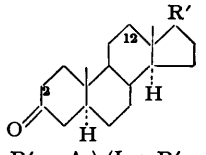
202. *Steroids and Related Compounds. Part XII. Some Heterocyclic Derivatives.*

By H. Antaki and V. Petrow.

Quinolino-, indolo-, pyrrolo-, thiazolo-, and triazafluoreno-steroids have been prepared for biological study. All the compounds obtained proved sparingly soluble with the exception of (II; R = NH_2), which failed to show appreciable activity against a variety of organisms.

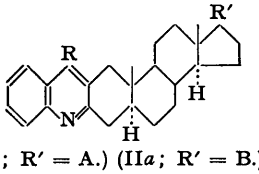
The preparation of basic derivatives of steroids for biological study has previously been recorded (cf. Haslewood, *Biochem. J.*, 1941, **35**, 1307; Barber, Dible, and Haslewood, *ibid.*, 1943, **37**, vi; Barnett, Ryman, and Smith, *J.*, 1946, 524, 528; James, Smith, Stacey, and Webb, *J.*, 1946, 665; Stacey and Webb, *Proc. Roy. Soc.*, 1947, *B*, **134**, 523, 538; Jones, Webb, and Smith, *J.*, 1949, 2164). Extension of this work to heterocyclic compounds, however, has not hitherto been described in the literature, in spite of the fact that some naturally occurring products of this type show interesting biological properties. Thus (i) the *Solanum* alkaloids β-dihydrosolasodine and tetrahydrosolasodine antagonise the positive chronotropic action of adrenaline in the anæsthetised dog (Krayer and Briggs, *Brit. J. Pharmacol.*, 1950, **5**, 118), (ii) the steroidal bases present in *Veratrum viride* appear to function as hypotensive agents in cases of essential hypertension (Wilkins, Stanton, and Freis, *Proc. Soc. Exp. Biol.*, 1949, **72**, 302), and (iii) conessine shows marked amœbicidal activity. Before considering the synthesis of such compounds, however, it seemed desirable to determine the limitations imposed by the steroidal ring system on established procedures for the preparation of the quinoline, pyrrole, indole, thiazole, and triazafluorene ring systems.

(A = ·CHMe·$[CH_2]_3$·Pr^i.)



(I; R′ = A.) (I*a*; R′ = B.)

(B = ·CHMe·CH_2·CH_2·CO_2H.)



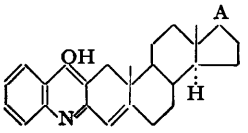
(II; R′ = A.) (II*a*; R′ = B.)

Borsche and Frank (*Ber.*, 1924, **57**, 1373) had previously recorded the condensation of 3 : 12-diketo- and of 3 : 7 : 12-triketo-cholanic acid with *o*-aminobenzaldehyde and with isatin, obtaining thereby the corresponding quinolino-derivatives, but they failed to comment on their biological activity. We now find that the reaction with *o*-aminobenzaldehyde may be extended to cholestan-3-one (I) and to 3-keto*allo*cholanic acid (I*a*), to give the corresponding quinolino-derivatives in good yield. Although both linear and angular formulations are possible for these compounds, the linear structures (II; R = H) and (II*a*; R = H), respectively, are preferred in view of the established reactivity of the $C_{(2)}$ methylene group in ketones of this type (cf. Butenandt and Wolff, *Ber.*, 1935, **68**, 2091; Rosenheim and Stiller, *J.*, 1938, 353; Windaus and Kuhr, *Annalen*, 1937, **532**, 52). Unfortunately both these compounds proved too insoluble for biological study, and we had perforce to turn our attention to the synthesis of basically-substituted analogues.

