273. *The Reaction of Ketocholanic Acids with Bromoacetic Ester and Cyanoacetic Ester.*

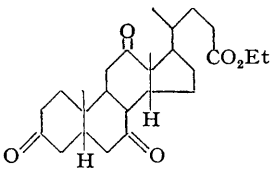
By F. Smith and M. Webb.

Isomeric (unsaturated) dicarboxylic acids have been obtained by the condensation of ethyl 3 : 7 : 12-triketocholanate with ethyl cyanoacetate and ethyl bromoacetate. It is believed that the C_3 keto-group of the triketocholanic acid molecule is involved in these condensations.

In previous papers (Barnett, Ryman, and Smith, *J.*, 1946, 524, 526, 528; James, Smith, Stacey, and Webb, *ibid.*, p. 665) preliminary reports on the investigation of basic derivatives of steroids have been recorded. Some of these compounds, particularly those with a basic group at C_{23} and C_{24} in the side chain of the norcholane and cholane structures, display antibacterial activity *in vitro*. Greater activity appears to be shown by those derivatives having an amino-group in the side chain than by those with an amino-group attached directly to the perhydro*cyclo*pentenophenanthrene nucleus. Thus 23-amino-3 : 7 : 12-trihydroxynorcholane is more active than 7-amino-3 : 12-dihydroxycholanic acid. Interest was then directed to an examination of compounds having a side chain at C_3 into which basic groups could be introduced so that this basic group would be separated from the nucleus by one or more carbon atoms.

In order to produce intermediates for the synthesis of these compounds we have subjected to preliminary examination the reaction of ketocholanic acids with bromoacetic ester, malonic ester, and cyanoacetic ester.

When ethyl 3 : 7 : 12-triketocholanate (I) is allowed to react with ethyl cyanoacetate in acetic acid solution in the presence of acetamide (cf. Cope, *J. Amer. Chem. Soc.*, 1937, **59**, 2327) a smooth reaction takes place with the production of *ethyl* 7 : 12-*diketo*-3-*cyanocarbethoxymethylenecholanate* (II) in good yield. This reaction is analogous to that occurring when ethyl cyanoacetate reacts with *cyclo*hexanone with sodium ethoxide as condensation reagent, whereby there is formed a mixture of two isomers which affords upon hydrolysis *cyclo*hexeneacetic acid and *cyclo*hexylideneacetic acid (Harding, Haworth, and Perkin, *J.*, 1908, **93**, 1943). In the condensation recorded in this paper the conditions seem to favour the formation of (II) which possesses the double bond in the side chain. Support for the structure (II) assigned to the condensation product is afforded by the fact that treatment of it with osmium tetroxide effects hydroxylation (see Criegee, *Annalen*, 1936, **522**, 75; Butenandt *et al.*, *Ber.*, 1939, **72**, 1112) to give (III), subsequent oxidation of which with periodic acid (Malaprade, *Bull. Soc. chim.*, 1928, **43**, 683; 1934, **1**, 833) gives ethyl 3 : 7 : 12-triketocholanate (I).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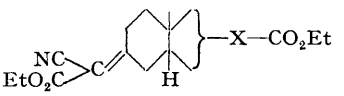
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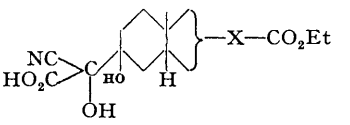
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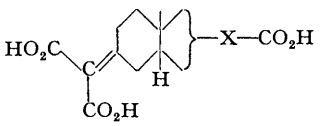
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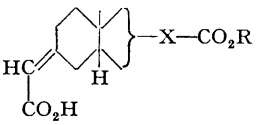
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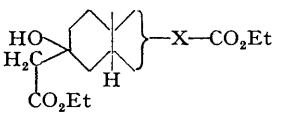
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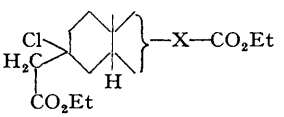
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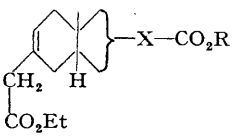
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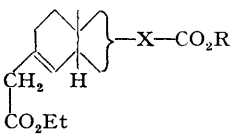
(IX.)

