The Partial Synthesis of 6-Oxo-Oestriol and 6' α '-Hydroxyoestriol

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Mueller & Rumney (1957) showed that when [16- ^{14}C]oestradiol-17 β was incubated under aerobic conditions with mouse-liver microsomes and reduced triphosphopyridine nucleotide a number of radioactive metabolic products more 'polar' than oestradiol-17 β were formed. One of these proved to be identical with 6-oxo-oestradiol-17 β (Longwell & Wintersteiner, 1940); a second was shown to be identical with an authentic preparation of a 6-hydroxyoestradiol-17 β supplied by Dr O. Wintersteiner which was described as '6 β -hydroxyoestradiol-17 β '; while a third was identified as '6 β -hydroxyestrone' since on reduction with sodium borohydride it yielded '6 β -hydroxyoestradiol-17 β ' and on oxidation it yielded 6-oxo-oestrone. Since the publication of this work Wintersteiner & Moore (1959) have given reasons for believing that the 6-hydroxyl group in the 6-hydroxyoestradiol-17 β which is formed by metal-hydride reduction of 6-oxo-oestradiol-17 β may have a quasi-equatorial configuration; accordingly these authors suggest that the reference compound supplied to Mueller & Rumney should be tentatively described as 6' α '-hydroxyoestradiol-17 β . Breuer, Nocke & Knuppen (1958, 1959) have detected what are apparently the same 6-hydroxy oestrogen metabolites as were described by Mueller & Rumney among the products formed on incubating rat-liver slices with oestradiol-17 β or oestrone.

No 6-oxo or 6-hydroxy oestrogen metabolites have yet been shown with certainty to occur in human urine, but unpublished and as yet incomplete work by K. H. Loke and G. F. Marrian has suggested that the Kober chromogen in the urine of pregnant women, which was designated KC-6B and which is similar in its chromatographic behaviour to 18-hydroxyoestrone in the Bush-type solvent systems used (Loke, Watson & Marrian, 1957; Loke, Marrian & Watson, 1959), may be a 6-hydroxyoestrone. The possibility that human urine might contain 6-oxo and 6-hydroxy derivatives of oestriol was therefore considered. Accordingly, as a preliminary to examining the urine of pregnant women for these compounds, 6-oxo-oestriol and 6' α '-hydroxyoestriol (terminology of Wintersteiner & Moore, 1959) were prepared from oestriol by the methods used for the preparation of

6-oxo-oestradiol-17 β and 6' α '-hydroxyoestradiol-17 β from oestradiol-17 β by Longwell & Wintersteiner (1940) and Wintersteiner & Moore (1959).

EXPERIMENTAL AND RESULTS

Methods

Melting points of acetates were determined on a microscope hot-stage: those of the unacetylated compounds were determined in sealed evacuated capillaries. All melting points were determined on the same thermometer and are uncorrected for emergent stem.

Samples for C and H analysis, optical-rotation determinations and determinations of ultraviolet spectra were dried to constant weight at 100° *in vacuo*.

Optical rotations were determined in a 0.5 dm. tube with a diameter of 2.5 mm.

6-Oxo-oestriol triacetate

Crude oestriol (1.96 g.), prepared from late-pregnancy urine, was acetylated with acetic anhydride and anhydrous pyridine at room temperature for 24 hr., and the product recrystallized once from hexane. The recrystallized oestriol triacetate (1.87 g.) was dissolved in 6.5 ml. of acetic acid, and to this solution was added 1.7 g. of CrO_3 dissolved in 9.7 ml. of acetic acid and 1.4 ml. of water. After standing at room temperature for 24 hr. the excess of CrO_3 was reduced by the addition of 2 ml. of ethanol, and, after dilution with 200 ml. of water, the mixture was extracted with ether. The extract was washed with 50 ml. portions of saturated aq. NaHCO_3 until the washings showed a pink colour, then with 50 ml. of aq. 5% (w/v) Na_2CO_3 saturated with NaHCO_3 , and finally with 50 ml. of water. After being dried over Na_2SO_4 the ether solution of the non-acidic oxidation products was evaporated to dryness.

