XLIX. SYNTHESIS OF STEROID GLUCURONIDES

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(Received 27 January 1939)

THE steroid glucuronides have acquired fresh interest since Marrian and his co-workers [Cohen & Marrian, 1936; Cohen *et al.* 1936] succeeded in isolating oestriol glucuronide from pregnancy urine, and Venning & Browne [1936] were able in a similar way to recover pregnandiol glucuronide from the urine of pregnant women. According to Marrian's data oestriol glucuronide is about 28 times weaker as an oestrogen than free oestriol. There is evidence that pregnandiol glucuronide is the form in which the corpus luteum hormone, progesterone, is eliminated; the latter cannot be condensed with glucuronic acid unless it is previously reduced to the hydroxylated pregnandiol.

The "male" sex hormones are also eliminated in urine in combined forms, although it is not yet known whether the excreted compounds are also glucuronides.

Glucuronides of steroids have not yet been made synthetically; in the present paper the synthesis of the following is recorded: methyl dehydroandrosterone-triacetylglucuronate; dehydroandrosterone-glucuronic acid; methyl testosterone-triacetylglucuronate; methyl α -oestradiol-3-benzoate-17-triacetylglucuronate; α -oestradiol-17-glucuronic acid; methyl oestrone-triacetylglucuronate; methyl cholesterol-triacetylglucuronate.¹

The synthetic preparation of glucuronides has become possible, since Goebel & Babers [1935] succeeded in preparing methyl acetobromoglucuronate, which is the necessary starting-point for the synthesis of these substances. When methyl acetobromoglucuronate and the appropriate sterol derivative are shaken in an indifferent medium, such as benzene with silver carbonate, glucuronide formation readily occurs.

The methyl steroid-triacetylglucuronates crystallize well. They are soluble in organic solvents, such as benzene and chloroform, and fairly readily soluble in hot absolute alcohol, from which they may be recrystallized.

The barium salts of the steroid-glucuronic acids can be prepared by hydrolysis of the acetylated methyl esters with baryta solution, and afford the steroid-glucuronic acids on treatment with acids. Dehydroandrosterone-glucuronic acid and α -oestradiol-17-glucuronic acid prepared in this way are characterized by their solubility in dilute alcohol; they are somewhat soluble also in boiling water.

Dehydroandrosterone-glucuronic acid can be recrystallized from absolute alcohol. α -Oestradiol-glucuronic acid crystallizes from very dilute alcohol with 1.5 mol. of water of crystallization, which are removed only on drying in a high vacuum at 115–120°. Also even after repeated recrystallization α -oestradiol-17-glucuronic acid always contains 0.86–1.1% of ash; further purification of the substance is accompanied by heavy losses of material.

¹ The hormones necessary for this investigation were very kindly placed at my disposal by the N. V. Organon, Oss (Holland). According to a private communication, Dr Luchs, of the Research Department N. V. Organon, Oss, tried to make esters and ethers of oestrone in 1934. This research could not be continued on account of Dr Luchs' illness.

EXPERIMENTAL

I. *Methyl dehydroandrosterone-triacetylglucuronate*. Methyl acetobromoglucuronate (700 mg.) and dehydroandrosterone (500 mg.) were dissolved in benzene (25 ml.) and the solution shaken for 24 hr. with 1 g. of dry Ag_2CO_3 . The solution, which was free from bromine, was centrifuged and the residue extracted repeatedly with warm benzene. The combined clear liquors from the centrifuge were evaporated *in vacuo* to a syrup, which crystallized spontaneously on rubbing with a little absolute alcohol. After standing in the ice chest for several hours, the crystals were filtered off, washed with a little cold alcohol and dried. Yield: 255 mg. (24 %), m.p. 193–196°,¹ unchanged after recrystallization from absolute alcohol. (Found: C, 63.88; H, 7.13 %; $\text{C}_{32}\text{H}_{44}\text{O}_{11}$ requires C, 63.54; H, 7.33 %.) The compound had $[\alpha]_D^{20} -19.7^\circ$ ($c=0.0033$ in CHCl_3) or -16.2° ($c=0.0063$ in benzene).²

220 mg. were dissolved in 20 ml. of hot absolute methyl alcohol and to the hot solution were added 4 ml. of 0.4 N $\text{Ba}(\text{OH})_2$. A fine, white precipitate was soon formed. The mixture was boiled for 45 min. under a reflux (protected by a soda-lime tube), cooled, treated with 20 ml. of water and kept at 0° for several hours. The crystalline precipitate, consisting of fine needles, was filtered off, washed well with water and dried. Yield: 178 mg. (92 %).

