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Preparation of Ethynyloestradiol-4-^{14}C

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SUMMARY

Oestrone-4-^{14}C in dimethyl sulphoxide solution has been treated with lithium acetylide (ethylene diamine complex) to give ethynyl-oestradiol-4-^{14}C.

INTRODUCTION.

Ethynyloestradiol-4-^{14}C of specific activity 40 μc/mg was required for clinical evaluation. Although the preparation of ethynyloestradiol-6,7-^{3}H and two of its 3 ethers has been reported [1], we are not aware of any publication dealing with ^{14}C- labelled ethynyloestradiol.

Classical methods employed for the condensation of acetylene with 17-keto steroids [2, 3, 4] are not easily adaptable to small scale studies and an alternative method of preparation was therefore sought. The use of the stable lithium acetylide-ethylene diamine complex [6] appeared to offer an attractive source of acetylide ion and, indeed, proved eminently satisfactory in the work to be described.

DISCUSSION.

The reaction between oestrone and the lithium acetylide complex was studied in a wide range of solvent systems. Notable amongst these were tetrahydrofuran, benzene/dimethylacetamide (50 : 50) and dimethyl sulphoxide (redistilled over calcium hydride). The last-named gave good yields of the required ethynyl compound.

The use of elevated temperatures (35-40 °C) brought about no increase in yield and in view of the known instability of the lithium acetylide complex at higher temperatures [6] all subsequent experiments were carried out at room temperature.

Variations of the molar proportions of the lithium acetylide complex relative to oestrone of between 1·5 moles and 9 moles were studied and a ratio of 1 mole oestrone to 6 moles of the complex found to give the optimum

yield of ethynyloestradiol. Reaction times of about 4 hr. were most suitable. Best results were obtained when the oestrone was dissolved in a quantity of dimethyl sulphoxide slightly in excess of the minimum volume required to effect complete solution and the lithium acetylide complex added portionwise with trituration so that a mix of gel-like consistency was ultimately obtained. After the appropriate reaction time, the product was isolated by addition of water and extraction with ether. Reaction mixtures were examined by thin-layer chromatography (unchanged oestrone being clearly distinguished from the required product by means of the colour reaction with chlorosulphonic acid-acetic acid) and by infrared spectral studies [7]. The oestrone content of the reaction mixtures proved to be about 8 % and successive recrystallisations failed to reduce this to less than 5 %. Recourse was therefore made to Girard separation [8]. Employing the Reagent P yields of between 50 and 75 % of pure ethynyloestradiol were consistently obtained from unlabelled oestrone. In the radioactive preparation a yield of 46 % ethynyloestradiol was obtained. Hydrolysis of the Girard complex led to the recovery of 23 % oestrone.

Experimental.

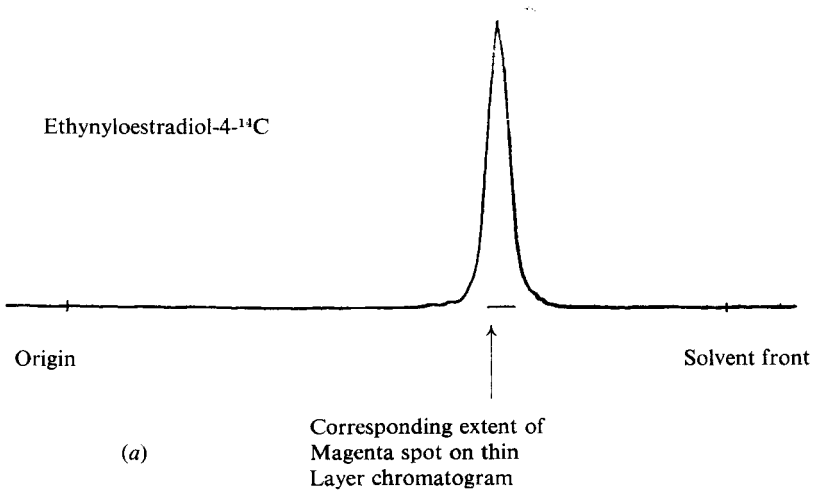
Oestrone-4-^{14}C (600 μc of specific activity 189 μc/mg; i.e. 3.165 mg) (purchased from the Radiochemical Centre, Amersham, England) was dissolved in dimethyl sulphoxide (0.3 ml) and unlabelled oestrone (10.412 mg) added. Lithium acetylide (29.041 mg) was added and the whole triturated to give an intimate mixture. After 4 hr., water (10 ml) was added dropwise and the solution allowed to stand for a few minutes. It was then extracted with six 10 ml portions of ether and the combined ether extracts washed with five 10 ml portions of water, dried (sodium sulphate) and evaporated to dryness. The partially crystalline gummy residue was dissolved in ethanol (0.72 ml) and acetic acid (0.072 ml), treated with recrystallised Girard's reagent P (13.940 mg) and stored overnight at room temperature. The solution was transferred to a separator in ether (20 ml) and water (20 ml) and shaken vigorously. The ether layer was washed with four portions of 10 ml water, dried (sodium sulphate) and evaporated to dryness.

