PREPARATION OF THE RADIOIODINATED HISTAMINE AMIDE OF 4-O-(CARBOXYPROPYL)-DIETHYLSTILBESTROL

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SUMMARY

The mixed anhydride synthesis provides a route to an iodinated form of the synthetic estrogen diethylstilbestrol (DES). Labelling of histamine by the oxidative Chloramine-T method prior to coupling to 4-O-(carboxypropyl)-diethylstilbestrol (1) minimizes radiation damage to the sensitive DES derivative. The radioiodinated derivative has demonstrated binding to an anti-DES serum, thus making possible a potentially useful iodine-based radioimmunoassay for DES.

Key Words: Diethylstilbestrol, Radioimmunoassay, Radioiodine Labelling, Mixed Anhydride Synthesis

INTRODUCTION

Analytical methods for detection of organic chemicals classed as carcinogens have attracted much attention in recent years. Present radioimmunoassay (RIA) procedures for the carcinogen diethylstilbestrol utilize a tritium-labelled tracer. [1,2]

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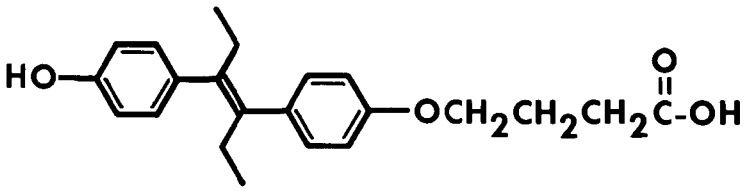
Problems inherent in the labelling procedure and in the storage of this sensitive chemical often result in costly delays to the user, as we have experienced. Thus, we have attempted to circumvent the problems associated with the tritium-based assay by investigating the possible development of a ^{125}I-based RIA. No such assay has been reported to our knowledge. We report in this paper the synthesis of a radioiodinated derivative of diethylstilbestrol prepared by the mixed anhydride coupling reaction.[3]

EXPERIMENTAL PROCEDURES

Synthesis of 4-O-(Carboxypropyl)-diethylstilbestrol (1).

To a solution of DES (2 g, 7.45 mmol) in 60 ml of 4:1 methanol/tetrahydrofuran (MeOH/THF) containing potassium carbonate (3.11 g, 22.51 mmol) were added dropwise, at 60 °C, 1.34 ml of ethyl 4-bromobutyrate (1.83 g, 9.39 mmol) in 60 ml of THF. The reaction was allowed to proceed in the dark for 92 hr at 25 °C.



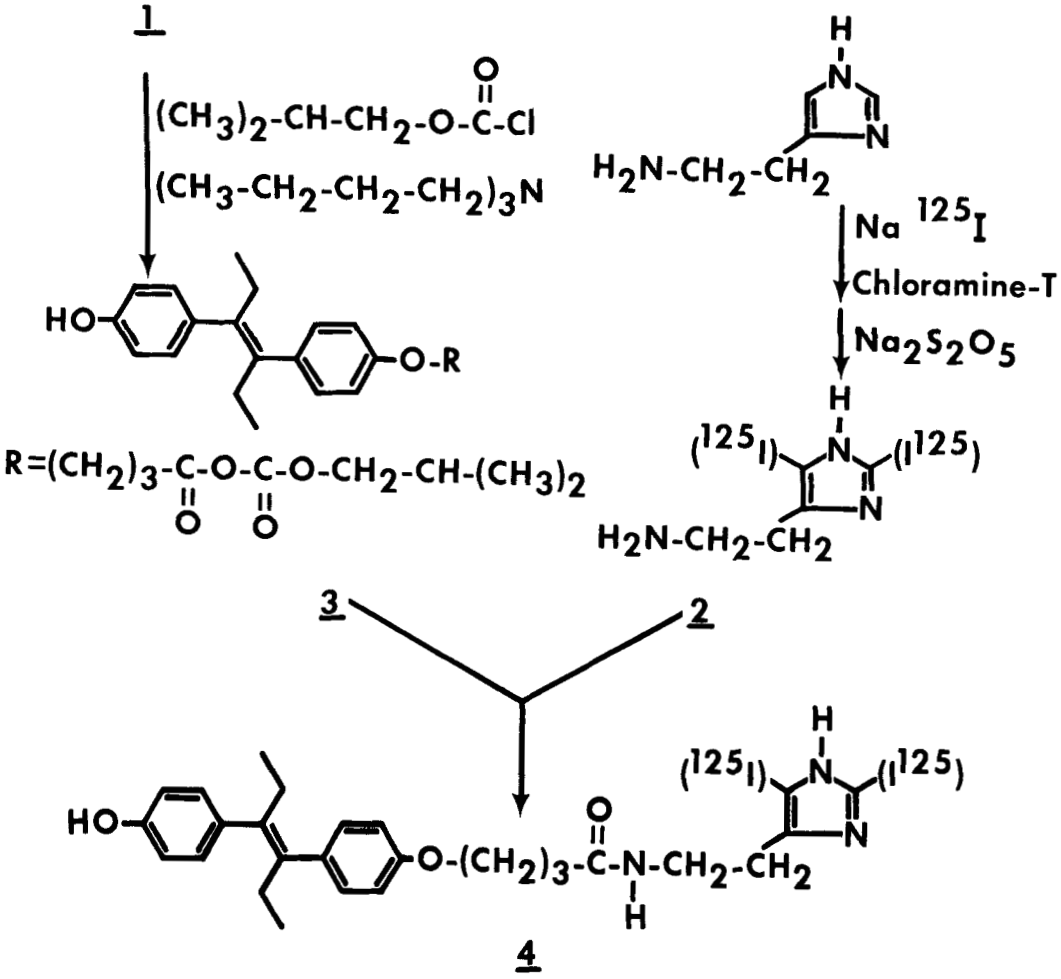
Following filtration to remove potassium carbonate, the filtrate was reduced to dryness under a stream of nitrogen. The residue was taken up in 250 ml of ethyl acetate, washed twice with water (150 ml ea.), dried over sodium sulfate (Na_2SO_4), and reduced to dryness in vacuo.