By treatment of this material (1.00 g.) with trimethylammonium hydrazide chloride in ethanolic acetic acid overnight at room temperature the ketonic fraction was obtained in the usual way. After reacylation of the product, decolorization with charcoal in methanolic solution and recrystallization from methanol, 0.24 g. of crystalline material, m.p. 135–138°, was obtained.

This product was combined with 0.49 g. of similarly prepared material, and after crystallization again from methanol 0.57 g. of crystals, m.p.

137–139°, $[\alpha]_D^{16.5} - 41^\circ$ in ethanol (c, 0.493), was obtained (Found: C, 67.3; H, 6.5. Calc. for $C_{24}H_{36}O_7$: C, 67.3; H, 6.6%).

The ultraviolet-absorption spectrum in ethanolic solution showed maxima at 249 m μ (ϵ 9150) and 298 m μ (ϵ 2260) and closely resembled that given for 6-oxo-oestradiol-17 β diacetate by Longwell & Wintersteiner (1940).

6-Oxo-oestriol

6-Oxo-oestriol triacetate (110 mg.) was hydrolysed with 2.0 ml. of 20% (w/v) KOH in methanol for 24 hr. at room temperature in an atmosphere of N_2 . After dilution with 30 ml. of water the solution was acidified to phenolphthalein with CO_2 and extracted three times with 10 ml. portions of ethyl acetate. After two washings with 5 ml. portions of water the extract was dried over Na_2SO_4 and evaporated to dryness. Crystallization of the product twice from methanol–benzene yielded 39 mg. of crystals, m.p. 240–242° (slight yellowing) and $[\alpha]_D^{16} - 7^\circ$ in ethanol (c, 0.504) (Found: C, 71.5; H, 7.1. Calc. for $C_{18}H_{22}O_4$: C, 71.5; H, 7.3%).

The ultraviolet-absorption spectrum in ethanolic solution showed maxima at 257 m μ (ϵ 7895) and 325 m μ (ϵ 3052) and closely resembled that given for 6-oxo-oestradiol-17 β by Longwell & Wintersteiner (1940).

The Kober reaction carried out by the method of Brown (1952) and Bauld (1954), with the former's 'oestriol reagent', was essentially negative (Fig. 1A).

6'α'-Hydroxyoestriol

A solution of 84.5 mg. of 6-oxo-oestriol in 8.0 ml. of methanol was added dropwise to a solution of 70.7 mg. of $NaBH_4$ in 6.5 ml. of methanol. After standing for 2 hr. at room temperature the solution was acidified to litmus with 10% (w/v) aq. acetic acid and evaporated nearly to dryness at room temperature under an air stream. To the residue was added 3 ml. of water; the suspended crystals were filtered off with suction, washed with water and dried *in vacuo*.

Some difficulty was experienced in crystallizing the product, but eventually it was found that by evaporating a solution in 50% (v/v) aq. methanol slowly to just under half the original volume well-formed needles separated on cooling at room temperature. These were filtered off with suction, washed with water and dried *in vacuo*. The recrystallized product had m.p. 242–245° with evolution of gas (change of form at 140–150°) and $[\alpha]_D^{14} + 84^\circ$ in ethanol (c, 0.498) (Found: C, 71.4; H, 7.6. Calc. for $C_{18}H_{24}O_4$: C, 71.0; H, 7.9%).

The ultraviolet-absorption spectrum in ethanolic solution showed a single maximum at 282 m μ (ϵ 2415) and closely resembled those of oestradiol-17 β , oestrone and oestriol.

In the Kober reaction the spectral characteristics of the colour produced were normal (Fig. 1, B), but the extinction at the absorption maximum was only 63% of that given by the same weight of oestriol under the same conditions.

6'α'-Hydroxyoestriol tetra-acetate

About 40 mg. of recrystallized 6'α'-hydroxyoestriol (m.p. 244–248°) was acetylated in the usual way with acetic anhydride and pyridine at room temperature for 24 hr. The crude acetate was purified by filtering in benzene solution through a short column of alumina (acid-washed; activated at 100°) and crystallizing twice from methanol. The product had m.p. 159–161° and $[\alpha]_D^{16} - 12^\circ$ in ethanol (c, 0.506) (Found: C, 66.2; H, 6.8. Calc. for $C_{26}H_{32}O_8$: C, 66.1; H, 6.8%).