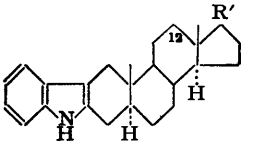
By heating cholestan-3-one (I) with anthranilic acid, 4′-hydroxyquinolino(3′ : 2′-2 : 3)-cholest-2-ene (II; R = OH) was readily obtained in excellent yield. With phosphorus pentachloride in phosphorus oxychloride this gave the corresponding 4′-chloro-compound (II; R = Cl), converted in *p*-cresol at 200° by gaseous ammonia into 4′-aminoquinolino(3′ : 2′-2 : 3)-cholest-2-ene (II; R = NH_2) (cf. Albert and Gledhill, *J. Soc. Chem. Ind.*, 1945, **64**, 169), and into the 4′-phenoxy-analogue by phenol at 130°. The action of anthranilic acid on cholest-4-en-3-one, however, appeared to follow a modified pattern. The product, it is true, gave analytical figures in good agreement with those for a 4′-hydroxyquinolino(3′ : 2′-2 : 3)cholesta-2 : 4-diene (III), but attempts at its conversion into the 4′-chloro-derivative failed. The formulation (III) assigned to this compound must, therefore, be accepted with reserve. Methyl 3-keto*allo*-cholanate, in contrast, gave methyl 4′-hydroxyquinolino(3′ : 2′-2 : 3)*allo*chol-2-enate, readily transformed into the 4′-chloro-acid (II*a*; R = Cl). Conversion of the latter product into the 4′-amino-acid (II*a*; R = NH_2), however, gave low yields. We were thus precluded from attempting a Curtius degradation.

Indolo-steroids were first described by Dorée (*J.*, 1909, 653), who prepared the " tetrahydro-

carbazole derivative from coprostan-3-one " by treating the latter with phenylhydrazine in acetic acid. Extending these observations to cholestan-3-one, Dorée and Petrow (*J.*, **1935**, 1391) obtained a compound to which they assigned the formulation of an indolo(2′ : 3′ : 3 : 4)cholestane.



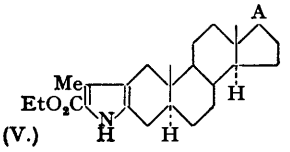
(III.)



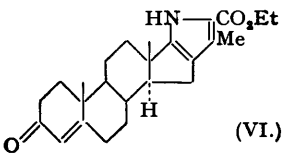
(IV; R′ = A.) (IV*a*; R′ = B.)

Evidence supporting this structure was furnished by surface-film measurements which, although not conclusive, appeared to favour a formulation of the angular type. There seems little doubt, however, that this conclusion must now be reversed and the compound assigned the linear structure (IV), the angular formulation being retained for the derivative from coprostan-3-one.

Biological study of (IV) was hampered by low solubility. We, therefore, examined the action of phenylhydrazine on methyl 3-keto*allo*- and 3 : 12-diketo-cholanate. Reaction occurred readily in acetic acid. The indolo-acids obtained by hydrolysis of the resulting methyl esters, however, proved insufficiently soluble to warrant further study. Attempts to convert the acid grouping of (IV*a*) into amino- *via* the hydrazide likewise proved disappointing. Reaction was ultimately achieved by heating the ester with 50% hydrazine hydrate at 140°, but the yield of hydrazide obtained was low.

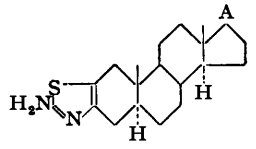


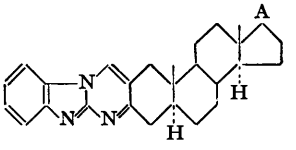
(V.)



(VI.)

(VII.)





(VIII.)

