584. *The Reaction between Thio-compounds and Keto-steroids.*

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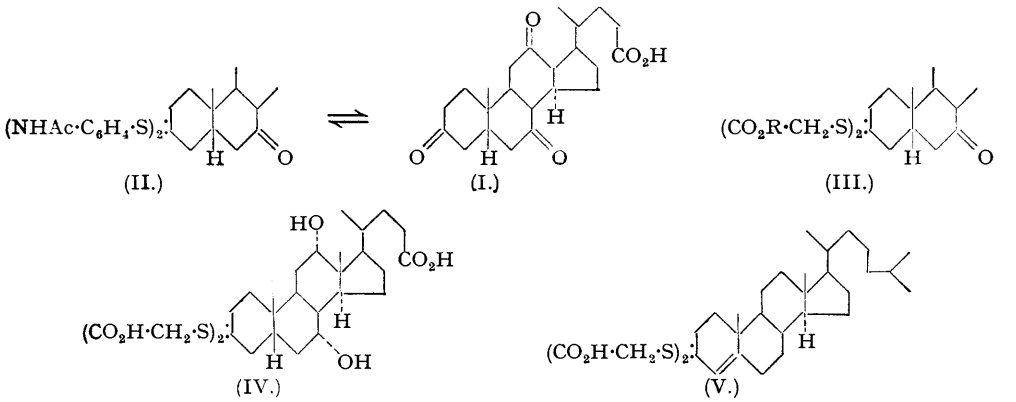
Thioglycollic acid appears to react preferentially with the $C_{(3)}$-keto-group of 3 : 7 : 12-triketocholanic acid (I) to give crystalline 7 : 12-*diketo*-3 : 3-*di*(*carboxymethylthio*)*cholanic acid* (III; R = H). The condensation of thioglycollic acid with 3-keto-steroids appears to be a general reaction. Thus, thioglycollic acid also reacts with cholest-4(5)-en-3-one to give (V) and with 7 : 12-dihydroxy-3-ketocholanic acid to give (IV). The latter compound is stable to dilute alkali, but treatment with dilute mineral acid regenerates the original 7 : 12-dihydroxy-3-ketocholanic acid. It also has been observed that *p*-acetamidothiophenol, as well as thiophenol, combine with 3 : 7 : 12-triketocholanic acid.

It is suggested that such thio-compounds may be of use in the characterisation of keto-steroids and in synthetic work.

Previous communications (Barnett, Ryman, and Smith, *J.*, 1946, 524, 526, 528; James, Smith, Stacey, and Webb, *J.*, 1946, 665; Jones, Webb, and Smith, this vol., p. 2164) have recorded the synthesis from the bile acids and the sterols of antibacterial compounds which contained basic groups. In view of the marked antibacterial action of certain sulphones, sulphoxides, thiols, and sulphonic acid (Dubos, *Ann. Rev. Biochem.*, 1942, **11**, 659) we now have extended this work to the synthesis and investigation of a series of water-soluble, sulphur-containing steroid derivatives.

Mylius (*Ber.*, 1887, **20**, 1968) first observed a reaction between dehydrocholic (3 : 7 : 12-triketocholanic) acid and thiophenol and isolated a sulphur-containing product which has been designated 3 : 3-bisphenylthiodehydrocholic acid by Sobotka ("Chemistry of the Sterids," Williams and Wilkins, Baltimore, 1937). We were unable to repeat Mylius's work using the conditions he specified, but found that condensation proceeds smoothly in either chloroform or dioxan in the presence of hydrogen chloride (Jones, Smith, and Webb, *Nature*, 1948, **162**, 857;

cf. Hauptmann, *J. Amer. Chem. Soc.*, 1947, **69**, 562). It is apparent that this is a general reaction as *p*-acetamidothiophenol combines with 3 : 7 : 12-triketocholanic acid (I) in an analagous fashion and gives rise to 7 : 12-*diketo*-3 : 3-*di*-p-*acetamidophenylthiocholanic acid* (II). Attempts to deacetylate this compound were unsuccessful, and hydrolysis with hot dilute hydrochloric acid regenerated 3 : 7 : 12-triketocholanic acid.



(II.) (I.) (III.) (IV.) (V.)