The crude ester (800 mg), an off-white solid, was taken up in 80:20 benzene/ethyl acetate, and chromatographed in the dark over silica gel (Mallinckrodt CC-7) using 80:20 benzene/ethyl acetate as eluting solvent. Fractions containing the ester were combined, solvents were evaporated in vacuo (keeping the bath temperature below 37 °C), and the solid was re-

crystallized from ethyl acetate/hexane, giving about 200 mg of the product: mp 128-130 °C (lit. mp [2] 118-119 °C).[Δ] Alternatively, thin layer chromatography of a part of the mixture on silica gel (Brinkman precoated glass-backed plates) using 2:1 hexane/ethyl acetate gave material of mp 122-125 °C from ethyl acetate/hexane.

Scheme 1.

Preparation of the Radioiodinated Histamine Amide of 4-O-(Carboxypropyl)-diethylstilbestrol



[Δ] Elemental analysis and spectral properties were in agreement with the assigned structure and calculated elemental composition.

The ester (100 mg, 0.261 mmol) was treated with 0.5 ml of 2 N sodium hydroxide in 3 ml of methanol at reflux for one hr. The mixture was acidified to pH 5.5 with glacial acetic acid and diluted with 10 ml of water. The mixture was extracted with ethyl acetate (2 x 10 ml), and the ethyl acetate was washed with water, dried over sodium sulfate, and reduced to dryness in vacuo. The residue was recrystallized from chloroform to yield 39 mg (42%) of a white product: mp 161-163 °C (Lit. mp [2] 166-168 °C).[Δ]

Synthesis of ^{125}I-labelled Histamine Derivative of 1.

Synthesis of the mixed anhydride 3 was begun first. (Scheme I) The carboxyl compound 1 (2.5 mg, 7 μmol, in 50 μl of dioxane) and 10 μl each of tri-n-butylamine (1:5 in dioxane) and isobutylchloroformate (1:10 in dioxane) were mixed at 10 °C for 20 min. The mixture was then diluted with 3.4 ml of dioxane.

The radioiodination of histamine can be timed such that its completion coincides with completion of the synthesis of the mixed anhydride. Histamine (2.2 μg, in 10 μl of 0.5 M sodium phosphate, pH 8.0) and sodium iodide ^{125}I (S.A. 17 Ci/mg, 1 mCi, 10 μl, in 0.1 M NaOH) were combined in a 1.5 ml stoppered polystyrene conical tube (Walter Sarstedt Co.). Reaction was initiated with Chloramine-T (50 μg in 10 μl of water) and quenched after 1-1.5 min by addition of sodium metabisulfite (300 μg in 10 μl of water).

To the iodination mixture were added 50 μl of the mixed anhydride 3, and the reaction tube was gently vortexed. The reaction tube was stoppered and left in a lead container at 0-4 °C for 2 hr. The reaction mixture was then diluted with 500 μl of phosphosaline buffer (0.01 M phosphate, 0.15 M NaCl, pH 7.0, with 0.5% gelatin) and chromatographed over a Sephadex G-25 column (1 x 30 cm), eluting twenty 35-drop fractions with the above buffer

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[Δ]Elemental analysis and spectral properties were in agreement with the assigned structure and calculated elemental composition.

to remove excess iodide. The eluting solvent was modified to contain dioxane (1:1) and thirty fractions (35-drop) were collected. Ten-microliter aliquots of each fraction were assayed for radioactivity in a Packard Model 5330 Auto-Gamma Scintillation Spectrometer. The ^{125}I-histamine derivative (4) generally appeared at about fraction 34 and was spread over several fractions.

DISCUSSION

Our objective in attempting to develop the ^{125}I-based radioimmunoassay was to eliminate the problems associated with the tritium-based assay. Introduction of the bulky iodine label directly into the aromatic rings of the DES molecule would reduce both the immunoreactivity and biological activity of the compound. The mixed anhydride synthesis thus offered the possibility of avoiding introducing iodine directly into the ring structures. The radioiodination of histamine, used often in the preparation of ^{125}I-labelled phenolic steriods, [4] was employed in this synthetic procedure. The histamine derivative was coupled to the O-carboxyl derivative (1), prepared by O-alkylation of DES with ethyl 4-bromobutyrate and alkaline hydrolysis of the ethyl ester. Reaction of the carboxyl derivative 1 with isobutylchloroformate in dioxane in the presence of tri-n-butylamine gave the mixed anhydride 3, which was then coupled to 2 to give the amide derivative 4.

The radioiodinated material (approximate specific activity 96 Ci/mmol) obtained in this way has demonstrated immunoreactivity when assayed with an antibody to DES (30% bound at a 1/1000 dilution of the antiserum). The iodinated derivative has been diluted to less than 500,000 cpm/10 µl aliquot with phosphosaline buffer which contained 0.1% Thimerosal (Sigma Chemical Co.) and 0.1% gelatin. When stored in this form at 4 °C in the dark, it has remained stable for at least one month.

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