LXXXVIII. METABOLISM OF STEROIDS

I. THE ISOLATION OF 7-HYDROXYCHOLESTEROL AND OF THE "HEPATOLS" FROM OX LIVER

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THE elucidation of the stages in the metabolism of the steroids clearly depends to a large extent upon the isolation and characterization of the intermediates in the processes involved. Many of the substances obtained by Reichstein, Wintersteiner, and Kendall and their colleagues in their work on the adrenal cortex must represent such intermediates; and comprehensive studies such as those of Marker *et al.* on steroid excretion products furnish essential data. Apart altogether from the search for physiologically active compounds, these experiments, together with a large number of more isolated observations [e.g. Marrian, 1929; Haslewood *et al.* 1934; Butenandt & Dannenbaum, 1937; Butler & Marrian, 1938; Burrows *et al.* 1937; Butenandt *et al.* 1937; Callow & Callow, 1938; Beall, 1938] emphasize the need for a complete examination of every type of animal tissue, with the object of the identification of all steroids and related substances present. With this end in view a study of animal liver is being made.

The work here described is the preliminary investigation of a large batch of ox liver. From the unsaponifiable portion of part of this material there has been isolated, as the pure dibenzoate, α -7-hydroxycholesterol (I). This compound has not been previously found in nature. Its dibenzoate, obtained after Ponndorf reduction of 7-ketocholesterol acetate [Windaus *et al.* 1935], is a well-known intermediate in the laboratory preparation of 7-dehydrocholesterol (provitamin D₃) which has itself been detected in natural sources [Windaus & Bock, 1937; Bock & Wetter, 1938]. β -7-Hydroxycholesterol was obtained by Barr *et al.* [1936] by direct oxidation of the hydrogen phthalate of cholesterol. Possibly the present work will have a bearing on the identification of the "oxycholesterol" repeatedly stated by Lifschütz [1906-9; 1913; 1914; 1921] to be present in animal tissues [cf. Rosenheim & Starling, 1937]. α -7-Hydroxycholesterol gives the colour reactions mentioned by Lifschütz [1914] as characteristic of "oxycholesterol". Its isolation from other animal sources is being attempted.

I

From the same liver fraction there has been obtained also a mixture containing at least two crystalline high-melting alcohols, the "hepatols". These compounds are digitonin-precipitable and can be partly acetylated. They have not yet been completely purified and characterized, but appear to be C_{21} steroids similar to some of the adrenal cortex substances. There seems little doubt that ox liver will yield other products of this type.

After suitable partition between solvents, 7-hydroxycholesterol and the "hepatols" were first obtained as the digitonides. Whilst the digitonide of the former sterol was readily decomposed by the pyridine method [Schoenheimer & Dam, 1933], treatment with boiling xylene was required for decomposition of the "hepatol" complex. The antimony trichloride reaction described by Barr *et al.* [1936] was of great value in the isolation of the hydroxycholesterol. A method used for the separation of "hepatols" from cholesterol was digitonin precipitation after treatment with excess of bromine, by which means Schoenheimer *et al.* [1930] separated dihydrocholesterol from gall-stone cholesterol.

EXPERIMENTAL

Optical rotations were carried out in a 0.5 dm. Fischer micro-tube. All melting points are uncorrected. Analyses were micro-analyses by Dr A. Schoeller.

Preparation of starting material. The starting material (supplied by Messrs Boots Ltd.) was dried liver marc, which is the residue left after liver has been extracted with 50 % alcohol, as described in the *British Pharmacopoeia* [1932], p. 171.

Extraction of marc. Powdered marc (1 kg.) was extracted with ether in a Soxhlet apparatus until the fresh extract was colourless. Evaporation of the ether gave an oily residue (approx. 200 g.).

Saponification. 500 g. of the above ether-soluble product in alcohol (500 ml.) were refluxed for 2 hr. with a solution of KOH (150 g.) in water (500 ml.). The dark solution was diluted to about 5 l. and continuously extracted with ether in a liquid extractor until the fresh extract was colourless. Evaporation of the washed ether gave a crystalline orange-coloured residue (*ca.* 50 g.). This was dissolved in hot methyl alcohol (*ca.* 1200 ml.) and the mixture kept at 0° for 24 hr. The crystalline precipitate was filtered off and washed with cold methyl alcohol, and the filtrate and washings evaporated to give a reddish brown gum (10–12 g.) which was *fraction A*.