Compounds possessing a condensed pyrrole residue were synthesised by Knorr and Lange's reaction (*Ber.*, 1902, **35**, 2998), as extended by Treibs and Dinelli (*Annalen*, 1935, **517**, 152). Thus by condensing (I) with ethyl oximinoacetoacetate in the presence of zinc dust and acetic acid, ethyl 4′-methylpyrrolo(3′ : 2′-2 : 3)cholest-2-ene-5′-carboxylate (V) was obtained, although in only 10% yield. Cholest-4-en-3-one failed to react under these conditions. With androst-4-ene-3 : 17-dione it was hoped that reaction might be confined to the ketomethylene residue present in ring D, and a nucleus isomeric with that present in conessine obtained. The expected product, $C_{25}H_{33}O_3N$, to which the formulation ethyl 3-keto-4′-methylpyrrolo(3′ : 2′-16 : 17)androsta-4 : 16-diene-5′-carboxylate (VI) is assigned, was formed in such low yield, however, as to rob the method of practical significance. The reverse condensation of 16-oximinoandrost-4-ene-3 : 17-dione (Stodola and Kendall, *J. Org. Chem.*, 1942, **7**, 336) with ethyl acetoacetate in the presence of zinc dust and acetic acid led only to 16-ketotestosterone.

2′-Aminothiazolo(5′ : 4′-2 : 3)cholest-2-ene (VII) was prepared by reaction between 2-bromo-cholestan-3-one and thiourea in boiling *iso*propyl alcohol. 1′ : 9′ : 11′-Triazafluoreno(3′ : 2′-2 : 3)cholest-2-ene (VIII) was obtained in good yield by condensing 2-aminobenziminazole with 2-hydroxymethylenecholestan-3-one (Rosenheim and Stiller, *J.*, 1938, 357).

Only (II; R = NH_2) of the above compounds proved sufficiently soluble for biological study. Examination against a variety of organisms, kindly carried out for us by Dr. S. W. F. Underhill (Physiological Laboratories, The British Drug Houses, Ltd.), failed to reveal appreciable activity.

Experimental.

M. p.s are uncorrected. Microanalyses are by Drs. Weiler and Strauss, Oxford. Optical rotations were measured in chloroform solution in a 2-dm. tube.

Methyl 3-*Keto*all*ocholanate.*—Methyl 3-hydroxy*allo*chol-5-enate was prepared from the acid by Wallis and Fernholtz's method (*J. Amer. Chem. Soc.*, 1935, **57**, 1504). Catalytic hydrogenation of this

ester (15 g.) in acetone (130 ml.), in the presence of 5% palladised charcoal (10 g.), gave methyl 3-hydroxy-*allo*cholanate, m. p. 148—150°. Oxidation to the 3-keto-derivative was effected by Wieland and Dane's procedure (*Z. physiol. Chem.*, 1933, **215**, 15).

Quinolino(3′ : 2′-2 : 3)*cholest-2-ene* (II; R = H).—Cholestan-3-one (1·5 g.), *o*-aminobenzaldehyde (500 mg.) and potassium hydroxide (2·0 g.) in aqueous ethanol (80 ml.) were shaken with gentle warming, dissolution occurring, followed by separation of the condensation product. After 10—15 minutes the mixture was cooled, and the solids collected and purified from benzene–alcohol. *Quinolino*(3′ : 2′-2 : 3)*cholest-2-ene* formed crystals, m. p. 184°, $[\alpha]_D^{23}$ +63·7° (*c*, 1·489) (Found: C, 86·1; H, 10·3; N, 3·0. $C_{34}H_{49}N$ requires C, 86·6; H, 10·4; N, 3·0%).

Methyl quinolino(3′ : 2′-2 : 3)allo*chol-2-enate*, prepared from methyl 3-keto*allo*cholanate as above, formed crystals (from benzene–alcohol), m. p. 264° (Found: C, 80·6; H, 8·8; N, 3·4. $C_{32}H_{43}O_2N$ requires C, 81·1; H, 9·0; N, 3·0%).

