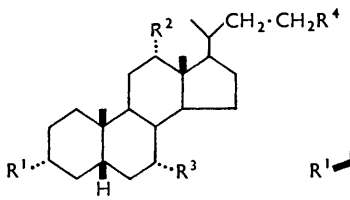
## 649. *The Steroid Series. Part IV.* Some Basic Derivatives.*

By A. F. Chaplin, D. H. Hey, and J. Honeyman.

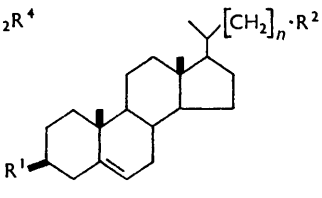
A number of amides of steroid acids has been prepared and converted into the corresponding amines by reduction. In this manner 24-aminocholan-3α-ol, 24-aminocholane-3α : 12α-diol, 24-aminocholane-3α : 7α : 12α-triol, 25-aminohomocholan-3α-ol, 22-aminobisnorchol-5-en-3β-ol, 23-aminonorchol-5-en-3β-ol and 24-aminochol-5-en-3β-ol were prepared. A preparation of 24-aminocholan-3-one is also described. A number of indolosteroids having the indole ring fused to ring A are described, and attempts to obtain indolosteroids having the indole ring fused to ring D are reported.

Interest in the biological activity of the steroidal alkaloids has stimulated the synthesis of simpler basic derivatives in this series by several groups of workers.[1] Gould and his co-workers [2] have reported that 3β-hydroxy-16α-piperidinopregn-5-en-20-one has the same kind of physiological properties as alkaloids of the veratrum group. The secondary amine 20-(5-methyl-2-piperidyl)pregn-5-ene-3β : 20-diol [3] has also been found to be active. Part of the present communication describes the preparation of steroids having a primary amino-group attached to the 17-side-chain.

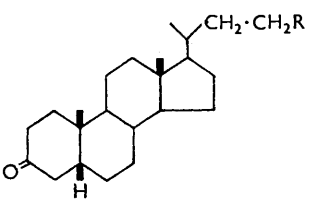
The acid chlorides of 3α-acetoxy-, 3α,12α-diformyloxy-, and 3α,7α,12α-triformyloxycholanic acid (I—III; $R^4 = CO_2H$), and 3β-acetoxybisnor- and 3β-acetoxy-chol-5-enic



| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| (I): | AcO | H | H |
| (II): | H·$CO_2$ | H·$CO_2$ | H |
| (III): | H·$CO_2$ | H·$CO_2$ | H·$CO_2$ |
| (IV): | HO | H | H |
| (V): | HO | HO | H |
| (VI): | HO | HO | HO |



| | $R^1$ | $R^2$ |
|---|---|---|
| (VII): | AcO | $CO_2H$ |
| (VIII): | AcO | COCl |
| (IX): | AcO | CO·$NH_2$ |
| (X): | OH | $NH_2$ |



(XI)

acid (VII; $n = 0$ and 2, respectively), have been converted into the amides in high yield. The chloride (I; $R^4 = COCl$) was also converted into 3α-acetoxyhomocholanamide (I; $R^4 = CH_2{\cdot}CO{\cdot}NH_2$), and the chloride (VIII; $n = 0$) into 3β-acetoxynorchol-5-enamide (IX; $n = 1$) by the Arndt–Eistert reaction. Each amide was reduced by lithium aluminium hydride to the hydroxy-amine, the acetyl or formyl groups being removed at the same time. The seven free hydroxy-amines (IV—VI; $R^4 = CH_2{\cdot}NH_2$), (IV; $R^4 = CH_2{\cdot}CH_2{\cdot}NH_2$) and (X; $n = 1$, 2, and 3) were not isolated but were precipitated from ethereal solution as hydrochlorides. The three amines (X; $n = 1$, 2, and 3), which form members of a homologous series, have the 3β-hydroxyl group and the 5 : 6-double bond, which is present in many of the steroidal alkaloids. Three of the other amines have the same side-chain attached to position 17 as the amine (X; $n = 3$) but show variations in other parts of the molecule. Thus they all have the 3α-hydroxy-A/B-*cis* ring fusion typical

* Part III, *J.*, 1954, 185.

[1] Barnett, Ryman, and Smith, *J.*, 1946, 524, 526, 528; James, Smith, Stacey, and Webb, *ibid.*, p. 665; Jones, Webb, and Smith, *J.*, 1949, 2164; Fieser and Huang, *J. Amer. Chem. Soc.*, 1953, **75**, 6306; Louw, Strating, and Backer, *Rec. Trav. chim.*, 1954, **73**, 667; 1955, **74**, 1540; Micheli and Bradsher, *J. Amer. Chem. Soc.*, 1955, **77**, 4788; Herzog, Payne, and Hershberg, *ibid.*, 1955, **77**, 5324, etc.

[2] Gould, Shapiro, and Hershberg, *J. Amer. Chem. Soc.*, 1954, **76**, 5567; Gould, Shapiro, Finckenor, Gruen, and Hershberg, *ibid.*, 1956, **78**, 3158.

[3] Uhle, *ibid.*, 1951, **73**, 883.

of the bile acids, whilst the amines (V and VI; $R^4 = CH_2 \cdot NH_2$) have the 12α-hydroxyl group as in the steroidal alkaloid rubijervine. A homologue of the amine (IV; $R^4 = CH_2 \cdot NH_2$) is also reported.

