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Synthesis of 3-Hydroxy-19-nor[21-¹⁴C]pregna-1,3,5(10)-trien-20-one from [¹⁴C]Methyl Iodide

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It is widely accepted that C₁₈ oestrogens are formed *in vivo* from C₁₉ steroids by steps that involve the aromatization of the A ring of the cyclopentenophenanthrene nucleus and the loss of the C-19 angular methyl group (Breuer, 1962). Corresponding changes in the pregnane series (C₂₁) would lead to the formation of C₂₀ steroids having the aromatic A-ring structure of the oestrogen series and the D-ring side-chain structures of progesterone and its derivatives. No compounds of this type have yet been found in mammalian sources.

The conversion of androgens (C₁₉) into oestrogens (C₁₈) *in vitro* in high yield has been reported with a system consisting of human placental microsomes, NADPH and oxygen (Ryan, 1959; Gual, Morato, Hayano, Gut & Dorfman, 1962). No phenolic products were detected by these workers with this system with either progesterone or 17 α -hydroxyprogesterone (C₂₁) as substrate. Nevertheless, if aromatization of the A ring of progesterone does

occur *in vivo*, it is reasonable to suppose that the compound 3-hydroxy-19-norpregna-1,3,5(10)-trien-20-one would be formed.

This C₂₀ steroid, a structural hybrid between oestrone and progesterone, has now been synthesized from oestrone by two independent routes. Although gram quantities have been prepared by following the route of Mills, Ringold & Djerassi (1958), this method did not lend itself to the introduction of ¹⁴C, and a second synthesis based on the formation of a cyanohydrin at C-17 (Ruggieri & Ferrari, 1959) has been devised. By this method 3-hydroxy-19-nor[21-¹⁴C]pregna-1,3,5(10)-trien-20-one has been prepared for metabolic studies. A preliminary account of this work has been presented (Schulster, Whitehead & Kellie, 1964).

MATERIALS AND METHODS

Ethanol, R.R. grade (James Burrough Ltd.), was used as supplied; all other solvents were distilled before use. Ether and benzene were dried over sodium. Acetone cyanohydrin (British Drug Houses Ltd.) was freshly distilled im-

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mediately before use. [^{14}C]Methyl iodide (specific activity 11.3 mc/m-mole; batch 57) was supplied by The Radiochemical Centre, Amersham, Bucks., and the palladium-charcoal catalyst (10%) by L. Light and Co. Ltd., Colnbrook, Bucks. Magnesium turnings for Grignard reaction (British Drug Houses Ltd.) were powdered, freed from grease by boiling with benzene and ethanol and stored in a desiccator over CaCl_2 .

Melting points were determined on a Kofler hot-stage and are uncorrected. Optical rotations were determined in chloroform and infrared-absorption spectra were recorded on a Perkin-Elmer model 21 recording spectrophotometer with sodium chloride optics. Radioactive samples were counted in a Packard Tri-Carb liquid-scintillation spectrometer (model 314X) with an efficiency of 57% for ^{14}C counting. Elementary analyses were performed by Weiler and Strauss, Oxford. Adsorption chromatography was carried out by gradient elution from alumina (nearly neutral; M. Woelm, Eschwege, Germany) with a moisture content of 4.5% (w/w).

EXPERIMENTAL

The title compound was synthesized from 3-benzoyloxy-oestra-1,3,5(10)-trien-17-one (I) by the route outlined in Scheme 1. The preparation of the cyanohydrin (II), based on the method of Ercoli & Ruggieri (1953), with acetone cyanohydrin was found to be the most efficient (cf. Velluz & Muller, 1950; Goldberg & Studer, 1941 *a, b*). The subsequent conversion into the unsaturated nitrile (III) and the unsaturated ketone (IV) followed the route described by Burn & Petrow (1962). The preparation of 3-hydroxy-19-norpregna-1,3,5(10)-trien-20-one (V) has been previously reported by Mills *et al.* (1958) and by Velluz & Muller (1950).