4′-*Hydroxyquinolino*(3′ : 2′-2 : 3)*cholest-2-ene* (II; R = OH).—An intimate mixture of cholestanone (8 g.) and anthranilic acid (3·5 g.) was heated in a 100-ml. flask at 140° until a homogeneous liquid had been obtained; the temperature was then slowly raised to 200—220° and maintained there until the reaction mass had solidified. The resulting yellow solid was powdered, extracted thoroughly with benzene and alcohol, and finally crystallised from pyridine or nitrobenzene. 4′-*Hydroxyquinolino*-(3′ : 2′-2 : 3)*cholest-2-ene* (6 g.) formed very small white needles, m. p. >300° (Found: C, 83·4; H, 10·1. N, 3·1. $C_{34}H_{49}ON$ requires C, 83·8; H, 10·0; N, 2·9%).

4′-*Chloroquinolino*(3′ : 2′-2 : 3)*cholest-2-ene* (II; R = Cl).—The foregoing compound (15 g.) was heated with phosphorus pentachloride (7 g.) and phosphorus oxychloride (30 ml.) for 2 hours under reflux at 120—130°. The *product*, isolated in the usual way, crystallised from benzene–alcohol in leaflets, m. p. 204°, $[\alpha]_D^{23}$ +40·7° (*c*, 1·311) (Found: N, 3·3; Cl, 7·4. $C_{34}H_{48}NCl$ requires N, 2·8; Cl, 7·0%).

4′-*Phenoxyquinolino*(3′ : 2′-2 : 3)*cholest-2-ene* (II; R = OPh).—The fore-going chloro-derivative (1·5 g.) and phenol (2·0 g.) were heated at 150—160° for 2 hours, after which the cooled mass was digested with sodium hydroxide solution and the product extracted with chloroform. Purification by adsorption on alumina, followed by elution with ethanol, gave 4′-*phenoxyquinolino*(3′ : 2′-2 : 3)*cholest-2-ene*, needles (from aqueous ethanol), m. p. 157° (softens at 112—113° with loss of $0·75H_2O$), $[\alpha]_D^{23}$ +36·3° (*c*, 0·716) (Found, on anhydrous material: C, 84·8; H, 9·1; N, 2·7. $C_{40}H_{53}ON$ requires C, 85·1; H, 9·5; N, 2·5%).

4′-*Aminoquinolino*(3′ : 2′-2 : 3)*cholestane* (II; R = NH_2).—The foregoing chloro-derivative (2·0 g.) dissolved in *p*-cresol (20 g.) was treated at 200° with a stream of dry ammonia for 70 minutes. The cooled mass was agitated with excess of sodium hydroxide solution, and the solids were extracted with hot benzene. Addition of light petroleum (b. p. 100—120°) to the hot concentrated extract gave the 4′-*amine* on cooling, as platelets, m. p. 217°, $[\alpha]_D^{23}$ +25·6° (*c*, 0·421) (Found: C, 84·1; H, 9·7; N, 5·8. $C_{34}H_{50}N_2$ requires C, 84·0; H, 10·2; N, 5·8%).

Methyl 4′-*hydroxyquinolino*(3′ : 2′-2 : 3)allo*cholenate*, prepared from methyl 3-keto*allo*cholanate (10·6 g.) and anthranilic acid (4·4 g.), formed a white powder, m. p. >300° (Found: C, 78·7; H, 8·2; N, 2·5. $C_{32}H_{43}O_3N$ requires C, 78·5; H, 8·7; N, 2·8%).

4′-*Chloroquinolino*(3′ : 2′-2 : 3)allo*chol-2-enic acid* (II*a*; R = Cl), obtained by treating the foregoing compound with phosphorus pentachloride–oxychloride, formed plates [from benzene–light petroleum (b. p. 80—100°)], m. p. 193°, $[\alpha]_D^{23}$ +34·7° (*c*, 0·748) (Found: C, 75·1; H, 8·1; N, 3·4; Cl, 7·7. $C_{31}H_{40}O_2NCl$ requires C, 75·3; H, 8·1; N, 2·8; Cl, 7·2%).