In order to obtain a keto-steroid having a basic group in the 17-side-chain, 3α-acetoxycholanamide (I; $R^4 = CO \cdot NH_2$) was converted into 3α-hydroxycholanamide (IV; $R^4 = CO \cdot NH_2$), which was oxidised to 3-oxocholanamide (XI; $R = CO \cdot NH_2$). The method of Heyl and Herr [4] was used for the protection of the keto-group. The pyrrolidyl derivative from the amide (XI; $R = CO \cdot NH_2$) was reduced to the amine, which was converted into 24-aminocholan-3-one hydrochloride (XI; $R = CH_2 \cdot NH_2, HCl$). The infrared spectrum of this salt has a peak at 1719 cm.$^{-1}$, which is consistent with a keto-steroid structure.[5] An amorphous product is deposited from an aqueous-ethanolic solution of this salt if it is boiled or kept for a few hours at room temperature. Such amorphous products, which are considered to arise from the condensation of the keto-group of one molecule with the amino-group of another, have been reported by other workers.[6]

A second approach to the synthesis of basic derivatives in the steroid series involved the preparation of indolo-steroids in which the nitrogen-containing ring of indole is fused to ring A of the steroid molecule. By employing the combination of an indole system with a second basic group in the 17-side-chain, it was hoped that salts soluble in water would be obtained. Antaki and Petrow [7] had attempted to prepare such a compound from methyl 3-oxo*allo*cholanate by the Curtius reaction on the carboxyl group of indolo(3′ : 2′-2 : 3)-*allo*chol-2-enic acid, but could only obtain the required intermediate hydrazide in very low yield.

It is now shown that when methyl 3-oxocholanate (XI; $R = CO_2Me$) is heated with phenylhydrazine in glacial acetic acid an indolo-steroid is obtained. This conclusion is supported by the elementary analysis and the infrared spectrum. Although a linear or an angular formulation, corresponding to ring-closure on to position 2 or 4 respectively, is possible for this compound, Antaki and Petrow [7] have concluded that indolo-steroids derived from 3-keto-steroids of the normal series have the angular formulation. The reactivity of the 4-methylene group of 3-keto-steroids in the normal series is well known, and it is reasonable to suppose that ring closure of the keto-steroid phenylhydrazone on to the 4-position will occur. Thus the indolo-steroid prepared from methyl 3-oxocholanate is tentatively assigned the angular structure methyl indolo(2′ : 3′-3 : 4)chol-3-enate (XII; $R^1 = CO_2Me$, $R^2 = R^3 = H$). This ester was hydrolysed to the acid (XII; $R^1 = CO_2H$, $R^2 = R^3 = H$), but attempts to convert the carboxyl group into the amide gave a product which could not be obtained pure. The infrared spectrum of the crude product has, however, two broad bands (one is of high intensity; the other, medium) in the 1600—1700 cm.$^{-1}$ region, indicating that a primary amide group may be present. In another attempt to prepare this amide, 3-oxocholanamide was heated with phenylhydrazine in acetic acid solution. Again the product could not be crystallised. Its infrared spectrum, with a very strong peak near 750 cm.$^{-1}$ and no peaks near 700 cm.$^{-1}$, indicates the absence of a monosubstituted benzene ring and hence the product may be the indolo-steroidal amide (XII; $R^1 = CO \cdot NH_2$, $R^2 = R^3 = H$). As the amide could not be obtained pure the crude product in ether was reduced with lithium aluminium hydride. After reaction dry hydrogen chloride was passed into an ethereal extract of the product, which precipitated 24-aminoindolo-(2′ : 3′-3 : 4)chol-3-ene hydrochloride (XII; $R^1 = CH_2 \cdot NH_2, HCl$; $R^2 = R^3 = H$). The same salt was also prepared from 24-aminocholan-3-one hydrochloride by reaction with phenylhydrazine in boiling methanol containing hydrochloric acid. The two methods of preparation of this salt gave products having identical infrared spectra.

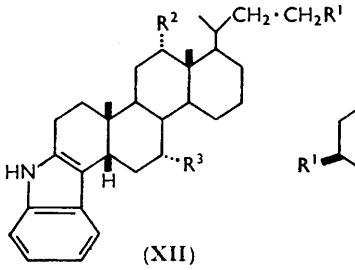
In an attempt to obtain an indolo-steroid with greater solubility in water the synthesis

[4] Heyl and Herr, *J. Amer. Chem. Soc.*, 1953, **75**, 1918.
[5] Jones, Humphries, and Dobriner, *ibid.*, 1950, **72**, 956.
[6] Julian, Meyer, Cole, and Magnani, U.S.P. 2,566,336.
[7] Antaki and Petrov *J.*, 1951, 901.

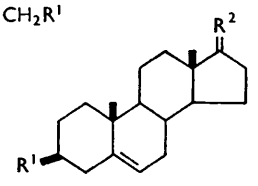
of an indolo-steroid containing two hydroxyl groups was undertaken. Methyl cholate was partially oxidised by the Oppenauer procedure to methyl 7α : 12α-dihydroxy-3-oxocholanate by following Pietra and Traverso's method.[8] Reaction of this compound with phenylhydrazine in acetic acid gave a product which did not crystallise, but, when the diacetylated ester was subjected to the Fischer indole synthesis, methyl 7α : 12α-diacetoxyindolo(2′ : 3′-3 : 4)chol-3-enate (XII; $R^1 = CO_2Me$, $R^2 = R^3 = OAc$) was readily obtained, which was hydrolysed to 7α : 12α-dihydroxyindolo(2′ : 3′-3 : 4)chol-3-enic acid (XII; $R^1 = CO_2H$, $R^2 = R^3 = OH$) in excellent yield. This acid was more soluble in water than indolo(2′ : 3′-3 : 4)chol-3-enic acid.

In order to confirm that no reaction other than hydrolysis of the ester groups of the indolo-steroid (XII; $R^1 = CO_2Me$, $R^2 = R^3 = OAc$) had occurred in the final stage of the above preparation an attempt was made to reconvert the dihydroxy-acid into methyl 7α : 12α-diacetoxyindolo(2′ : 3′-3 : 4)chol-3-enate. The dihydroxy-acid was acetylated by Whitman and Schwenk's method,[9] and the resulting product, after treatment with diazomethane, proved to be methyl 7α : 12α-diacetoxy-*N*-acetylindolo(2′ : 3′-3 : 4)chol-3-enate, identical with the product obtained by acetylation of methyl 7α : 12α-diacetoxyindolo-(2′ : 3′-3 : 4)chol-3-enate (XII; $R^1 = CO_2Me$, $R^2 = R^3 = OAc$).