In view of the greater reactivity of thioglycollic acid (cf. Hickinbottom, " Reactions of Organic Compounds," Longmans Green and Co., 1945, p. 108) we extended our investigations to the reaction between this thio-acid and keto-steroids. 3 : 7 : 12-Triketocholanic acid (I) was found to react smoothly with two molecular proportions of thioglycollic acid to give the crystalline 7 : 12-*diketo*-3 : 3-*di*(*carboxymethylthio*)*cholanic acid* (III; R = H), characterised by its equivalent weight and by the formation of the *trimethyl* ester (III; R = Me). That condensation occurs at $C_{(3)}$ and not at $C_{(7)}$ or $C_{(12)}$ is indicated by the fact that both ethyl 3 : 12-dihydroxy-7-ketocholanate and 3 : 7-dihydroxy-12-ketocholanic acid fail to react with thioglycollic acid, whilst 7 : 12-dihydroxy-3-ketocholanic acid behaves like 3 : 7 : 12-triketocholanic acid and affords the crystalline 7 : 12-*dihydroxy*-3 : 3-*di*(*carboxymethylthio*)*cholanic acid* (IV). In this connection it may be recalled that, whereas ethanedithiol reacts with the three keto-groups in 3 : 7 : 12-triketocholanic acid (Hauptmann, *loc. cit.*), both ethane- and toluene-ω-thiol react only with the 3-keto-group (Bernstein and Dorfman, *J. Amer. Chem. Soc.*, 1946, **68**, 1152; Hauptmann, *loc. cit.*). Similarly, cholest-4(5)-en-3-one combines with thioglycollic acid to give a 3 : 3-*dithio-compound* (V), in which reaction the double bond does not take part (cf. Cunneen, *J.*, 1947, 36; Bernstein and Dorfman, *loc. cit.*).

The thioglycollic acid residues at $C_{(3)}$ appear to be stable to sodium hydroxide, but they may be split off by dilute hydrochloric acid. Thus 7 : 12-dihydroxy-3 : 3-di(carboxymethylthio)-cholanic acid (IV) is unaffected by boiling 5N-sodium hydroxide, but, when heated under reflux with dilute hydrochloric acid, 7 : 12-dihydroxy-3-ketocholanic acid is regenerated (cf. Bernstein and Dorfman, *loc. cit.*; Hauptmann, *loc. cit.*). In view of the behaviour of these 3 : 3-dithio-compounds towards acids and alkalis, they may prove useful in synthetic experiments. Hauptman's observations (*loc. cit.*) on the reaction between keto-steroids and ethanedithiol have led to an excellent synthetic method for the preparation of the hitherto relatively inaccessible lithocholic acid.

The activities of the members of this series of compounds bacteriostatic for Gram-positive organisms have been recorded elsewhere (Jones, Smith, and Webb, *loc. cit.*).

Experimental.

The Condensation of p-*Acetamidothiophenol with* 3 : 7 : 12-*Triketocholanic Acid.*—Dry hydrogen chloride was passed through a suspension of 3 : 7 : 12-triketocholanic acid (1 g.) and *p*-acetamidothiophenol (Zincke and Jorg, *Ber.*, 1909, **42**, 3362) (0·6 g.) in dry dioxan (10 c.c.) at 10° for 3 hours. The suspended solids gradually dissolved and ultimately a white solid was precipitated. The contents of the reaction vessel were then poured slowly with stirring into water (600 c.c.) cooled in ice. The resulting precipitate was collected after 5 minutes, repeatedly washed with water, and dried at the pump. Two crystallisations from aqueous alcohol gave 7 : 12-*diketo*-3 : 3-*di*-(p-*acetamidophenylthio*)-*cholanic acid* (1·5 g.) as white needles, m. p. 165°, $[\alpha]_D^{18}$ +13·0° (*c*, 1·2 in ethyl alcohol) (Found, after correcting for ash: C, 66·8; H, 7·0; N, 4·2; Ac, 13·1. $C_{40}H_{50}O_6N_2S_2$ requires C, 66·8; H, 7·0; N, 3·9; Ac, 12·0%).

Alkaline Hydrolysis of 7 : 12-*Diketo*-3 : 3-*di*-(p-*acetamidophenylthio*)*cholanic Acid.*—(*a*) A solution of

7 : 12-diketo-3 : 3-di-(*p*-acetamidophenylthio)cholanic acid (0·2 g.) in 2N-sodium hydroxide (10 c.c.) was boiled under reflux for 1 hour and then evaporated to dryness under reduced pressure. The residual solid was dissolved in water and the solution neutralised (litmus) with 2N-hydrochloric acid. The dark brown solid which separated was filtered off, washed with water, and dried. The compound could not be induced to crystallise. Similar non-crystalline products were obtained when the hydrolysis was effected with 0·5N- and 0·1N-aqueous-alcoholic sodium hydroxide.

(*b*) A solution of 7 : 12-diketo-3 : 3-di-(*p*-acetamidophenylthio)cholanic acid (0·2 g.) in 0·05N-methyl alcoholic potassium hydroxide (60 c.c.) was boiled under reflux for 3 hours in an atmosphere of nitrogen. After cooling in ice, the light brown solution was neutralised with dry hydrogen chloride, filtered, and evaporated under reduced pressure at 40° (bath temp.). The residue on trituration with ethyl alcohol yielded a crystalline solid which, after crystallisation from aqueous ethyl alcohol, had m. p. 218° and contained a trace of sulphur. Recrystallisation from the same solvent yielded fine white needles which did not contain sulphur and had m. p. 232° alone and in admixture with an authentic specimen of 3 : 7 : 12-triketocholanic acid.