II. *Dehydroandrosterone-glucuronic acid*. 172 mg. of the above Ba salt were digested on the water bath for 30 min. with 1.3 ml. of 0.25 N H_2SO_4 and 5 ml. of 50 % methyl alcohol when the coarsely powdered Ba salt of the condensed glucuronic acid was replaced by finely divided BaSO_4 . The warm suspension was centrifuged and the residue repeatedly extracted with warm 50 % methyl alcohol. The combined centrifugates were evaporated in a vacuum to incipient turbidity. On cooling, the solution soon began to deposit a very finely divided crystalline precipitate, which was filtered off after 24 hr., washed with ice-cold water containing methyl alcohol and dried in a vacuum over phosphorus pentoxide. It formed irregular scales with a mother of pearl lustre. Yield: 114 mg. (76 %).

After recrystallization from absolute alcohol, thick, glittering fragments of prisms were obtained with m.p. 253–254°. Two further recrystallizations from absolute alcohol afforded 49 mg. melting at 254–257°. Finally the substance was recrystallized from 3.5 ml. of 80 % alcohol, when the m.p. rose to 262–264° (constant). The crystals became yellow at about 185–190°, opaque at about 210° and finally melted with decomposition. Yield of pure substance: 25 mg. (Found: C, 64.05; H, 7.79 %. $\text{C}_{25}\text{H}_{36}\text{O}_8$ requires C, 64.62; H, 7.82 %.)

III. *Methyl testosterone-triacetylglucuronic acid*. Methyl acetobromoglucuronate (700 mg.) and testosterone (500 mg.) were dissolved in benzene (25 ml.) and the solution shaken for 24 hr. with 1 g. of dry Ag_2CO_3 . The debrominated solution was worked up as described under (I). The syrupy residue was dissolved in a little alcohol and the solution diluted with water. Crystallization occurred after a long time in the ice chest. The crystals were filtered off, washed with cold dilute alcohol and dried. After two recrystallizations from absolute alcohol and one from 96 % alcohol, 26 mg. of a substance were finally obtained which melted at 186–189° and crystallized in beautiful needles.

¹ All melting points were determined in a micro-melting point apparatus according to Kofler.

² The value for the rotation, which we have recently given in *Nature, Lond.*, **142**, 1036 (1938), was the result of a single determination and can only be considered as preliminary.

A crystalline mixture was obtained from the mother liquors, from which fractional crystallization from 50 % and finally from 70 % alcohol afforded a further 84 mg. (about 10.5 %) of pure substance.

Methyl testosterone-triacetylglucuronate crystallizes with 1 mol. of alcohol of crystallization, which is lost at 78°, the crystals becoming opaque. (Found: C, 62.49; H, 7.51 %. $C_{32}H_{44}O_{11}$, C_2H_5OH requires C, 62.73; H, 7.75 %.) The compound had $[\alpha]_D^{20} + 28.3^\circ$ ($c = 0.006$ in $CHCl_3$).

IV. *Methyl α -oestradiol-3-benzoate-triacetylglucuronate*. Methyl acetobromoglucuronate (500 mg.) and the equivalent amount of α -oestradiol-3-benzoate were dissolved in benzene (20 ml.), shaken for 24 hr. with 1 g. of dry Ag_2CO_3 , and the product worked up as described in (I). The resultant syrup crystallized spontaneously when rubbed with a little absolute alcohol. Yield of crude product: 303 mg.; m.p. 165–175°.

After two recrystallizations from absolute alcohol, about 234 mg. (27 %) of pure substance were obtained, crystallizing in fine, felted needles, m.p. 188–191.5°. (Found: C, 65.73; H, 6.40 %. $C_{38}H_{44}O_{12}$ requires C, 65.88; H, 6.51 %.) $[\alpha]_D^{20} + 9.2^\circ$ ($c = 0.0065$ in $CHCl_3$).

223 mg. were dissolved in 30 ml. of methyl alcohol and 5 ml. of 0.33 *N* $Ba(OH)_2$ and 5 ml. of water added to the solution. The Ba salt soon crystallized out in fine needles. The solution was boiled gently for 1 hr. under reflux (soda-lime tube) and, after cooling and diluting with water, the precipitate was filtered off and washed with hot water until the smell of methyl benzoate had disappeared. The salt was then dried in a vacuum over phosphorus pentoxide. Yield: 125 mg. (75 %).

V. *α -Oestradiol-17-glucuronic acid*. 125 mg. of the above Ba salt were dissolved in 25 ml. of warm alcohol with the addition of 3.5 ml. of 0.1 *N* HCl and the warm solution was treated drop by drop with 0.1 *N* H_2SO_4 until no further turbidity was produced. In this connexion it was observed that the precipitation of the Ba must take place hot, since in the cold an excess of acid precipitates the unchanged Ba salt. Nevertheless a slight excess of H_2SO_4 is an advantage. The hot liquid was centrifuged and the residue extracted repeatedly with hot dilute alcohol. The combined, clear, acid centrifugates were carefully evaporated in a vacuum, after which crystallization soon occurred in the weakly alcoholic solution in the cold. After a long time in the ice chest, the precipitate was filtered off with suction and washed with ice-cold water containing methyl alcohol. The crystals were extremely thin, long, platelets arranged in rosettes. Yield: 87.5 mg. (80 %).