3-Benzoyloxyoestra-1,3,5(10)-trien-17-one (I). Oestrone

(10.0 g.) was refluxed for 1.5 hr. with redistilled benzyl chloride (15 ml.) in ethanol (475 ml.) and water (25 ml.) containing a suspension of potassium carbonate (24 g.). The hot mixture was diluted with hot water (160 ml.) and left overnight. The colourless crystals were collected and washed with ice-cold 70% (v/v) ethanol. The yield of 3-benzoyloxyoestra-1,3,5(10)-trien-17-one was 12.78 g. (96%), m.p. 130–131° [Kereszty & Wolff (1943) reported m.p. 135–136°]. A sample crystallized twice from ethanol had m.p. 131–132° and $\nu_{\text{max}}^{\text{CH}_2}$ 692, 730, 1025 (benzyl ether), 1005, 1053 and 1745 cm^{-1} (17-ketone).

3-Benzoyloxy-17 ξ -cyano-17 ξ -hydroxyoestra-1,3,5(10)-triene (II). Method (a). 3-Benzoyloxyoestrone (I) (4.7 g.) was dissolved in hot ethanol (100 ml.), and acetic acid (50 ml.) was added to the cooled solution. To this acid solution, cooled in an ice bath, KCN (45 g.) was added in small portions, and the centrifuge pot containing the reaction mixture was tightly stoppered and shaken for 18 hr. at 65°. The crude cyanohydrin was precipitated with water, centrifuged, collected and dried. This precipitate was dissolved in benzene and filtered to remove the solid black KCN degradation products. Evaporation of the filtrate yielded 3.66 g. of white material, which was chromatographed on alumina (145 g.) with a gradient elution system of 2% (v/v) ethanol in benzene (600 ml.) into a reservoir of benzene (500 ml.). Two compounds were eluted from the column. The first was unchanged starting material (2.9 g.). The second compound was 3-benzoyloxy-17 ξ -cyano-17 ξ -hydroxyoestra-1,3,5(10)-triene obtained after crystallization from methanol as colourless plates (0.55 g.; 11%), m.p. 186–186.5°.

The analytical sample, crystallized twice from methanol, had m.p. 186.5–187.5°, $[\alpha]_{\text{D}}^{20} + 65 \pm 3^\circ$ (c 0.45 in CHCl_3) and $\nu_{\text{max}}^{\text{CH}_2}$ 3640 cm^{-1} (hydroxyl), 1025, 730 and 692 cm^{-1} (benzyl ether), without absorption at 1745, 1053 and 1005 cm^{-1} (Found: C, 81.07; H, 7.52; N, 3.75. $\text{C}_{26}\text{H}_{28}\text{NO}_3$ requires: C, 80.58; H, 7.54; N, 3.62%).

Scheme 1. Reaction sequence used for the synthesis of 3-hydroxy-19-nor[21- ^{14}C]pregna-1,3,5(10)-trien-20-one (V). Details are given in the text.

Method (b). 3-Benzylxyoestrone (I) (9.1 g.) was dissolved in ethanol (180 ml.) and refluxed for 5 hr. with freshly distilled acetone cyanohydrin (67 ml.) containing a few drops of 20% (w/v) K_2CO_3 . All solvents were evaporated from the orange solution, and the crude cyanohydrin was dried and used without further purification for the preparation of the unsaturated nitrile (III). A sample of this cyanohydrin, crystallized three times from methanol, had m.p. 185–186.5° and $\nu_{max}^{CS_2}$ 3640, 1025, 730 and 692 cm^{-1} , without absorption at 1745, 1053 and 1005 cm^{-1} (Found: C, 80.98; H, 7.55; N, 3.86. $C_{26}H_{29}NO_2$ requires: C, 80.58; H, 7.54; N, 3.62%).

