Preparation of Equilin ^{3}H and Determination of Tritium Distribution

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Received on 5th August 1967

SUMMARY

Tritiated equilin of specific activity 6.85 × 10^{8} *dpm/mg has been prepared by catalytic exchange with tritium oxide. It was found after a series of chemical reactions, that at least* 90 % *of the radioactivity remained in the B-ring of the molecule. Of this,* 75 % *was found to be present at C-6.*

INTRODUCTION

Equilin [1] is a component of estrogenic mixtures of wide therapeutic applications [2]. The radioactive form of equilin, however, was not available for its metabolic studies, partly because of synthetic difficulties connected with this substance. We wish now to report a simple preparation of radioactive equilin.

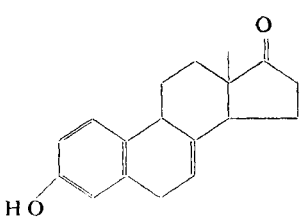

FIG. 1. Equilin

Wilzbach's gaseous exchange method [3] for introducing tritium in a molecule is well known. This method, however, has some limitations, since it gives random labelling and also that decomposition of the substance during the gas exposure renders its purification more difficult. The base catalyzed exchange of tritium and the hydrogens of aromatic compounds yielding nuclear labelled compounds is also well known [4]. For metabolic studies,

however, equilin labeled in the aromatic ring would not be very suitable. For this reason, the exchange method of introducing tritium using tritium oxide in presence of a catalyst was used for the present studies. Thus equilin, in dioxan solution was heated in a closed vessel with undiluted tritium oxide in presence of platinum oxide. After removal of the catalyst, the product was treated with base to remove all labile tritium. The total incorporation of tritium with respect to tritium oxide was 0.4 %. This crude product was found to contain some equilenin, which was removed by partition chromatography on sodium hydroxide impregnated celite, using benzene as the mobile phase. Although random tritiation could have taken place under the exchange condition, the hydrogens at position C-9 and C-6, which are doubly activated being allylic as well as benzylic are clearly more prone to exchange. In order to determine the distribution of tritium in the molecule, the product was treated with one equivalent of dichloro-dicyano-benzoquinone in dioxane to yield equilenin. Equilenin thus obtained by the removal of the α-hydrogens at C-9 and C-6 in a concerted process, contained only 10 % of the radioactivity of the starting material. The remaining activity (10 %) could either be at C-6, C-7 or elsewhere. Equilin was osmilated according to procedure described by Baran [5] to yield 7α, 8α-dihydroxy-8-isoestrone [6]. When this diol was sublimed under reduced pressure, it dehydrated to the enol which spontaneously ketonized to yield 7-ketoestrone [6] without loss of radioactivity, indicating that tritium was not present at C-7. Treatment of the latter compound with base for 24 hours resulted in loss of 75 % of radioactivity indicating that the same amount was located at position C-6.

EXPERIMENTAL

Preparation of tritiated equilin

To a solution of 150 mg of equilin in 5 ml purified dioxane containing 10 C of tritium oxide, 75 mg of platinum oxide was added and the mixture was heated in a sealed tube at 110° C for 18 hours. The tube was cooled, seal broken and contents diluted with methylene chloride. It was filtered from the catalyst and the solvent was removed under reduced pressure. The residue was dissolved in 10 ml of methanol containing 2 ml of 1N NaOH solution and kept at room temperature in dark for 24 hours. This was acidified with cold 2N HCl, diluted with water and extracted with ethyl acetate. Ethyl acetate extract was washed with water and was dried over anhydrous sodium sulfate. Removal of solvent yielded 135 mg of brownish semi-crystalline product having activity of 6.66×10^8 dpm/mg. Approximately 1γ of this product was put on a thin layer chromatography plate along with 20γ each of equilin and equilenin. The plate was developed in triethylamine-isopropylether-toluene system and scanned for location of radioactivity and subsequently sprayed with phenol reagent for visual location of equilenin and equilin

(Fig. II). From the scannogram it was evident that the product was contaminated with some equilenin. This crude product was chromatographed on 100 g of Celite soaked with a mixture of 100 ml of 1.2N NaOH and 100 ml of benzene and packed into a column 2 cm $\times$ 30 cm. The column was eluted with benzene collecting 10 ml fractions. Hold up volume for this column was 110 ml. Each fraction was checked for the presence of radioactivity corresponding to equilin by thin layer chromatography and scanning. Thus identical fractions containing equilin (Fractions 27 to 50) were mixed together. Removal of solvent gave 76 mg of pure equilin of specific activity 6.85×10^{8} dpm/mg. The radiochemical purity of this was checked by putting 1γ of it on thin layer plate along with equilin and equilenin and developing it in TEA system, scanning the plate and subsequently visually locating equilin and equilenin by spraying with phenol reagent (Fig. III). A little of this was mixed with 950 mg of cold equilin to give a product of specific activity 5 100 dpm/mg. This product was recrystallized three times from ethyl acetate, hexane and specific activity of both crystals and mother liquors were determined and is shown in Table I.