Acid Hydrolysis of 7 : 12-*Diketo*-3 : 3-*di*-(p-*acetamidophenylthio*)*cholanic Acid.*—A solution of 7 : 12-diketo-3 : 3-di-(*p*-acetamidophenylthio)cholanic acid (0·1 g.) in ethyl alcohol (15 c.c.) and 5N-hydrochloric acid (5 c.c.) was left at room temperature for 24 hours and then poured into water (200 c.c.). The fine white needles which separated were collected after 18 hours and recrystallised from aqueous ethyl alcohol. The sulphur-free product had m. p. 232° alone and in admixture with an authentic specimen of 3 : 7 : 12-triketocholanic acid.

7 : 12-*Diketo*-3 : 3-*di*(*carboxymethylthio*)*cholanic Acid.*—A solution of 3 : 7 : 12-triketocholanic acid (1·0 g.) in thioglycollic acid (10 c.c.) was left for 24 hours at room temperature and then diluted with 2N-sodium hydroxide (600 c.c.). The resulting clear solution was acidified (Congo-red) with 5N-hydrochloric acid. The white, flocculent precipitate which separated was collected after 4 hours, washed with water, and dried in a vacuum over phosphoric oxide. The compound crystallised from acetone–light petroleum as white needles (0·65 g.), m. p. 200°. Recrystallisation from aqueous ethyl alcohol gave 7 : 12-*diketo*-3 : 3-*di*(*carboxymethylthio*)*cholanic acid* as long white needles, m. p. 203°, $[\alpha]_D^{24}$ +55° (*c*, 1·0 in ethyl alcohol) (Found : C, 58·2; H, 7·0; S, 11·6; H_2O, 1·7; equiv., 187. $C_{28}H_{40}O_8S_2,\frac{1}{2}H_2O$ requires C, 58·2; H, 7·1; S, 11·1; H_2O, 1·6%; equiv., 192).

Methyl 7 : 12-*Diketo*-3 : 3-*di*(*carbomethoxymethylthio*)*cholanate.*—A solution of 7 : 12-diketo-3 : 3-di-(carboxymethylthio)cholanic acid (0·55 g.) in dry methyl alcohol (10 c.c.) was esterified with ethereal diazomethane. Crystallisation of the product from aqueous ethyl alcohol gave the *trimethyl* ester (0·45 g.) as white plates, m. p. 78°, $[\alpha]_D^{24}$ +51·3° (*c*, 1·0 in ethyl alcohol) (Found : C, 61·0; H, 7·8; S, 10·2. $C_{31}H_{46}O_8S_2$ requires C, 61·0; H, 7·6; S, 10·3%).

7 : 12-*Dihydroxy*-3 : 3-*di*(*carboxymethylthio*)*cholanic Acid.*—A solution of 7 : 12-dihydroxy-3-keto-cholanic acid (Jones, Webb, and Smith, *loc. cit.*) (1·2 g.) in thioglycollic acid (5 c.c.) was kept at room temperature for 24 hours. The resulting solution was then diluted with 2N-sodium hydroxide and the product isolated as described above. After drying in a vacuum over phosphoric oxide the compound (1·3 g.) was crystallised twice from acetone–light petroleum (1 : 1). From this solvent it separated as white prisms, m. p. 132—135°. Recrystallisation from aqueous ethyl alcohol gave 7 : 12-*dihydroxy*-3 : 3-*di*(*carboxymethylthio*)*cholanic acid* as white needles (0·9 g.), m. p. 141°, $[\alpha]_D^{20}$ +35·7° (*c*, 1·0 in ethyl alcohol), which contained one molecule of water of crystallisation (Found : C, 56·9; H, 7·4; S, 10·8; H_2O, 3·0; equiv., 197. $C_{28}H_{44}O_8S_2,H_2O$ requires C, 56·9; H, 7·8; S, 10·9; H_2O, 3·0%; equiv., 195).