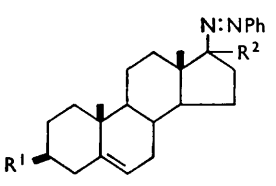
In an attempt to obtain a steroid with a nitrogen-containing heterocyclic ring fused to positions 16 and 17 of ring D, a structure which is common in steroidal alkaloids, dehydro-*epi*androsterone was heated with phenylhydrazine in acetic acid. The product, which crystallised from benzene–light petroleum in *yellow* needles, m. p. 151—151·5° (decomp.), was neither an indolo-steroid nor the phenylhydrazone. Oxidation of the phenylhydrazone is presumed to have taken place to give the hydroperoxide (XIV; $R^1 = OH$, $R^2 = O{\cdot}OH$). Similar reactions have been previously reported by Pausacker.[10] When dehydro*epi*-androsterone was heated with phenylhydrazine in ethanol containing a few drops of concentrated hydrochloric acid, the cooled solution deposited *colourless* needles which, on recrystallisation from benzene, became tinged with yellow. Analysis indicated that they could consist of a mixture of the phenylhydrazone (XIII; $R^1 = OH$, $R^2 = N{\cdot}NHPh$) and the hydroperoxide (XIV; $R^1 = OH$, $R^2 = O{\cdot}OH$). Repeated recrystallisation from ethanol gave the pure hydroperoxide. When, however, dehydro*epi*androsterone acetate was caused to react with phenylhydrazine in ethanol containing hydrochloric acid, *colourless*




(XII)




(XIII)




(XIV)

needles, m. p. 177—178°, separated from the reaction mixture. Rapid recrystallisation of this product from ethanol gave colourless needles, m. p. 137—142°, which gave correct analyses for dehydro*epi*androsterone acetate phenylhydrazone (XIII; $R^1 = OAc$, $R^2 = N{\cdot}NHPh$). The infrared spectra of the products of m. p. 177—178° and m. p. 137—142° are identical. Repeated recrystallisation of the phenylhydrazone (XIII; $R^1 = OAc$, $R^2 = N{\cdot}NHPh$) from benzene–light petroleum gave yellow leaflets, m. p. 154·5—155° (decomp.), identical with the hydroperoxide (XIV; $R^1 = OAc$, $R^2 = O{\cdot}OH$) obtained from the oxidation of the phenylhydrazone of dehydro*epi*androsterone acetate. Reduction of the hydroperoxide in ether with lithium aluminium hydride and crystallisation of the product

[8] Pietra and Traverso, *Gazzetta*, 1951, **81**, 687.
[9] Whitman and Schwenk, *J. Amer. Chem. Soc.*, 1946, **68**, 1865.
[10] Pausacker, *J.*, 1950, 3478.

from dilute ethanol gave 17ξ-phenylazoandrost-5-en-3β-ol (XIV; $R^1$ = OH, $R^2$ = H) in *yellow* needles, m. p. 214—216°. As reduction of a hydroperoxide with lithium aluminium hydride generally gives the corresponding hydroxy-compound, the formation of this product is difficult to interpret. Acetylation of the azo-compound (XIV; $R^1$ = OH, $R^2$ = H), m. p. 214—216°, with acetic anhydride in pyridine gave yellow needles of the acetate (XIV; $R^1$ = OAc, $R^2$ = H), m. p. 173—174°, depressed on admixture with the isomeric phenylhydrazone (XIII; $R^1$ = OAc, $R^2$ = N·NHPh). In the presence of mineral acid mixed azo-compounds readily change into the isomeric hydrazone. Thus acetylation of the *yellow* azo-compound (XIV; $R^1$ = OH, $R^2$ = H), m. p. 214—216°, by Whitman and Schwenk's method [9] gave *colourless* needles, m. p. 137—142°, of dehydro*epi*androsterone acetate phenylhydrazone. A solution of the yellow azo-steroid (XIV; $R^1$ = OH, $R^2$ = H) in ethanol became colourless when acidified. From this mixture, after it had been heated for one hour and then kept overnight, dehydro*epi*androsterone was isolated. Presumably the azo-steroid (XIV; $R^1$ = OH, $R^2$ = H), when acidified, changes rapidly into the more stable phenylhydrazone (XIII; $R^1$ = OH, $R^2$ = N·NHPh), which is then hydrolysed to the keto-steroid (XIII; $R^1$ = OH, $R^2$ = O).

Dehydro*epi*androsterone acetate phenylhydrazone was recovered unchanged after being heated in boiling acetic acid for 18 hours or shaken at room temperature with hydrochloric acid; when it was heated in a boiling solution of hydrochloric acid, it was hydrolysed to dehydro*epi*androsterone.

## Experimental

Unless otherwise stated, the light petroleum has b. p. 60—80°. Solutions were dried, when appropriate, over anhydrous sodium sulphate before being evaporated under reduced pressure. The chromatography column, of internal diameter 2 cm., was packed with " neutral " alumina, type H, 200/250 mesh, supplied by P. Spence and Sons Ltd., Widnes. When required dry, benzene and ether were kept over sodium wire; chloroform was kept over calcium chloride. Ultraviolet absorption spectra were determined with a Unicam S.P. 500 spectrophotometer. Infrared absorption spectra were of Nujol mulls in a Grubb-Parsons double-beam S3 instrument. In all determinations of $[\alpha]_D$ the temperature was about 20° and *c* about 1.

*Preparation of the Amides.*—3α-Acetoxycholanic acid [11] (3·8 g.), 3α : 12α-diformyloxycholanic acid [12] (4 g.), 3α : 7α : 12α-triformyloxycholanic acid [12] (5 g.), 3β-acetoxybisnorchol-5-enic acid [13] (3 g.), and 3β-acetoxychol-5-enic acid [14] (1 g.) were converted into the corresponding amides by the method described below.