Crystallisation from aqueous methanol gave ethynyloestradiol as colourless needles 5.382 mg, m.p. 139-143 °C. The aqueous methanolic filtrate gave a further 1.434 mg, m.p. 135-140 °C.

The first and second crops of crystalline ethynyloestradiol-4-^{14}C were separately stored in benzene solution containing 5 % ethanol at a concentration of 1 mg steroid per 1 ml solution. Aliquots of these solutions were assayed by means of the Nuclear Chicago liquid scintillation system, 720 series. The scintillator solution was 30 % ethanol in toluene containing PPO (2,5-diphenyloxazole) and POPOP (1,4-di-2-(5-phenyloxazolyl)-benzene) as primary and secondary scintillators. The specific activities of the two samples were identical at 40 μc/mg.

Thin layer chromatography was carried out on plates spread with Kieselgel G (Merck A. G.) or on Silica Gel F_{254} pre-coated, abrasion resistant plates (Merck A. G.). The last named (5 × 20 cm) were used exclusively for the chromatography of the radioactive samples. Benzene/ethyl acetate (3 : 1) was used as solvent system. Assays were carried out on the Packard Model 7200 Radiochromatogram Scanner.

Reproductions of the thin layer chromatographic scans of (*a*) pure ethynyloestradiol-4-^{14}C and (*b*) ethynyloestradiol-4-^{14}C containing about 8 % oestrone-4-^{14}C are shown in figure 1. Spraying with a mixture of chlorosulphonic acid/acetic acid (1 : 3) showed ethynyloestradiol and oestrone as magenta and orange spots respectively.



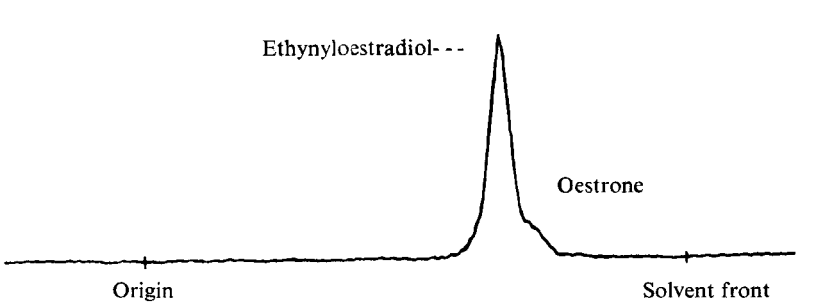


Fig. 1 Radiochromatographic scans of

(*a*) Ethynylestradiol-4-^{14}C

(*b*) Ethynyloestradiol-4-^{14}C containing oestrone-4-^{14}C as impurity

Recovery of unreacted Oestrone-4-^{14}C.

The aqueous layer resulting from the Girard separation (ca. 60 ml) was treated dropwise whilst stirring, with concentrated hydrochloric acid (3.0 ml) to bring the concentration of the resulting solution to approximately 0.5 N [8]. The mixture was stored at room temperature for 1 hr. and the crystalline precipitate isolated with ether. In this way oestrone-4-^{14}C (3.10 mg) was recovered.

We are grateful to Dr. A. E. Kellie, The Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London, W.1, England for hitherto unpublished details of a preparation of ethynyloestradiol-6,7-^{3}H carried out in his laboratories. The method used was based on that of Inhoffen *et al.*[5]

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