α-7-Hydroxycholesterol dibenzoate

20 g. of *fraction A* were mixed with 250 ml. of 90 % (by vol.) methyl alcohol, and the mixture extracted 6 times with light petroleum (*ca.* 1 l. in all, of B.P. 40–60°). Evaporation of the petroleum gave *fraction B* (18 g.). The alcohol was evaporated and the residue (1 g.), in 70 % (by vol.) ethyl alcohol, extracted 4 times with benzene. The benzene was evaporated, and the residue (1 g.) in 90 % alcohol (20 ml.) treated with a solution of digitonin (1 g.) in 90 % alcohol. Next day the mixture was filtered and the precipitate collected, washed with 90 % alcohol and ether and dried. The product (0.7 g.) was dissolved in dry pyridine (10 ml.) and the solution treated with excess of ether and filtered. The filtrate, washed with dil. HCl and water, was evaporated and the residue washed with light petroleum. A solution of the precipitate (0.2 g.) in dry pyridine (1 ml.) with benzoyl chloride (0.4 ml.) was kept 24 hr. at 0°. The diluted mixture was then ether-extracted and the washed ether evaporated. The residue crystallized from methyl alcohol with a little light petroleum; giving white needles (0.1 g.). After recrystallization from the same solvents the dibenzoate formed white needles, which, alone or mixed with authentic *α-7-hydroxycholesterol dibenzoate*

(M.P. 171–172°, $[\alpha]_D^{15} = +96^\circ$, in chloroform) had M.P. 172.5–173.5° $[\alpha]_D^{30} = +95^\circ$ ($c = 0.544$ in chloroform). (Found: C, 80.3; H, 8.85%. $C_{41}H_{54}O_4$ requires C, 80.6; H, 8.9%.) The dibenzoate, the digitonide and the crude 7-hydroxycholesterol each gave an intense blue colour with antimony trichloride in chloroform. Crude 7-hydroxycholesterol gave an intense blue-violet colour with acetic acid and conc. H_2SO_4 (Lifschütz reaction). By no means all the 7-hydroxycholesterol was separated from fraction A by the above method. In experiments with alumina column adsorption followed by benzene washing of the column, 7-hydroxycholesterol was detected only in the upper third of the column.

Isolation of the "hepatols"

A solution of 20 g. of fraction B in approximately 200 ml. of light petroleum (B.P. 40–60°) was extracted 8 times with 90% methyl alcohol (1 l. in all). The alcohol was washed twice with small portions of light petroleum, evaporated, diluted and ether-extracted. Evaporation of the washed extract gave a gummy residue (ca. 5 g.), which was dissolved in 80% (by vol.) alcohol and treated with a solution of digitonin (8 g.) in 80% alcohol. Excess of bromine, dissolved in 80% alcohol, was now added, when part of the precipitate dissolved. The mixture (ca. 500 ml.) was kept overnight and then filtered. The precipitate, washed with 80% alcohol and ether, was dried and the product (4 g.) powdered and refluxed for 1½ hr. with purified xylene (25 ml.). The cooled mixture, diluted with ether, was filtered and the residue after evaporation of the filtrate kept for 16 hr. at 0° with a mixture of 10 ml. each of ether and light petroleum. The insoluble material was collected, washed with ice-cold ether/light petroleum, and the white solid (0.6 g.) sublimed at 190–230°/0.05 mm.

Partial purification of "hepatols". The "hepatol" sublimate in a little alcohol was heavily diluted with light petroleum and the solution kept at 0° overnight. White needles (0.2 g.) having M.P. 277–279° were obtained. The compound gave a yellow-orange colour in the Liebermann-Burchard reaction, and was precipitable by digitonin from 80% alcoholic solution. (Found (a) sample dried *in vacuo* at room temperature C, 71.2; H, 9.9%; (b) sample dried at 100° *in vacuo* C, 71.8; H, 9.7%. $C_{21}H_{36}O_3$, H_2O requires C, 71.2; H, 10.8%.) A sample was acetylated with pyridine (1 ml.) and acetic anhydride (1 ml.) at 100° for 15 min. The product, precipitated with water, was collected and recrystallized twice from methyl alcohol; from which it formed white needles, M.P. 229–231°. (Found: C, 71.2; H, 9.2%. $C_{21}H_{34}O$, $(OCOCH_3)_2$ requires C, 71.4; H, 9.6%.) On hydrolysis, this product gave white needles, M.P. 284–285°. A sample of "hepatol" digitonide from the brominated mother liquors gave, on decomposition, sublimation and crystallization by the above procedure, followed by a second sublimation, white crystals, M.P. 266°. (Found C, 67.6; H, 9.0%.)

SUMMARY

Examination of the unsaponifiable fraction of ox-liver residue (after 50% alcoholic extraction) has resulted in the isolation, for the first time from a natural source, of the following compounds:

- (1) α -7-Hydroxycholesterol, characterized as the dibenzoate.
- (2) At least two (partially purified) digitonin-precipitable alcohols, the "hepatols", M.P. ca. 285 and 265°.

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