Redistilled thionyl chloride (2 ml. per g. of acid) and pyridine (0·1 ml.) were added to the acid in dry benzene (10 ml. per g. of acid). The mixture was left at room temperature for 4 hr., moisture being excluded. The solution was evaporated below 30°. More benzene was then added, and removed similarly. Excess of gaseous ammonia was passed into a cooled solution of the freshly prepared acid chloride in dry benzene, and the mixture was kept overnight at room temperature. The mixture was then diluted with methylene chloride and washed successively with water, dilute sulphuric acid, 3% aqueous sodium carbonate, and with water. Evaporation of the solution yielded the crude amide. In this manner, 3α-acetoxycholanamide (90%), m. p. 193—196° (Ercoli and De Ruggieri [15] recorded m. p. 193°), 3α : 12α-diformyloxycholanamide (93%), gum, 3α : 7α : 12α-triformyloxycholanamide (98%), m. p. 183—187° (Cortese and Bauman [16] recorded m. p. 187°), 3β-acetoxybisnorchol-5-enamide (90%), m. p. 223—225° (Cole and Julian [17] recorded m. p. 227—229°), and 3β-acetoxychol-5-enamide (95%), m. p. 215—218°, were prepared and were used without further purification. Crystallisation of 3β-*acetoxychol-5-enamide* from dilute ethanol gave plates, m. p. 217—218° (Found: C, 75·0; H, 10·05. $C_{26}H_{41}O_3N$ requires C, 75·1; H, 9·9%).

3α-*Acetoxyhomocholanamide.*—Redistilled thionyl chloride (5 ml.) and pyridine (1 drop) were

[11] Reindel and Niederländer, *Ber.*, 1935, **68**, 1969.
[12] Hughes, Smith, and Webb, *J.*, 1949, 3437.
[13] Smith and Wallis, *J. Org. Chem.*, 1954, **19**, 1628.
[14] Ruzicka and Wettstein, *Helv. Chim. Acta*, 1935, **18**, 986.
[15] Ercoli and De Ruggieri, *Farm. Sci. e tec (Pavia)*, 1951, **6**, 547.
[16] Cortese and Bauman, *J. Amer. Chem. Soc.*, 1935, **57**, 1393.
[17] Cole and Julian, *ibid.*, 1945, **67**, 1369.

added to 3α-acetoxycholanic acid (3·7 g.) in dry benzene (50 ml.), and the mixture was left at room temperature for 4 hr. with the exclusion of moisture. The solution was evaporated below 30°. More benzene was then added and removed similarly, yielding the crude acid chloride, which was suspended in dry ether (50 ml.) and stirred into a solution at 0° of diazomethane (*ca.* 7 g.) in ether (200 ml.). After being stirred at 0° for 3 hr. the mixture was left at room temperature overnight. Evaporation below 30° of the filtered solution yielded the crude diazo-ketone, m. p. 127·5—128·5° (decomp.), which was immediately dissolved in dioxan (30 ml.) and then stirred into dioxan (90 ml.) and water (2 ml.) saturated with gaseous ammonia. Ammoniacal silver nitrate (10 ml.) was added dropwise to the vigorously stirred solution, which was slowly heated to 60° and maintained at that temperature for 2 hr. The mixture was then heated to 80° for ½ hr., cooled, and centrifuged. The mother-liquor, diluted with methylene chloride, was washed successively with 2N-nitric acid (200 ml.), 5% aqueous sodium carbonate (200 ml.), and water (200 ml.). Evaporation of the dry solution yielded a brown solid which, in benzene (20 ml.), was chromatographed on alumina (20 g.). Elution with benzene (150 ml.) gave a gum, which crystallised from light petroleum (b. p. 100—120°) in needles (0·55 g.), m. p. 162—164°. Further elution with benzene (1800 ml.), followed by benzene–ether (9 : 1; 300 ml.), gave a solid (1·3 g.), m. p. 163—166°, and ether–benzene (1 : 1; 200 ml.) afforded a solid (0·29 g.), m. p. 160—163°. Finally, elution with ether (500 ml.) and with ethanol (500 ml.) produced a gum, which did not crystallise. The solid isolated from the chromatogram crystallised from light petroleum (b. p. 100—120°) to give 3α-*acetoxyhomocholanamide* (1·8 g.) in needles, m. p. 166·5—167·5°, $[\alpha]_D$ +47° (in chloroform) (Found, after drying at 80°/15 cm. for 3 hr.: C, 75·2; H, 10·4. $C_{27}H_{45}O_3N$ requires C, 75·1; H, 10·5%).

3β-*Acetoxynorchol-5-enamide*.—A suspension in dry ether (50 ml.) of the crude acid chloride, prepared from 3β-acetoxybisnorchol-5-enic acid (2 g.) and thionyl chloride (5 ml.) as described in the experiment above, was added to cooled ethereal diazomethane (*ca.* 5 g. in 200 ml.). The mixture was left for 3 hr. at −10° and 14 hr. at room temperature and then filtered. Evaporation of the filtrate gave a pale yellow solid, which was added to dioxan (150 ml.) and water (2 ml.) saturated with gaseous ammonia. The stirred mixture at 60° was treated with ammoniacal silver nitrate (10 ml.) for 2 hr., and then the product was isolated as in the previous experiment. Elution from alumina (20 g.) with benzene (800 ml.) yielded no solid. Benzene–ether (4 : 1, 300 ml.; 1 : 1, 100 ml.) removed a gum (0·165 g.) which crystallised from a mixture of methylene chloride, light petroleum, and benzene in prisms (0·08 g.), m. p. 278—280° (decomp.). Elution with ether (300 ml.) and ether–ethanol (9 : 1, 200 ml.; 7 : 3, 1250 ml.) gave crystals (0·66 g.), m. p. 279—282° (decomp.). Final elution of the column with chloroform (300 ml.) and with ethanol (500 ml.) gave no crystalline products. The amide (36%), m. p. 278—282° (decomp.), recrystallised from methylene chloride–light petroleum–benzene in stocky prisms, m. p. 281—283° (decomp.). Herr and Heyl [18] prepared this amide, m. p. 283—285° (decomp.), by a different method.

3α-*Hydroxycholanamide* (*Lithocholamide*).—3α-Acetoxycholanamide (4 g.), m. p. 193—196°, was hydrolysed with boiling methanolic potassium hydroxide (5%; 80 ml.) to give 3α-hydroxycholanamide (3·4 g.) in needles (from methanol), m. p. 214·5—216·5°, $[\alpha]_D$ +35° (in ethanol). Louw *et al.*[19] recorded m. p. 211·5—213·5°.