(X = $C_{16}H_{20}O_2$)

Hydrolysis of (II) with aqueous alcoholic sulphuric acid yields a mixture of the tricarboxylic acid (7 : 12-diketo-3-dicarboxymethylenecholanic acid) (IV) and the dicarboxylic acid (7 : 12-*diketo*-3-*carboxymethylenecholanic acid*) (V; R = H). The conversion of (IV) into (V; R = H) can be brought about by heating (IV) above its melting point. (V; R = H) was also characterised by its transformation into the crystalline diethyl ester (V; R = Et).

No reaction occurred when a solution of 3 : 7 : 12-triketocholanic acid in acetic anhydride was boiled for 18 hours in the presence of ethyl malonate.

Ethyl 3 : 7 : 12-triketocholanate (I) reacts, however, with ethyl bromoacetate in the presence of zinc under the usual Reformatzky reaction conditions to give ethyl hydroxydiketocarbethoxymethylcholanate (VI). In view of the fact that (I) contains three keto-groups, its reaction with bromoacetic ester might involve any one of the three groups. Experimental facts, however, demonstrate that the 7-keto-group in the cholane nucleus does not take part in the Reformatzky

reaction inasmuch as ethyl 3 : 12-dihydroxy-7-ketocholanate failed to react with ethyl bromoacetate under the prescribed conditions. Hence it follows that the keto-group concerned in the reaction must be either at C_3 or C_{12}. Of these, that at C_3 is known to be the more reactive (Sobotka, " Chemistry of the Steroids ", Williams and Wilkins, 1937, p. 420). Furthermore a Grignard reagent, such as phenylmagnesium bromide, does not react with the C_{12} keto-group (Riegel and Moffett, *J. Amer. Chem. Soc.*, 1943, **65**, 1971). It is therefore tentatively concluded that, in the reaction between ethyl 3 : 7 : 12-triketocholanate and bromoacetic ester with zinc, it is the C_3 keto-group which is affected.

Such a condensation will result in the introduction at C_3 of a new asymmetric centre with the possible formation of two enantiomorphs. This may explain why the product failed to crystallise. When this dissymmetry at C_3 was eliminated from (VI) by the removal of the elements of water, the resulting unsaturated *compound* (IX or VIII; R = Et) crystallised readily. The double bond in (IX) and (VIII) is believed to be in the ring and not in the side chain for the reason that the ethyl ester (IX or VIII; R = Et) is different from the ethyl ester (V; R = Et) the structure of which is known (cf. Harding, Haworth, and Perkin, *loc. cit.*; Haberland, *Ber.*, 1943, **76**, 621). Furthermore, oxidation of (IX or VIII; R = H) by potassium permanganate fails to give triketocholanic acid, a product which might be expected to be formed if the double bond were in the side chain as in (V).

Direct conversion of (VI) into (IX or VIII; R = Et), by loss of the elements of water, occurred when (VI) was distilled in high vacuum.

When (VI) was dehydrated by treatment with thionyl chloride, (IX or VIII; R = H) was obtained. This procedure probably gives rise to (VII) which upon saponification followed by treatment with acid affords (IX or VIII; R = H) (cf. Bachmann, Cole, and Wilds, *J. Amer. Chem. Soc.*, 1940, **62**, 824). The crystalline unsaturated acid (IX or VIII; R = H) undergoes smooth esterification with ethereal diazoethane to give the corresponding ethyl ester (IX or VIII; R = Et). This unsaturated ethyl ester was not identical with the crystalline unsaturated ethyl ester obtained by vacuum distillation of the saturated ethyl ester (VI); furthermore it also differed from the unsaturated ester (V; R = Et) which has its double bond in the side chain. It is suggested therefore that the difference between the two isomeric unsaturated ethyl esters derived from (VI) and represented by (IX and VIII; R = Et) is due to the fact that the double bond occupies a different position in the ring.

The tentative conclusion is thus reached that the two esters represented by formulæ (IX and VIII; R = Et) and that to which formula (V; R = Et) has been assigned are isomers.

Further examination of these unsaturated compounds will be made in order to confirm the above results and to explore the possibilities of the preparation of basic derivatives.

Experimental.