3-Benzylxy-17-cyano-oestra-1,3,5(10),16-tetraene (III). The cyanohydrin (II) (2.0 g.), prepared by method (b) above, was refluxed gently with $POCl_3$ (7.0 ml.) and pyridine (20 ml.) for 15 min. The precipitate obtained after pouring the cooled reaction mixture into water (400 ml.) was filtered, washed extensively with water and dried. The precipitate was chromatographed on alumina (160 g.; 2 cm. $\times$ 53.5 cm.) with a gradient elution system of benzene–light petroleum (b.p. 80–100°) (1:1, v/v; 600 ml.) into a recipient reservoir of benzene–light petroleum (b.p. 80–100°) (3:17, v/v; 400 ml.). The column was packed in the latter solvent. The eluted fractions (9.5 ml.) were numbered to include the first retention volume. Examination of the eluate by infrared spectroscopy showed the presence of three distinct compounds. Combination of fractions 65–77, evaporation to dryness and crystallization from methanol yielded long fine needles of 3-benzylxy-17-cyano-oestra-1,3,5(10),16-tetraene (0.44 g.; 22%), m.p. 168–168.5°, $[\alpha]_D^{20} + 56.5 \pm 1.5$ (c 1.9 in $CHCl_3$), $\nu_{max}^{CCl_4}$ 2224 cm^{-1} ($\alpha\beta$ -unsaturated nitrile) and characteristic $\nu_{max}^{CS_2}$ 1161, 1135, 1117, 1025, 959, 833, 780, 730 and 692 cm^{-1} (Found: C, 84.63; H, 7.40; N, 3.61. $C_{26}H_{27}NO$ requires: C, 84.51; H, 7.37; N, 3.79%).

The two other compounds were eluted from the column in later fractions. One compound crystallized from methanol as thick hexagonal needles (0.03 g.; 1.5%), m.p. 140.5–142.5°, characteristic $\nu_{max}^{CS_2}$ 1161, 1132, 1038, 1025, 730 and 692 cm^{-1} and $\nu_{max}^{CCl_4}$ 2230 cm^{-1} (Found: C, 84.96; H, 7.45; N, 3.59%). The other compound crystallized from methanol as square flat plates (0.10 g.; 5%), m.p. 156–157.5°, characteristic $\nu_{max}^{CS_2}$ 1161, 1099, 1025, 832, 804, 793, 730 and 692 cm^{-1} and $\nu_{max}^{CCl_4}$ 2231 cm^{-1} (Found: C, 83.95; H, 7.04; N, 3.70; Cl, 0.0%). These two compounds were not further identified.

3-Benzylxy-19-nor[21- ^{14}C]pregna-1,3,5(10),16-tetraen-20-one (IV). The [^{14}C]methyl iodide (5.5 μ l.) supplied in the break-seal vial A, and a solution of unlabelled methyl iodide (56.7 μ l.) in dry redistilled ether (2.0 ml.) sealed under vacuum into the break-seal vial C, were incorporated into the apparatus (Fig. 1) for the preparation of the ^{14}C -labelled Grignard reagent. Dry grease-free finely-powdered magnesium (30 mg.) was placed in the centre vial B. The apparatus was evacuated (10^{-3} mm. Hg) on a high-vacuum manifold, and sealed off at the upper constriction D. The [^{14}C]methyl iodide was frozen by immersing vial A in liquid nitrogen and the seals of vials C and A were broken by twisting their respective break-rods. The unlabelled methyl iodide and ether distilled rapidly into vial A, diluting the [^{14}C]methyl iodide to a specific activity of 1.00 mc/m-mole. The ethereal solution of methyl iodide was then distilled into vial B by transferring the liquid nitrogen from vial A to vial B and leaving for 10 min. Vial B was sealed at

the constriction E and pulled off the apparatus, and the contents were left under vacuum at room temperature for 2 days.

The vial B containing the ^{14}C -labelled Grignard reagent was placed, break-seal uppermost, inside a tube containing a solution of the unsaturated nitrile (III) (156.7 mg.) in dry benzene (3.0 ml.). The tube was then constricted 10 cm. above the top of the vial, evacuated (10^{-3} mm. Hg) while its contents were frozen in liquid nitrogen, and sealed at the constriction. Inversion of the tube broke the seal of the enclosed vial. The benzene and ethereal solutions were thoroughly mixed within the tube, and left under vacuum at 33–35° for 2½ days to allow formation of the ketimine.