3-*Oxocholanamide*.—Sodium chromate (1 g.) in acetic acid (20 ml.) was added to a solution of 3α-hydroxycholanamide (2 g.) in glacial acetic acid (40 ml.). The mixture was kept at 35° ± 1° for 6 hr., and then stirred into iced water (500 ml.). The precipitate was filtered off, washed with water, and dried. Crystallisation from aqueous ethanol gave leaflets (1·6 g.) of 3-*oxocholanamide*, m. p. 191—192°, $[\alpha]_D$ +34° (in chloroform) (Found: C, 73·4; H, 10·2. $C_{24}H_{39}O_2N,H_2O$ requires C, 73·6; H, 10·5%).

*Preparation of the Amine Hydrochlorides*.—The amides described above were reduced to the amines and isolated as hydrochlorides by the following method: The amide, in a Soxhlet extractor, was extracted with ether into lithium aluminium hydride (2—3 mol. excess) in boiling ether (450 ml.). The base was isolated in the normal manner in ethereal solution into which dry hydrogen chloride was passed until precipitation was complete. The crude amine hydrochloride was collected, washed with ether, and dried in a vacuum-desiccator over phosphorus pentoxide. In this manner, 3β-acetoxybisnorchol-5-enamide (0·2 g.) was converted into 22-*aminobisnorchol-5-en*-3β-*ol hydrochloride* (76%), which crystallised from ethanol–ether

[18] Herr and Heyl, *J. Amer. Chem. Soc.*, 1950, **72**, 1753.
[19] Louw, Strating, and Backer, *Rec. Trav. chim.*, 1954, **73**, 671.

in needles (Found: C, 71·6; H, 10·2. $C_{22}H_{37}ON,HCl$ requires C, 71·8; H, 10·3%). The hydrochloride decomposes when heated, but does not melt below 300°. 3β-Acetoxynorchol-5-enamide (0·2 g.), subjected to the same sequence of reactions, gave *23-aminonorchol-5-en-3β-ol hydrochloride* (49%), which crystallised from ethanol–ether in needles, m. p. *ca.* 300° (decomp.) (Found: C, 69·2; H, 10·1. $C_{23}H_{39}ON,HCl,H_2O$ requires C, 69·3; H, 10·5%).

In similar manner 3β-acetoxychol-5-enamide (0·4 g.) gave *24-aminochol-5-en-3β-ol hydrochloride* (75%), which crystallised from aqueous ethanol in needles, m. p. *ca.* 300° (decomp.) (Found: C, 70·1; H, 10·8; N, 3·55. $C_{24}H_{41}ON,HCl,H_2O$ requires C, 69·65; H, 10·7; N, 3·4%). 3α-Acetoxycholanamide (1·5 g.) gave the crude hydrochloride (73%), crystallisation of which from ether–ethanol afforded 24-aminocholan-3α-ol hydrochloride in needles, m. p. *ca.* 300° (decomp.), $[\alpha]_D$ +33·7° (in ethanol). Wesseley and Swoboda [20] reported m. p. *ca.* 300° with sintering at 260°, $[\alpha]_D$ +32·4° (in ethanol). 3α : 12α-Diformyloxycholanamide (1·7 g.) gave 24-aminocholane-3α : 12α-diol hydrochloride (61%) in prisms, m. p. 275—277° (decomp.), $[\alpha]_D$ +52·7° (in ethanol). Wesseley and Swoboda [20] recorded m. p. 276—278°, $[\alpha]_D$ +53·2° (in ethanol). 24-Aminocholane-3α : 7α : 12α-triol hydrochloride, formed from 3α : 7α : 12α-triformyloxycholanamide (0·4 g.), separated from ether–ethanol in needles (64%), m. p. 270° (decomp.), $[\alpha]_D$ +33° (in ethanol). Wesseley and Swoboda [20] recorded m. p. 270—271°, $[\alpha]_D$ +33·1° (in ethanol). Similarly, 3α-acetoxyhomocholanamide (0·22 g.) gave *25-aminohomocholan-3α-ol hydrochloride* (58%), which decomposed above 250°, Crystallisation from ether–ethanol afforded prisms (Found: C, 72·4; H, 11·3. $C_{25}H_{45}ON,HCl$ requires C, 72·9; H, 11·2%).

*24-Aminocholan-3-one Hydrochloride.*—3-Oxocholanamide (0·5 g.), m. p. 189·5—191°, in " AnalaR " benzene (50 ml.), was boiled under reflux until no more moisture was deposited in a Dean and Stark moisture trap. The solution was cooled and pyrrolidine (0·6 g.) was added (cf. ref. 4). The mixture was then vigorously stirred and boiled under reflux overnight; water was collected in the trap. Removal of the solvents, by distillation under reduced pressure, left a gum which solidified on trituration with ether. The crude enamine was separated from the ether, immediately placed in a sintered-glass crucible inside a Soxhlet extractor, and reduced with lithium aluminium hydride (1 g.) in boiling ether (250 ml.). When all of the amide had dissolved, the reduction was continued for a further 8 hr., after which the mixture was cooled and water (3 ml.) was added cautiously. After the contents of the flask had been boiled under reflux for 0·5 hr. and filtered, the ethereal layer was collected. The precipitate was twice extracted with more boiling ether in a similar manner. Dry hydrogen chloride was passed into the combined and dried ($Na_2SO_4$) ethereal solution until precipitation was complete. The crude amine hydrochloride was filtered off, washed with ether, and dried. Crystallisation from aqueous ethanol furnished *24-aminocholan-3-one hydrochloride* (57%) in needles which began to char at about 200°, but did not melt below 300° (Found: Cl, 9·2. $C_{24}H_{41}ON,HCl$ requires Cl, 9·0%). The infrared spectrum of this compound has a prominent peak at 1719 $cm.^{-1}$. When a solution of the salt in aqueous ethanol was left at room temperature, or boiled for a few minutes, an amorphous solid was precipitated.