Ethyl 7 : 12-*Diketo*-3-*cyanocarbethoxymethylenecholanate* (II).—A solution of ethyl triketocholanate (ethyl dehydrocholate) (20 g., 0·046 moles), ethyl cyanoacetate (15·8 g., 0·14 moles), and acetamide (1·0 g.) in glacial acetic acid (170 c.c.) was slowly distilled from a Claisen flask fitted with a fractionating column such that the temperature of the vapours distilling was maintained at 105—110°. After 9 hours, when the volume of the distillate had reached 150 c.c., the product was diluted with glacial acetic acid (200 c.c.) and the solution poured with stirring into water (2 l.). After 16 hours the solid precipitate was collected, washed with water, and dried at the pump. After two recrystallisations from ethyl alcohol (a large volume of alcohol is necessary, as otherwise the product separates as a gel) the ethyl 7 : 12-*diketo*-3-*cyanocarbethoxymethylenecholanate* (II), which formed fine needles, had m. p. 150—153° (yield, 15 g.). A further recrystallisation from benzene–light petroleum (1 : 1) afforded pure (II), m. p. 155—157°, $[\alpha]_D^{19°}$ + 54·5° in chloroform (*c*, 2·0). It was sparingly soluble in ether and alcohol, soluble in benzene, ethyl acetate, chloroform, and acetone. It decolourised bromine in carbon tetrachloride rapidly (Found : C, 71·0; H, 7·7; N, 2·6. $C_{31}H_{43}O_6N$ requires C, 70·8; H, 8·2; N, 2·7%).

Hydrolysis. A suspension of ethyl 7 : 12-diketo-3-cyanocarbethoxymethylenecholanate (II) (4 g.) in water (75 c.c.) and 5N-sulphuric acid (10 c.c.) was warmed to 60—70°, and ethyl alcohol was then added until complete solution was obtained. The solution was boiled under reflux for 8 hours, cooled, and left at room temperature for 16 hours. The amorphous solid which separated was filtered off and combined with the precipitate formed when the mother liquors were poured with stirring into water (1 l.). After removal of sulphuric acid by washing, the combined solids were dried in a vacuum. The compound was soluble in ethyl alcohol, methyl alcohol, benzene, acetone, ethyl acetate, and chloroform, and sparingly soluble in ether. From aqueous ethyl alcohol or aqueous acetone it separated as a gel, and from benzene–light petroleum it separated in an amorphous condition. From ether–light petroleum (1 : 1), the flocculent precipitate, on standing, gradually formed fine needles (1·8 g.), m. p. 141°. The analytical figures and equivalent weight determinations indicated that this material was a mixture of the di- (V, R = H) and tri- (IV) carboxylic acids. [Found: equiv., 180. Calc. for (V, R = H): equiv., 163. Calc. for (IV) : equiv., 222).

The above mixture (1·8 g.) was gradually heated to 150—160° and kept there until the evolution of gas had ceased. After cooling and trituration with methyl alcohol, the product crystallised. The solid

was collected and recrystallised first from methyl alcohol and then from aqueous methyl alcohol, giving 7 : 12-*diketo*-3-*carboxymethylenecholanic acid* (V, R = H) (0·4 g.) as fine needles, m. p. 131—133°, $[\alpha]_D^{21°}$ + 48·5° in chloroform (*c*, 4·0). It was soluble in chloroform, ethyl acetate, glacial acetic acid and benzene, less soluble in methyl alcohol, and sparingly soluble in ether; it decolourised a solution of bromine in carbon tetrachloride rapidly [Found: C, 70·2; H, 7·9; equiv. (by titration), 223. $C_{24}H_{34}O_2(CO_2H)_2$ requires C, 70·2; H, 8·2%; equiv., 222).

Treatment of a solution of (V, R = H) in methyl alcohol with a slight excess of ethereal diazoethane gave the *diethyl* ester (V; R = Et) which separated from aqueous ethyl alcohol as fine needles, m. p. 250°. This product decolourised bromine in carbon tetrachloride rapidly (Found: C, 71·8; H, 9·1. $C_{30}H_{44}O_6$ requires C, 71·9; H, 8·9%).