Fig. 1. Apparatus constructed for the preparation of ^{14}C -labelled Grignard reagent. Details are given in the Experimental section. A, Vial containing [^{14}C]methyl iodide (5.5 μ l.; 11.3 mc/m-mole); B, vial containing magnesium (30 mg.); C, vial containing unlabelled methyl iodide (56.7 μ l.) and ether (2.0 ml.); D, upper constriction; E, lower constriction.

The tube was opened and the steroid ketimine hydrolysed by boiling gently with 20% (v/v) acetic acid (6.0 ml.) and acetone (40 ml.) in the fume cupboard. The solution was evaporated to dryness on the rotary evaporator and the residue dissolved in benzene (25 ml.) and water (25 ml.). The benzene layer was washed three times with water and the combined aqueous washings were back-extracted twice with benzene. The combined aqueous washings (70 ml.) contained only 1% of the radioactivity found in the combined benzene extract (55 ml.), and were discarded.

The combined benzene extract was evaporated to dryness and the residue chromatographed on alumina (33 g.; 1.1 cm. $\times$ 38 cm.) with a gradient elution system of benzene-light petroleum (b.p. 80–100°) (9:1, v/v; 105 ml.) into a recipient reservoir of benzene-light petroleum (b.p. 80–100°) (1:3, v/v; 210 ml.). The column was packed in the latter solvent. The eluted fractions (2 ml.) were numbered to include the first retention volume. On the basis of infrared spectroscopy and radioactivity estimations on the eluate (Fig. 2), fractions 96–128 of the column were combined, evaporated and dried, yielding the unsaturated ketone (IV) (41.6 mg.). All characteristic infrared-absorption frequencies were identical with those for the unlabelled analytical sample of 3-benzyloxy-19-norpregna-1,3,5(10),16-tetraen-20-one. The analytical sample crystallized from methanol had m.p. 127–128.5°, $[\alpha]_D^{20} + 97.5 \pm 2^\circ$ (c 0.5 in CHCl_3) and $\nu_{\text{max}}^{\text{CH}_3}$ 1666, 1132, 971, 730 and 692 cm^{-1} (Found: C, 83.2; H, 7.99. Calc. for $\text{C}_{27}\text{H}_{30}\text{O}_2$: C, 83.9; H, 7.83%).

By combining fractions 63–86, evaporating to dryness and crystallizing from methanol, long fine needles of the unsaturated nitrile (III) (66 mg.) were recovered.

3-Hydroxy-19-nor[21- ^{14}C]pregna-1,3,5(10)-trien-20-one (V). A solution of the unsaturated ketone (IV) (41.6 mg.) in acetone (1.7 ml.) containing a drop of conc. HCl was shaken in an atmosphere of hydrogen with 10% palladium-on-charcoal catalyst (68 mg.) until the gas uptake stopped (2.5 hr.). Filtration, evaporation to dryness and crystallization from methanol (2.7 ml.) yielded colourless hexagonal plates of 3-hydroxy-19-nor[21- ^{14}C]pregna-1,3,5(10)-trien-20-one (18.0 mg.; 1.03 mc/mg.), m.p. 242–245°. A further yield of colourless hexagonal plates (4.3 mg.) obtained from the residual liquor had m.p. 232–245°.

Identity was further confirmed by comparing the infrared-absorption spectrum of an acetylated sample with that of a specimen prepared by the method of Mills *et al.* (1958) having $\nu_{\text{max}}^{\text{CH}_3}$ 1765, 1705, 1205 and 1009 cm^{-1} .

The unlabelled analytical sample had m.p. 247.5–248.5° and $[\alpha]_D^{25} + 164 \pm 0.9^\circ$ (c 0.68 in CHCl_3). The mixed m.p. with an authentic sample (m.p. 247–249°; $[\alpha]_D + 164^\circ$) prepared according to the method of Mills *et al.* (1958) was 247–248.5° (Found: C, 80.1; H, 8.73. Calc. for $\text{C}_{20}\text{H}_{26}\text{O}_2$: C, 80.5; H, 8.78%).