*Methyl Indolo(2′ : 3′-3 : 4)chol-3-enate.*—Methyl 3-oxocholanate [21] (1·5 g.) and phenylhydrazine (15 g.) were heated with glacial acetic acid (50 ml.) on a steam-bath for 1 hr. The mixture was then stirred into iced water (300 ml.) containing hydrochloric acid (*d* 1·18; 5 ml.). The precipitated pale yellow solid (1·6 g.) crystallised from ethanol, to give needles (1·2 g.) of *methyl indolo(2′ : 3′-3 : 4)*chol-3-enate, m. p. 158—160°, $[\alpha]_D$ +152° (in chloroform) (Found: C, 80·6; H, 9·3; N, 2·7. $C_{31}H_{43}O_2N$ requires C, 80·7; H, 9·4; N, 3·0%). The infrared absorption spectrum has a strong band at 746 $cm.^{-1}$, but no bands near 700 $cm.^{-1}$.

*Indolo(2′ : 3′-3 : 4)chol-3-enic Acid.*—Methyl indolo(2′ : 3′-3 : 4)chol-3-enate (0·3 g.) and potassium hydroxide (14 g.) in 1 : 1 aqueous methanol (70 ml.) were boiled under reflux for 4 hr. The solution was diluted with water, concentrated under reduced pressure, and acidified with dilute hydrochloric acid. Crystallisation of the resulting precipitate from aqueous ethanol gave a pale yellow powder (0·22 g.), m. p. 184—186°. Repeated recrystallisations from the same solvent gave stocky needles of *indolo(2′ : 3′-3 : 4)chol-3-enic acid*, m. p. 209° with softening below 200° (Found: C, 76·7; H, 9·2; N, 3·1. $C_{30}H_{41}O_2N,H_2O$ requires C, 77·4; H, 9·3; N, 3·0%).

*Indolo(2′ : 3′-3 : 4)chol-3-enamide.*—(i) Thionyl chloride (5 ml.) and pyridine (1 drop) were added to indolo(2′ : 3′-3 : 4)chol-3-enic acid (0·3 g.) in dry ether (30 ml.), and the mixture kept

[20] Wesseley and Swoboda, *Monatsh.*, 1951, **82**, 437.
[21] Fieser and Ettorre, *J. Amer. Chem. Soc.*, 1953, **75**, 1700.

at room temperature for 4 hr. The solvent was removed below 30° and benzene was then added to the residue and removed similarly. The resultant gum was stirred with ammonia solution (*d* 0·88; 50 ml.) for 1 hr. and the mixture was then filtered. Attempts to crystallise the precipitate of crude amide, m. p. 130—150°, were unsuccessful. It could not be purified by chromatography. The infrared absorption spectrum of the compound has two prominent peaks in the 1600—1700 cm.$^{-1}$ range, a peak near 750 cm.$^{-1}$, but no peak near 700 cm.$^{-1}$.

(ii) 3-Oxocholanamide (0·2 g.) and phenylhydrazine (0·2 g.) were heated with glacial acetic acid (30 ml.) on a steam-bath for 1 hr., and then the mixture was stirred into iced 0·2N-hydrochloric acid (150 ml.). The precipitated solid (0·24 g.) was collected, but it did not crystallise. The infrared spectrum has prominent peaks in the same positions as those of the spectrum of the amide prepared by method (i).

24-*Aminoindolo*(2′ : 3′-3 : 4)*chol*-3-*ene Hydrochloride*.—(i) The crude amide of indolo(2′ : 3′-3 : 4)chol-3-enic acid (0·2 g.), in dioxan (20 ml.), was added dropwise to a boiling solution of lithium aluminium hydride (0·5 g.) in dioxan (150 ml.). The mixture was boiled under reflux for 12 hr., then cooled, and water (2 ml.) was cautiously added. When effervescence had ceased, the mixture was boiled under reflux for 30 min. and cooled. The precipitate was filtered off and extracted several times with boiling ether. The combined ethereal and dioxan solutions, concentrated by distillation under reduced pressure, were stirred into water (1 l.), and the precipitated amine was extracted with ether. Dry hydrogen chloride was passed into the ethereal extract, and the resulting precipitate of crude amine hydrochloride was collected. This pale yellow solid, which did not crystallise from any of the common solvents, was shaken with warm water (100 ml.), and the mixture was filtered. Sodium hydrogen carbonate (2% solution) was added to the filtrate until no more precipitation occurred. The mixture was extracted with ether. Passage of dry hydrogen chloride into the dried ethereal extract caused precipitation of the *amine hydrochloride*. This salt, which was discoloured, does not melt below 300° but chars when heated (Found: C, 73·7; H, 9·2; N, 6·0; Cl, 8·0. $C_{30}H_{44}N_2$,HCl,$H_2O$ requires C, 74·0; H, 9·7; N, 6·0; Cl, 7·6%). (ii) A solution of 24-aminocholan-3-one hydrochloride (0·1 g.) and phenylhydrazine (0·1 g.) in ethanol (30 ml.) containing hydrochloric acid (*d* 1·18; 0·1 ml.) was boiled under reflux for 30 min. More hydrochloric acid (4N; 10 ml.) was added to the reactants, which were then boiled for a further hour before they were concentrated to a small volume and stirred into iced water (30 ml.). The precipitate which was filtered off did not crystallise from any of the common solvents. The infrared spectrum of the compound appeared to be identical with that of the amine hydrochloride prepared by method (i).