Oxidation. Ethyl 7 : 12-diketo-3-cyanocarbethoxymethylenecholanate (II) (1 g.) was suspended in dry ether (175 c.c.), and osmium tetroxide (1 g.) added. The suspension was left at room temperature for 4 days; most of the ester had then dissolved. The solution was filtered and evaporated under reduced pressure. Ethyl alcohol (60 c.c.) and water (120 c.c.) were added to the residue, and the resulting solution was boiled under reflux for 6 hours with anhydrous sodium sulphite (10 g.) and then evaporated to dryness under reduced pressure. The residue was extracted 4 times with ethyl alcohol, and the combined extracts evaporated under reduced pressure. The residual solid was washed with water to remove any sodium sulphite and then dissolved in methyl alcohol (20 c.c.). A solution of periodic acid (from sodium metaperiodate, 0·2 g.) in water (4 c.c.) was added, and the solution left at room temperature for 16 hours. After addition of water (100 c.c.) as much methyl alcohol as possible was removed under reduced pressure at room temperature. The precipitated solid thus produced was collected after 24 hours, washed with water, and crystallised from aqueous ethyl alcohol. After a second crystallisation from the same solvent, the compound (fine needles) (75 mg.) had m. p. 216—218° alone or in admixture with ethyl 3 : 7 : 12-triketocholanate (I).

Treatment of the compound (m. p. 216—218°) with hydroxylamine gave a trioxime, m. p. 227—229°, not depressed by admixture with an authentic specimen of the trioxime of ethyl 3 : 7 : 12-triketocholanate.

Treatment of 3 : 7 : 12-*Triketocholanic Acid with Ethyl Malonate.*—A solution of 3 : 7 : 12-triketocholanic acid (4 g.), ethyl malonate (4·8 g.), and zinc chloride (0·2 g.) in acetic anhydride (25 c.c.) was boiled for 18 hours under reflux. After cooling, the product was poured with stirring into water (500 c.c.). The precipitated syrup had solidified after 48 hours at room temperature. Filtration followed by washing with water and decolourisation with charcoal in hot aqueous alcohol gave a light brown solution from which a product was separated by pouring the mixture into water with stirring. The precipitate was repeatedly extracted with sodium hydrogen carbonate solution until the extracts no longer gave a precipitate upon acidification. The combined extracts were acidified by addition of dilute hydrochloric acid, and the precipitate (1·0 g.) washed with water, dried, and esterified by boiling for 5 hours with ethyl alcohol (50 c.c.) containing sulphuric acid (2·0 c.c.). The mixture was cooled and poured with stirring into water (600 c.c.). The turbid solution was extracted thrice with ether, and the combined extracts washed with water (twice), sodium hydrogen carbonate solution (twice), and finally water. After the ethereal solution had been dried ($CaCl_2$) and the ether removed, ethyl 3 : 7 : 12-triketocholanate (0·6 g.) readily crystallised; m. p. and mixed m. p. 220—221° (after recrystallisation from ethyl alcohol).

Treatment of the recovered ethyl 3 : 7 : 12-triketocholanate (0·07 g.) with hydroxylamine hydrochloride (0·04 g.) and anhydrous sodium acetate (0·17 g.) in methyl alcohol (2·8 c.c.) and water (1·7 c.c.) on the boiling water-bath for 1 hour gave the crystalline trioxime, which separated directly from the reaction mixture, m. p. and mixed m. p. 228—230° (after recrystallisation from aqueous ethyl alcohol).

Ethyl 3-*Hydroxy*-7 : 12-*diketo*-3-*carbethoxymethylcholanate* (VI).—A mixture of ethyl 3 : 7 : 12-triketocholanate (I) (dried in a vacuum at 100°, 14 g.), dry benzene (200 c.c.), ethyl bromoacetate (10 g.), activated zinc (6 g.), and dry copper powder (0·5 g.) was boiled under reflux with precautions to prevent ingress of moisture. After 2 hours, ethyl bromoacetate (1·5 g.) was added and the boiling continued for a further 5 hours. The product was cooled and poured with stirring into ice-cold 2N-sulphuric acid (250 c.c.). The benzene layer was separated, and the acid solution extracted with benzene (twice) and ether (twice). The combined extracts were washed with water (3 times), dried ($CaCl_2$), and evaporated under reduced pressure. The residual *compound* (7·95 g.) formed a syrup which failed to crystallise [Found: OEt, 17·9. $C_{24}H_{36}O_3(CO_2Et)_2$ requires OEt, 17·4%].