RESULTS AND DISCUSSION

For the purpose of metabolic study it is preferable to introduce ^{14}C into the steroid nucleus (Fujimoto, 1951; Levitz, 1953) rather than into the side chain at the C-21 position, but cost and practical difficulties precluded this possibility. The present synthesis provided an opportunity for the introduction of ^3H at the C-16 position by the method of Pearlman (1957a), and very much higher specific activities could have been achieved by using carrier-free ^3H for the catalytic reduction of the unsaturated C-20 ketone (IV). Introduction of ^3H in this position has the disadvantage that, after oxidative removal of the side chain and the formation of a C-17 ketone, a change known to occur in progesterone metabolism, the ^3H at C-16 would become chemically labile (Pearlman, 1957b). The [21- ^{14}C]-labelled compound has no superiority over the [16- ^3H]-labelled compound in this respect, as loss of the side chain involves the total loss of radioactivity. The ^{14}C -labelled compound was preferred as it can be counted with a higher efficiency and because the purpose of this work is the study of compounds in which the side chain is intact.

A second feature of the synthesis was the use of a benzyloxy group to protect the C-3 position during the formation of the C-17 side chain. Reduction of the unsaturated ketone (IV) simultaneously reduced the C-16,17 double bond and removed the C-3-protecting group with the minimum loss of radioactive material. It was found that a considerable amount of radioactivity remained associated with the palladium-on-charcoal hydrogenation catalyst,

Fig. 2. Radioactivity in fractions (2 ml.) of the eluate after adsorption chromatography of the hydrolysed products of the Grignard reaction. Details are given in the Experimental section. (IV), 3-Benzyloxy-19-nor[21- ^{14}C]pregna-1,3,5(10),16-tetraen-20-one.

even after repeated washing with acetone. In retrospect it is thought that losses on the last stage of the synthesis would have been smaller if less catalyst had been used.

Chromatography of the Grignard reaction products followed by radioactivity measurements on the eluted fractions showed the presence of at least two other minor products with chromatographic characteristics different from the unsaturated ketone (IV) (Fig. 2). The less polar of these by-products (column fractions 138–149; 3.9 mg.) had m.p. 225–226.5°, $\nu_{\text{max}}^{\text{CHCl}_3}$ 1603 cm^{-1} and $\nu_{\text{max}}^{\text{CS}_2}$ 1233, 730, 692 and 667 cm^{-1} , without absorption between 1800 and 1650 cm^{-1} . The other, more polar, compound (column fractions 158–168; 5.5 mg.) had m.p. 238–241°, $\nu_{\text{max}}^{\text{CHCl}_3}$ 1606 and 1573 cm^{-1} and $\nu_{\text{max}}^{\text{CS}_2}$ 1233, 730 and 692 cm^{-1} , without absorption between 1800 and 1650 cm^{-1} . The specific activity of both by-products was very similar to that of the final product (V), thus precluding the possibility that the former are C-16-methyl derivatives produced by further reaction with ^{14}C -labelled Grignard reagent (cf. Maksimov, Luri & Morozova, 1963). These compounds have not been further identified.

The compound (V) prepared by the method of Mills *et al.* (1958) and a sample prepared by the route described have been compared in the following properties: m.p., mixed m.p., infrared-absorption spectrum of their acetylated derivatives, ultraviolet-absorption spectrum, optical rotation, gas-liquid-chromatographic retention time and R_F value in two paper-chromatography systems. All these criteria confirm that the products of these two different routes are identical.

The biological activity of the non-radioactive compound (V) has been examined in the rat and the mouse. In confirmation of the findings of Djerassi, Rosenkranz, Iriarte, Berlin & Romo (1951), it was inactive as an oestrogen at a dose level of 1 mg. in the rat. However, the oestrogenic activity of a 2 mg. dose, estimated by the uterine-weight assay on immature mice, was found to be comparable with that of a 0.1 μg . dose of oestrone.

SUMMARY

1. 3-Hydroxy-19-norpregna-1,3,5(10)-trien-20-one, a possible product of aromatization in the pregnane series, was synthesized from oestrone by two independent methods.

2. The 21- ^{14}C -labelled steroid (1 mc/m-mole) was prepared for metabolic studies by one of these methods from [^{14}C]methyl iodide.

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