7α : 12α-*Diacetoxyindolo*(2′ : 3′-3 : 4)*chol*-3-*enic Acid*.—Methyl 7α : 12α-diacetoxy-3-oxocholanate [22] (1·2 g.) and phenylhydrazine (1 g.) were heated with glacial acetic acid (50 ml.) on a water-bath for 1 hr., and then stirred into iced water (300 ml.) containing hydrochloric acid (*d* 1·18; 5 ml.). The precipitate (1·25 g.) was filtered off and crystallised (carbon) from benzene–light petroleum to afford *methyl* 7α : 12α-*diacetoxyindolo*(2′ : 3′-3 : 4)*chol*-3-*enate* (0·98 g.) in needles, m. p. 238·5—240°, $[\alpha]_D$ +218° (in chloroform) (Found: C, 72·7; H, 8·1; N, 2·5. $C_{35}H_{47}O_6N$ requires C, 72·8; H, 8·2; N, 2·4%). A solution of the methyl ester (0·3 g.) and potassium hydroxide (16 g.) in 1 : 1 aqueous methanol (80 ml.) was boiled under reflux for 4 hr., diluted with water, and then concentrated under reduced pressure. Subsequent acidification with dilute hydrochloric acid yielded a precipitate, which crystallised from aqueous ethanol to give needles (0·28 g.) of 7α : 12α-*dihydroxyindolo*(2′ : 3′-3 : 4)*chol*-3-*enic acid*, m. p. 172°, resolidifying and remelting at 210—212° (Found: C, 72·8; H, 8·8. $C_{30}H_{41}O_4N$,$H_2O$ requires C, 72·4; H, 8·7%).

*Acetylation of* 7α : 12α-*Dihydroxyindolo*(2′ : 3′-3 : 4)*chol*-3-*enic Acid* (*cf. ref.* 9).—Perchloric acid (0·1 ml.) was added to a mixture of 7α : 12α-dihydroxyindolo(2′ : 3′-3 : 4)chol-3-enic acid (0·14 g.), acetic anhydride (1·5 ml.) and acetic acid (5 ml.) at 18°. After 30 min. at <30° the reactants were cooled to 18° and ice was added. The mixture was then stirred into water, and the precipitate (0·15 g.), m. p. 247—257° (decomp.), was filtered off. Crystallisation from aqueous ethanol gave needles of 7α : 12α-*diacetoxy*-N-*acetylindolo*(2′ : 3′-3 : 4)*chol*-3-*enic acid*, m. p. 260—263° (decomp.) (Found: C, 70·8; H, 7·7. $C_{36}H_{47}O_7N$ requires C, 71·4; H, 7·8%).

*Methyl* 7α : 12α-*Diacetoxy*-N-*acetylindolo*(2′ : 3′-3 : 4)*chol*-3-*enate* (*cf. ref.* 9).—(i) Methyl 7α : 12α-diacetoxyindolo(2′ : 3′-3 : 4)chol-3-enate was treated with acetic acid, acetic anhydride, and perchloric acid. The product, isolated in the usual manner, crystallised from aqueous ethanol to yield *methyl* 7α : 12α-*diacetoxy*-N-*acetylindolo*(2′ : 3′-3 : 4)*chol*-3-*enate* in needles, m. p.

[22] Jones, Webb, and Smith, *J.*, 1949, 2164.

222—224° (Found: C, 71·2; H, 7·9. $C_{37}H_{49}O_7N$ requires C, 71·7; H, 7·9%). (ii) 7α : 12α-Diacetoxy-*N*-acetylindolo(2′ : 3′-3 : 4)chol-3-enic acid was esterified with diazomethane in ether. Crystallisation of the product from aqueous ethanol afforded needles, m. p. 222—224°, undepressed when mixed with the *N*-acetyl derivative prepared by method (i). The products have identical infrared absorption spectra.

*Reaction between Dehydro*epi*androsterone and Phenylhydrazine.*—(i) Dehydro*epi*androsterone (0·3 g.) and phenylhydrazine (0·3 g.) were heated together in glacial acetic acid (20 ml.) on a boiling-water bath for 1 hr. The mixture was kept for 1 hr. at room temperature before it was stirred into iced water (200 ml.) containing hydrochloric acid (*d* 1·18; 2 ml.). The precipitated solid crystallised from benzene–light petroleum, to give yellow needles (0·13 g.) of 17ξ-*hydroperoxy*-17ξ-*phenylazoandrost*-5-*en*-3β-*ol*, m. p. 151—151·5° (decomp.) (Found: C, 73·8; H, 8·2; N, 7·3. $C_{25}H_{34}O_3N_2$ requires C, 73·2; H, 8·3; N, 6·8%). The crystals darkened on exposure to light and the atmosphere. (ii) A solution of dehydro*epi*androsterone (0·2 g.) and phenylhydrazine (0·1 g.) in ethanol (30 ml.) containing concentrated hydrochloric acid (0·1 ml.) was boiled under reflux for 30 min., and then evaporated. The residual jelly crystallised from aqueous ethanol in needles (0·16 g.), m. p. 128—141°, which, when recrystallised from the same solvent, were tinted yellow (Found: C, 75·4; H, 9·1. Calc. for $C_{25}H_{34}ON_2$: C, 79·3; H, 9·0. Calc. for $C_{25}H_{33}ON_2{\cdot}O{\cdot}OH$: C, 73·2; H, 8·3%). The crystals are probably a mixture of the phenylhydrazone and 17ξ-hydroperoxy-17ξ-phenylazoandrost-5-en-3β-ol. The latter compound, m. p. 151—151·5°, was obtained when the mixture was repeatedly crystallised from ethanol.

*Reaction between Dehydro*epi*androsterone Acetate and Phenylhydrazine.*—Concentrated hydrochloric acid (0·2 ml.) was added to a solution of dehydro*epi*androsterone acetate (2·5 g.) and phenylhydrazine (2 g.) in ethanol (80 ml.) at 70°. When crystals began to be formed, the mixture was cooled and then filtered. The crystalline precipitate (2·1 g.), m. p. 177—178° (decomp.), by rapid recrystallisation from ethanol, afforded colourless needles (1·8 g.) of *dehydro*epi*androsterone acetate phenylhydrazone*, m. p. 137—142° (decomp.), $[\alpha]_D$ +37° (in dioxan) (Found: C, 76·5; H, 8·6. $C_{27}H_{36}O_2N_2$ requires C, 77·1; H, 8·6%), $\lambda_{max.}$ 272 mμ (log ε 4·34) in dioxan. The infrared absorption spectrum of the product of m. p. 177—178° and that of the phenylhydrazone, m. p. 137—142°, were identical.