Dehydration. (*a*) A fraction of the syrup (VI) was subjected to high vacuum distillation. No distillation occurred below 320° (0·03 mm.). Above this temperature, distillation, with some decomposition, occurred giving a product which crystallised readily on trituration with methyl alcohol. After recrystallisation from methyl alcohol, the small prisms (IX or VIII; R = Et) (10 mg.) had m. p. 215°. The compound was unsaturated and decolourised bromine in carbon tetrachloride rapidly in the cold.

(*b*) A solution of the dry Reformatzky condensation product (VI) (7·0 g.) in a mixture of dry benzene (100 c.c.) and dry pyridine (3·4 c.c.) was treated for 30 minutes at room temperature with thionyl chloride (6·8 c.c.) (purified by distillation first from quinoline and then from linseed oil according to the method of Velick, White, and Lewis, *J. Biol. Chem.*, 1939, **127**, 477). During this period an oil separated. The mixture was evaporated under reduced pressure, first at room temperature and then at 40° (bath temp.). Methyl alcohol (100 c.c.) containing potassium hydroxide (4 g.) was added to the residue and the resulting solution boiled under reflux for 30 minutes. A reddish-brown precipitate separated, and was redissolved by addition of 45% potassium hydroxide (50 c.c.) and water (100 c.c.). The solution was boiled under reflux for a further 4 hours, cooled, and neutralised (litmus) with 2N-hydrochloric acid. The solution was then boiled with charcoal (2—3 g.) for 10 minutes, filtered, and concentrated in an open vessel on a boiling water-bath to remove methyl alcohol. After cooling, the solution was acidified with 2N-hydrochloric acid and the light brown solid which separated was collected after 48 hours, dried in

a vacuum over phosphoric oxide, powdered, and extracted with ether to remove coloured impurities. After recrystallisation from aqueous methyl alcohol, the *compound* (IX or VIII; R = H) (1·6 g.) formed extremely fine needles, m. p. 278°. It decolourised bromine in carbon tetrachloride rapidly. It was insoluble in light-petroleum, sparingly soluble in benzene, chloroform, acetone, and ether, slightly soluble in cold methyl alcohol, ethyl alcohol, and ethyl acetate. It showed $[\alpha]_D^{21°}$ + 43° in water (sodium salt) (*c*, 1·8) [Found, for the acid dried in a vacuum at 100° for 6 hours: C, 69·7; H, 8·35; equiv. (by titration), 218. $C_{26}H_{36}O_6$ requires C, 70·2; H, 8·2%; equiv., 222].

Treatment of the acid (0·6 g.) in dry methyl alcohol (300 c.c.) with excess of ethereal diazoethane followed by removal of the solvent under reduced pressure gave a crystalline residue which on recrystallisation from dilute alcohol gave the ethyl ester (IX or VIII; R = Et) (0·4 g.) in the form of long colourless needles, m. p. 150—151°, $[\alpha]_D^{21°}$ + 56° in chloroform (*c*, 1·1). This *ester* was readily soluble in ether, chloroform, warm ethyl alcohol, and benzene, less soluble in ethyl acetate and acetone, sparingly soluble in light petroleum (Found: C, 72·4; H, 9·35. $C_{30}H_{44}O_6$ requires C, 71·9; H, 8·9%).

Reformatzky Reaction with Ethyl 3 : 12-*Dihydroxy-7-ketocholanate.*—To activated zinc (2·2 g.) in dry benzene (50 c.c.) dry ethyl 3 : 12-dihydroxy-7-ketocholanate (Haslewood, *loc. cit.*) (5·4 g.) and ethyl bromoacetate (4 g.) were added. After the addition of a trace of dry copper powder, the mixture was boiled for 4 hours. The cooled solution was poured with stirring into ice-cold N-hydrochloric acid (500 c.c.). The benzene layer was separated and the lower acid layer extracted with ether (3 times). The combined ether and benzene extracts were washed with water, then with dilute sodium hydrogen carbonate solution, and dried ($MgSO_4$). Evaporation under reduced pressure gave a thick syrup which crystallised on trituration with methyl alcohol. Two recrystallisations from methyl alcohol afforded the original compounds as colourless prisms (3 g.), m. p. 154° not depressed by mixture with ethyl 3 : 12-dihydroxy-7-ketocholanate, m. p. 154°. The compound formed an oxime, m. p. 93°, alone or in admixture with the oxime of ethyl 3 : 12-dihydroxy-7-ketocholanate.

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