4′-*Aminoquinolino*(3′ : 2′-2 : 3)allo*chol-2-enic acid* (II*a*; R = NH_2) separated from benzene–light petroleum (b. p. 100—120°) in pale yellow crystals, m. p. 198° (Found: C, 78·5; H, 9·2; N, 5·9. $C_{31}H_{42}O_2N_2$ requires C, 78·4; H, 8·8; N, 5·9%).

4′-*Hydroxyquinolino*(3′ : 2′-2 : 3)*cholesta*-2 : 4-*diene* (III) separated from nitrobenzene in a yellow felted mass of very small needles, m. p. >300° (Found: C, 85·0; H, 9·3; N, 2·9. $C_{34}H_{47}ON$ requires C, 84·1; H, 9·6; N, 2·9%).

Methyl Indolo(3′ : 2′-2 : 3)allo*chol-2-enate*.—Methyl 3-keto*allo*cholanate (2·5 g.) and redistilled phenylhydrazine (5 g.) were heated with acetic acid (80 ml.) on the water-bath for 1 hour. The mixture was then poured into water. The precipitated pale yellow solids were collected and crystallised from benzene–ethanol, giving *methyl indolo*(3′ : 2′-2 : 3)allo*chol-2-enate* in white crystals, m. p. 194°, $[\alpha]_D^{23}$ +55·6° (*c*, 1·025) (Found: C, 80·6; H, 9·1; N, 3·1. $C_{31}H_{43}O_2N$ requires C, 80·6; H, 9·5; N, 3·0%).

Indolo(3′ : 2′-2 : 3)allo*chol-2-enic acid hemihydrate* (IV*a*), prepared by hydrolysis of the foregoing ester, formed from aqueous ethanol crystals which melted at 180°, resolidified, and finally melted at 220° (Found: C, 78·3; H, 9·6; N, 3·6. $C_{30}H_{41}O_2N,\frac{1}{2}H_2O$ requires C, 78·6; H, 9·2; N, 3·0%).

Methyl 12-*ketoindolo*(3′ : 2′-2 : 3)*chol-2-enate*, prepared from methyl 3 : 12-diketocholanate, formed crystals (from benzene–ethanol), m. p. 197°, $[\alpha]_D^{22}$ +224·8° (*c*, 0·889) (Found: C, 77·7; H, 8·7; N, 2·6. $C_{31}H_{41}O_3N$ requires C, 78·3; H, 8·6; N, 2·9%).

The corresponding *acid monohydrate*, obtained by hydrolysis of the foregoing ester and crystallised from aqueous ethanol, had m. p. 232° (decomp.) (Found: C, 75·1; H, 8·1. $C_{30}H_{39}O_3N,H_2O$ requires C, 75·1; H, 8·5%).

Indolo(3′ : 2′-2 : 3)allo*chol-2-enoylhydrazide*, prepared by heating the corresponding methyl ester (2·5 g.) and hydrazine hydrate (10 ml. of 50%) in a sealed tube at 140° for 5 hours, separated from aqueous alcohol in crystals, m. p. 240—242° (Found: C, 77·3; H, 9·3; N, 8·8. $C_{30}H_{43}ON_3$ requires C, 78·0; H, 9·3; N, 9·1%).

Ethyl 4′-*Methylpyrrolo*(3′ : 2′-2 : 3)*cholest-2-ene-5′-carboxylate* (V).—Cholestanone (8 g.) and ethyl oximinoacetoacetate (3·2 g.) in acetic acid (170 ml.) were heated gently under reflux, and zinc dust (6 g.) was gradually added with efficient mechanical stirring during 20 minutes. Refluxing was continued for a further 3 hours, whereafter unreacted zinc was removed and washed with acetic acid and

ethanol, and the bulked filtrate and washings were poured into water (1 l.) with stirring. The precipitated solids (10 g.) were collected, dried, extracted with alcohol (which removed unchanged cholestanone), and crystallised from benzene–light petroleum (b. p. 60—80°), to give the desired *product*, (700 mg.), m. p. 212°, $[\alpha]_D^{26}$ +34·1° (*c*, 0·468) (Found: C, 79·9; H, 10·2; N, 3·2. $C_{33}H_{53}O_2N$ requires C, 80·0; H, 10·7; N, 2·8%).