17ξ-*Hydroperoxy*-17ξ-*phenylazoandrost*-5-*en*-3β-*yl Acetate.*—(i) The phenylhydrazone, prepared as above, gave on repeated recrystallisation from benzene–light petroleum yellow leaflets of 17ξ-*hydroperoxy*-17ξ-*phenylazoandrost*-5-*en*-3β-*yl acetate*, m. p. 154·4—155° (decomp.), $[\alpha]_D$ −118° (in dioxan) (Found: C, 71·7; H, 8·1; N, 6·75. $C_{27}H_{36}O_4N_2$ requires C, 71·7; H, 8·0; N, 6·2%), $\lambda_{max.}$ 269, 419 mμ (log ε 4·1, 2·2), $\lambda_{min.}$ 347 mμ (log ε 1·4). The crystals darkened when exposed to light and the atmosphere. (ii) Oxygen was bubbled for 2 hr. *via* a sintered-glass sparger into a solution of dehydro*epi*androsterone acetate phenylhydrazone (1 g.) in benzene (150 ml.) which contained benzoyl peroxide (10 mg.). The solution became yellow and, finally, deep red. Benzene (approx. 100 ml.) was distilled off until precipitation occurred from the solution. The mixture was then cooled, and the hydroperoxide (0·76 g.) was isolated. It crystallised from ethanol in yellow leaflets, m. p. and mixed m. p. with the compound prepared by method (i), 154·5—155° (decomp.). The products have identical infrared absorption spectra.

17ξ-*Phenylazoandrost*-5-*en*-3β-*ol.*—(i) A solution of 17ξ-hydroperoxy-17ξ-phenylazoandrost-5-en-3β-yl acetate (0·2 g.) in dry ether (75 ml.) was added dropwise to a boiling solution of lithium aluminium hydride (0·2 g.) in ether (50 ml.). This mixture was boiled under reflux for 2 hr. and the product of the reduction was isolated, as previously described, as a pale yellow solid, which crystallised from ethanol in needles (0·16 g.) of 17ξ-*phenylazoandrost*-5-*en*-3β-*ol*, m. p. 214—216°, $[\alpha]_D$ −8° (in dioxan) (Found: C, 79·2; H, 9·0; N, 7·2. $C_{25}H_{34}ON_2$ requires C, 79·3; H, 9·0; N, 7·4%), $\lambda_{max.}$ 267, 409 mμ (log ε 4·0, 2·2), $\lambda_{min.}$ 332 mμ (log ε 0·93) in dioxan. (ii) 17ξ-Hydroperoxy-17ξ-phenylazoandrost-5-en-3β-ol (0·4 g.), in a sintered-glass crucible in a Soxhlet extractor, was reduced for 3 hr. with lithium aluminium hydride (0·5 g.) in boiling ether (200 ml.). The product, which was isolated as in method (i), on crystallisation from ethanol, furnished 17ξ-phenylazoandrost-5-en-3β-ol in needles (0·3 g.), m. p. and mixed m. p. with the compound prepared by method (i), 214—216°.

*Acetylation of* 17ξ-*Phenylazoandrost*-5-*en*-3β-*ol.*—(i) *With acetic anhydride–pyridine mixtures.* The azo-steroid (100 mg.) was kept for 24 hr. at room temperature in a mixture of pyridine (5 ml.) and acetic anhydride (5 ml.). Sufficient ice was added to destroy the anhydride, and

then the mixture was stirred into water (100 ml.). The resulting precipitate crystallised from ethanol to give 17ξ-*phenylazoandrost-5-en-3β-yl acetate* (75 mg.), pale yellow needles, m. p. 173—174° (Found: C, 77·4; H, 8·4. $C_{27}H_{36}O_2N_2$ requires C, 77·1; H, 8·6%). (ii) *With acetic acid–acetic anhydride–perchloric acid* (cf. ref. 9). Perchloric acid (50%; 1 drop) was added to a solution of the azo-steroid (100 mg.) in acetic acid (10 ml.) and acetic anhydride (3 ml.). After 30 min. at $\nearrow$30°, the reactants were cooled and sufficient ice was added to destroy the anhydride. The mixture was then stirred into water (100 ml.), and the resulting precipitate (0·1 g.) was collected. Rapid crystallisation of the product from ethanol gave colourless needles, m. p. 135—140°, having an infrared spectrum identical with that of dehydro*epi*androsterone acetate phenylhydrazone.

*Hydrolysis of* 17ξ-*Phenylazoandrost-5-en-3β-ol.*—Concentrated hydrochloric acid (5 ml.) was added to a solution of the azo-steroid (0·1 g.) in ethanol (20 ml.). This solution was boiled under reflux for 1 hr.; it became deep crimson and then the colour faded. After the solution had been kept overnight at room temperature it was poured into water. The resulting precipitate was crystallised, with difficulty, from dilute methanol to give prisms, m. p. 149—151°. The m. p. was undepressed when the compound was mixed with an authentic specimen of dehydeo*epi*androsterone, and the compound has an infrared spectrum which is identical with that of the keto-steroid.

*Attempted Conversion of Dehydro*epi*androsterone Acetate Phenylhydrazone into an Indolosteroid.*—(i) The phenylhydrazone (100 mg.) in glacial acetic acid (20 ml.) was heated for 1 hr. on a boiling-water bath and then added to dilute hydrochloric acid, and filtered. Crystallisation from ethanol gave dehydro*epi*androsterone acetate phenylhydrazone (85 mg.), m. p. 135—145°. (ii) A solution of the phenylhydrazone (100 mg.) in glacial acetic acid (50 ml.) was boiled under reflux for 18 hr., then poured into dilute hydrochloric acid (200 ml.). Crystallisation from ethanol gave dehydro*epi*androsterone acetate phenylhydrazone, m. p. 137—142°. (iii) A solution of the phenylhydrazone (100 mg.) in ethanol (40 ml.) containing concentrated hydrochloric acid (10 ml.) was boiled under reflux for 1 hr. The mixture was then stirred into water (100 ml.) and kept overnight. Crystallisation of the precipitate from dilute methanol gave dehydro*epi*androsterone, m. p. 142—143°. The phenylhydrazone was recovered unchanged after being shaken with cold dilute hydrochloric acid for 10 min.

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KING'S COLLEGE (UNIVERSITY OF LONDON),
STRAND, LONDON, W.C.2.

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