The Action of Thioglycollic Acid on Ethyl 3 : 12-*Dihydroxy*-7-*ketocholanate.*—Ethyl 3 : 12-dihydroxy-7-ketocholanate (Haslewood, *Biochem. J.*, 1943, **37**, 109) (0·5 g.) was dissolved in thioglycollic acid (5 c.c.) and left for 3 days at room temperature. The solution was then poured into water (200 c.c.) and the resulting emulsion extracted with ether (3 times). The ethereal extract was washed with water (6 times), dried ($MgSO_4$), and concentrated under reduced pressure. The resulting syrup, which contained much thioglycollic acid, was dissolved in ethyl alcohol (50 c.c.) and the solution poured into water (600 c.c.). The semi-solid precipitate was triturated with water until completely solid and then filtered off, washed with water, and dried in a vacuum over phosphoric oxide. The resulting dry, amorphous material crystallised on trituration with cold methyl alcohol. Recrystallisation from acetone–light petroleum (1 : 1) gave large white plates which did not contain sulphur and had m. p. 156° alone or in admixture with an authentic specimen of ethyl 3 : 12-dihydroxy-7-ketocholanate.

The Action of Thioglycollic Acid on 3 : 7-*Dihydroxy*-12-*ketocholanic Acid.*—3 : 7-Dihydroxy-12-ketocholanic acid (Wieland and Kapitel, *Z. physiol. Chem.*, 1932, **212**, 269) (0·5 g.) was dissolved in thioglycollic acid (10 c.c.) and left at room temperature for 48 hours. The product was poured into water (100 c.c.) and 5N-sodium hydroxide added until the solution was only faintly acid (litmus). The solution was then saturated with sodium chloride and the precipitate which separated was filtered off, washed with water, and dissolved in N-sodium hydroxide (10 c.c.). Acidification of this solution with 5N-hydrochloric acid gave a partially crystalline solid which, after crystallisation first from ethyl alcohol and then from aqueous ethyl alcohol, formed white prisms, m. p. 216° alone and in admixture with an authentic specimen of 3 : 7-dihydroxy-12-ketocholanic acid.

3 : 3-*Di*(*carboxymethylthio*)*cholest*-4-*ene.*—The clear solution which resulted when cholest-4-en-3-one (Windaus, *Ber.*, 1906, **39**, 518) (0·75 g.; m. p. 79—79·5°) was shaken with thioglycollic acid (3 c.c.) was left at room temperature for 18 hours. Most of the thioglycollic acid was removed from the resulting semi-solid mass by suction, and the residual crystals were washed with a little cold ethyl alcohol and then with water until the presence of thioglycollic acid was no longer detected in the washings. The crystalline residue was dried *in vacuo* over phosphoric oxide and recrystallised by slowly cooling a solution of the compound in benzene saturated at 65°. 3 : 3-*Di*(*carboxymethylthio*)*cholest*-4-*ene* (0·5 g.) separated as white prisms, m. p. 142°, $[\alpha]_D^{22}$ +111° (*c*, 1·0 in ethyl alcohol) (Found : C, 68·05; H, 9·1; S, 11·7. $C_{31}H_{50}O_4S_2$ requires C, 68·05; H, 9·2; S 11·6%).

The Action of Alkali on 7 : 12-*Dihydroxy*-3 : 3-*di*(*carboxymethylthio*)*cholanic Acid.*—A solution of 7 : 12-dihydroxy-3 : 3-di(carboxymethylthio)cholanic acid (0·2 g.) in 5N-sodium hydroxide (6 c.c.) was

boiled under reflux for 2 hours. The solution was diluted with water and acidified with 5N-hydrochloric acid, and the resulting precipitate collected after 18 hours. After being washed with water, the solid was crystallised from aqueous ethyl alcohol. Recrystallisation from the same solvent gave fine white needles of a sulphur-containing compound which had m. p. 140—141° alone and in admixture with a specimen of the original compound, m. p. 141°.

Acid Hydrolysis of 7 : 12-*Dihydroxy*-3 : 3-*di*(*carboxymethylthio*)*cholanic Acid.*—A solution of 7 : 12-dihydroxy-3 : 3-di(carboxymethylthio)cholanic acid (0·2 g.) in ethyl alcohol (5 c.c.) and 5N-hydrochloric acid (5 c.c.) was boiled under reflux for 4 hours. The cooled solution was diluted with water, made alkaline with 5N-sodium hydroxide, and extracted with ether. The aqueous layer was left until it was free from ether and then acidified with 5N-hydrochloric acid. The small precipitate which separated could not be induced to crystallise. After drying ($MgSO_4$), the ethereal extract was evaporated to dryness, and the residue crystallised from absolute ethyl alcohol. A solution of the product (m. p. 163—165°) in aqueous ethyl alcohol (charcoal) was evaporated to dryness, and the residue twice crystallised from benzene–light petroleum (1 : 1). The sulphur-free product (0·1 g.) had m. p. 178° alone and in admixture with an authentic specimen of ethyl 7 : 12-dihydroxy-3-ketocholanate, m. p. 178°.

Thanks are due to Professor M. Stacey for his interest in this work, and to the Medical Research Council for a grant in aid of expenses.

THE UNIVERSITY, EDGBASTON, BIRMINGHAM 15. [*Received, June* 1*st*, 1949.]