Ethyl 3-*Keto*-4′-*methylpyrrolo*(3′ : 2′-16 : 17)*androsta*-4 : 16-*diene*-5′-*carboxylate* (VI).—When androst-4-ene-3 : 17-dione (5 g.) and ethyl oximinoacetoacetate (3·2 g.) in acetic acid (170 ml.) were treated with zinc dust (10 g.) under reflux, a very low yield of this *pyrroloandrostadiene* was obtained, as crystals [from benzene–light petroleum (b. p. 60—80°)], m. p. 204—220° (partial decomp.) (Found: C, 75·7; H, 8·2; N, 3·5. $C_{25}H_{33}O_3N$ requires C, 75·9; H, 8·3; N, 3·5%).

16-*Oximinoandrost*-4-*ene*-3 : 17-*dione*.—The following method was employed (cf. Stodola and Kendall, *loc. cit.*): Androst-4-ene-3 : 17-dione (10 g.) and amyl nitrite (4·6 g.) were added to sodium (1·0 g.) in absolute ethanol (80 ml.) and the mixture heated at 60—70° for 30 minutes. After 2 days at room temperature the dark red solution was poured into ice-water (750 ml.) and extracted with ether, and the aqueous layer acidified with acetic acid. Crystallisation of the precipitated solids from benzene–light petroleum (b. p. 60—80°) gave 16-oximinoandrost-4-ene-3 : 17-dione as a yellow-brown solid, darkening above 220° and melting at 244° (decomp.), $[\alpha]_D^{26}$ +240·8° (*c*, 0·745) (Found: C, 72·1; H, 7·5; N, 4·2. Calc. for $C_{19}H_{25}O_3N$: C, 72·3; H, 7·9; N, 4·4%). Stodola and Kendall (*loc. cit.*) give 230° and 237—238°, respectively.

16-*Ketotestosterone*.—Reduction of 16-oximinoandrost-4-ene-3 : 17-dione (2·4 g.) and ethyl acetoacetate (8 g.) in 95% acetic acid (100 ml.) with zinc dust (7 g.) under reflux gave 16-*ketotestosterone*, needles (from benzene–light petroleum), m. p. 189° (Found: C, 75·1; H, 8·6. $C_{19}H_{26}O_3$ requires C, 75·5; H, 8·6%).

2′-*Aminothiazolo*(5′ : 4′-2 : 3)*cholest*-2-*ene* (VII).—2-Bromocholestanone (1·5 g.) and thiourea (250 mg.) were heated under reflux in *iso*propyl alcohol (30 ml.) for 40 minutes. After cooling, the mixture was treated with hot sodium carbonate solution, and the solids were collected and crystallised from benzene. 2′-*Aminothiazolo*(5′ : 4′-2 : 3)*cholest*-2-*ene* formed crystals, m. p. 266°, $[\alpha]_D^{26}$ +64·4° (*c*, 0·496) (Found: C, 76·0; H, 10·1; N, 5·8; S, 6·9. $C_{28}H_{46}N_2S$ requires C, 76·0; H, 10·4; N, 6·3; S, 7·2%).

1′ : 9′ : 11′-*Triazafluoreno*(3′ : 2′-2 : 3)*cholestane* (VIII), prepared by heating 2-hydroxymethylenecholestan-3-one (1·7 g.) with 2-aminobenziminazole (500 mg.) at 190° and finally at 220°, crystallised from chloroform–alcohol in yellow plates, m. p. 295°, $[\alpha]_D^{24}$ +78·1° (*c*, 0·467) (Found: C, 81·1; H, 9·6; N, 8·7. $C_{35}H_{49}N_3$ requires C, 82·2; H, 9·6; N, 8·2%).

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