Partition of oestriol, 6-oxo-oestriol and 6'α'-hydroxyoestriol between organic solvents and water

A chance observation made when 6-oxo-oestriol was first prepared by hydrolysis of its acetate suggested that this compound might be much less readily extracted from aqueous solution by ether than oestriol. In view of this the partition of oestriol, 6-oxo-oestriol and 6'α'-hydroxyoestriol in the systems ether–water and ethyl acetate–water

Fig. 1. Kober colour reactions on: A, 60.0 μ g. of 6-oxo-oestriol; B, 4.03 μ g. of 6'α'-hydroxyoestriol; C, 4.06 μ g. of oestriol. Final vol. of reaction mixture, 3.3 ml.

Table 1. *Partition of oestriol, 6-oxo-oestriol and 6'α'-hydroxyoestriol in the systems ether-water and ethyl acetate-water*

	$K = \left(\frac{\text{concn. in organic phase}}{\text{concn. in aqueous phase}} \right)$	
	Ether-water	Ethyl acetate-water
Oestriol	7.7	24
6-Oxo-oestriol	0.66	5.6
6'α'-Hydroxyoestriol	0.12	0.90

was determined quantitatively in the following way:

The steroid (100–300 μg.) spread in a thin film on the inside of a flask was shaken at 25° with 10 ml. of each phase (pre-equilibrated) until all had dissolved. The mixture was then transferred to a separating funnel and, after separation of the phases, 6.0 ml. of each was removed and evaporated to dryness in a vacuum desiccator. Each residue was dissolved in 3.0 ml. of ethanol and the concentration of steroid determined from the extinction of the solution at 281 mμ (oestriol and 6'α'-hydroxyoestriol) or 325 mμ (6-oxo-oestriol). The results (Table 1) show that the substitution of either an oxo or an 'α'-hydroxyl group at C-6 in oestriol has a surprisingly great effect in increasing the relative solubility in water.

DISCUSSION

Although neither 6-oxo-oestriol nor 6'α'-hydroxy-oestriol has yet been shown to be present in the urine of pregnant women it is by no means improbable that they, or other highly oxygenated oestrogen derivatives with similar distribution characteristics in the systems ether-water and ethyl acetate-water, may be metabolic products of the oestrogenic hormone. In studies on the metabolism of radioactive oestrogens *in vivo* or *in vitro* it may therefore be dangerous to draw any conclusions about the nature of the metabolites formed (whether 'free', conjugated, or degraded to acidic substances) on the basis of the extractability of

radioactive products from aqueous media by arbitrarily fixed proportions of ether or of other organic solvents (cf. Beer & Gallagher, 1955; Valcourt, Thayer, Doisy, Elliott & Doisy, 1955; Jellinck, 1959).

SUMMARY

1. 6-Oxo-oestriol, 6'α'-hydroxyoestriol and their acetates have been prepared from oestriol by methods based on those of Longwell & Wintersteiner (1940) and Wintersteiner & Moore (1959) for the preparation of 6-oxo-oestradiol-17β and 6'α'-hydroxyoestradiol-17β from oestradiol-17β.

2. Determinations of the distribution of 6-oxo-oestriol, 6'α'-hydroxyoestriol and oestriol in the systems ether-water and ethyl acetate-water showed that the substitution of an oxo or hydroxy group at C-6 in the oestriol molecule has a very great effect in increasing the relative solubility of these derivatives in water.

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The Sedimentation of Myosin

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A wide range of reported values for the sedimentation coefficient of myosin at infinite dilution, S_{20}^0 (termed henceforth the extrapolated sedimentation coefficient), exists in the literature. Values of 7.3 (Snellman & Tenow, 1948), 7.1 (Weber, 1950), 7.1 (Johnson & Landolt, 1951), and 6.7s (Mommarts & Parrish, 1951) were earlier reported.

More recently values of 6.38 and 6.43 (Holtzer & Lowey, 1956), 6.15 (Laki & Carroll, 1955) and 6.20s (Miller, Golder, Eitelman & Miller, 1952) have been obtained, the last two values referring to low temperatures. Moreover, the work of Laki & Carroll (1955) and Parrish & Mommarts (1954) has suggested that the observed value may be a