After recrystallizing twice from aqueous methyl alcohol, 56 mg. (about 52 %) were obtained, melting at 191–194.5° after drying in a high vacuum at 90° (the crystals sintered at 140–150°). (Found (ash-free): C, 61.02; H, 7.41 %; $C_{24}H_{32}O_8$, 1.5 H_2O requires C, 60.60; H, 7.41 %.)

The substance was dissolved in alcohol, and the solution treated with 0.1 *N* HCl and a few drops of 0.1 *N* H_2SO_4 in the cold. After 24 hr. in the ice chest the solution was scarcely turbid; it was clarified by filtration and part of the alcohol removed very carefully in a vacuum. Crystallization soon began in the form of small, irregular platelets. The gelatinous nature of the precipitate made filtration extremely slow. The pasty precipitate was again dissolved in a little alcohol and precipitated with water. The operation was repeated until the filtrates were free from acid. The substance was then dried, first in an ordinary vacuum and then in a high vacuum at 115–120°, when a faint tinge of yellow appeared. At this stage the carbon content was found to be too low and the hydrogen content too high; the substance was therefore again dried to constant weight in a high vacuum at

117°, when it lost a further 3.41 % of water and the slight yellowing did not increase. (Found (ash-free): C, 64.19; H, 7.57 %. $C_{24}H_{32}O_8$ requires C, 64.25; H, 7.19 %.)

VI. *Methyl oestrone-triacetylglucuronate*. Oestrone (142 mg.) and methyl acetobromoglucuronate (208 mg.) were dissolved in warm benzene (50 ml.) and the solution shaken overnight with 0.5 g. of dry Ag_2CO_3 , after which the mixture was warmed for 30 min. on the water bath. The suspension was then centrifuged and the clear centrifugate worked up as usual. 52 mg. of substance were obtained, which was very sparingly soluble in cold absolute alcohol. After four recrystallizations from absolute alcohol-chloroform the m.p. was 225.5–228°. (Found: C, 63.28; H, 6.64 %. $C_{31}H_{38}O_{11}$ requires C, 63.45; H, 6.53 %.) $[\alpha]_D^{25} + 57.1^\circ$ ($c = 0.0063$ in $CHCl_3$).

VII. *Methyl cholesterol-triacetylglucuronate*. Methyl acetobromoglucuronate (150 mg.) and cholesterol (146 mg.) were dissolved in benzene (8 ml.) and shaken overnight with 300 mg. of Ag_2CO_3 . The product was worked up as usual and the resultant syrup crystallized spontaneously when rubbed with alcohol. The yield of crude product was 40 mg. (15 %). After three recrystallizations from absolute alcohol the substance melted at 162–164.5° after softening at about 152°. Yield of pure product: 18 mg. (about 7 %).

The substance crystallizes in fine platelets with a mother of pearl lustre. (Found: C, 68.06; H, 8.88 %. $C_{40}H_{62}O_{10}$ requires C, 68.33; H, 8.89 %.)

Biological activities of products

The following data may be given in the biological activities of the glucuronides.

I. A daily dose of 100 μ g. of methyl dehydroandrosterone-triacetylglucuronate (containing 47.6 μ g. of dehydroandrosterone) per capon, applied for 4 days dissolved in propylene glycol by smearing on the comb, was inactive.

II. A daily dose of 20 μ g. of dehydroandrosterone-glucuronic acid (containing 12.4 μ g. of dehydroandrosterone) per capon, applied for 4 days by smearing a solution in propylene glycol on the comb, was inactive. 77 μ g. (containing 47.7 μ g. of dehydroandrosterone) gave an average comb growth after 5 days of 14 %.

III. A daily dose of 50 μ g. of methyl testosterone-triacetylglucuronate (containing 22 μ g. of testosterone), applied to each capon in the same way for 4 days, was inactive.

IV. 12 μ g. of methyl α -oestradiol-3-benzoate-17-triacetylglucuronate (containing 6.5 μ g. of α -oestradiol-3-benzoate) were inactive in castrated mice. 60 μ g. (containing 32.6 μ g. of α -oestradiol-3-benzoate) produced oestrus when injected subcutaneously during 24 hr. in three lots of 20 μ g. each.

V. 12 μ g. of α -oestradiol-17-glucuronic acid (containing 6.8 μ g. of α -oestradiol) were inactive. 60 μ g. (containing 34.3 μ g. of α -oestradiol), injected during 24 hr. in three lots of 20 μ g., produced oestrus.

VI. 30 μ g. of methyl oestrone-triacetylglucuronate (containing 13.8 μ g. of oestrone), injected subcutaneously in 24 hr. in three lots of 10 μ g., produced oestrus.

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