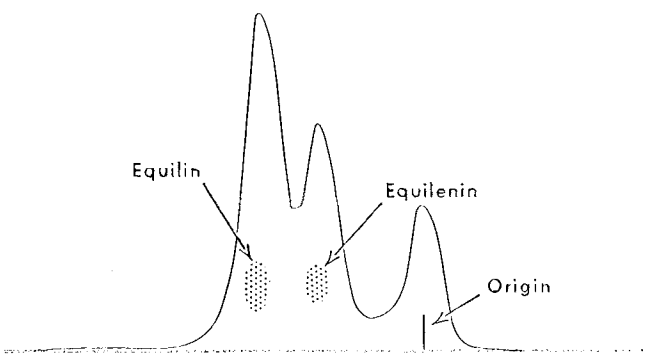

Fig. II. Scannogram of crude radioactive equilin after thin layer chromatography.

TABLE 1

	Crystals		Mother Liquors	
	Weight	Sp. activity	Weight	Sp. activity
Original	950 mg	5 100 dpm/mg		
I	545 mg	5 117 dpm/mg	410 mg	5 000 dpm/mg
II	250 mg	5 105 dpm/mg	285 mg	5 095 dpm/mg
III	150 mg	5 100 dpm/mg	95 mg	5 090 dpm/mg

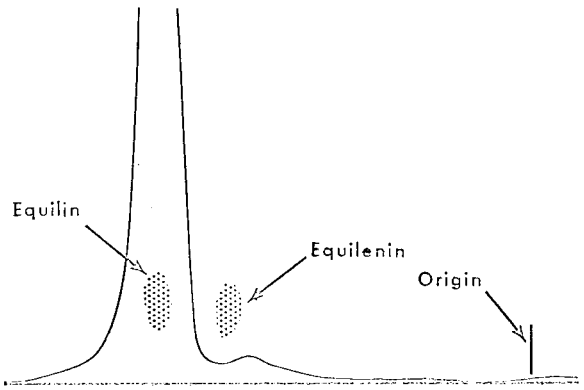

Fig. III. Scannogram of purified radioactive equilin after thin layer chromatography.

TRANSFORMATION OF EQUILIN (FIG. IV)

Dehydrogenation of equilin ^3H.

To a solution of 30 mg of equilin ^3H (S. A. 571600 dpm/mg) in 2 ml dioxane, a solution of 27 mg of dichloro-dicyano benzoquinone in 1 ml dioxane was added. There was a transient green colour followed by precipitation of hydroquinone. After keeping this at room temperature for 10 min., the precipitate was filtered off and filtrate was taken to dryness to give 25 mg of residue which in ethyl acetate solution was passed through a small column containing 8 g of alumina Act III. The column was eluted with ethyl acetate. The eluates were mixed together and removal of solvent yielded 20 mg of equilenin. This was recrystallized from ethyl acetate to give 11 mg of pure equilenin m.p. 258-259 °C.
Specific activity : 59 600 dpm/mg.
Therefore loss of activity *ca.* 90 %.

Acetylation of equilin ^3H

A solution of equilin ^3H, 335 mg (S. A. 325,100 dpm/mg) in 5 ml pyridine and 2 ml acetic anhydride was kept at room temperature for 18 hours. Excess of anhydride was decomposed by adding methanol and poured into ice containing 2N HCl. The precipitated solids were extracted with ethyl acetate. Ethyl acetate extract was washed with water and was dried over anhydrous sodium sulfate. Removal of solvent yielded 343 mg of crystalline equilin acetate ^3H (2), m.p. 126-127 °C. S. A. 325,000 dpm/mg.

3-Acetoxy-7α,8α-dihydroxy-8-iso-estrone ^3H (3)

To 300 mg of equilin acetate (2) in 4 cc pyridine 250 mg of osmium tetroxide in 2 ml pyridine was added and the mixture was stirred at room temperature for 18 hours. After that 500 mg of sodium bisulfite in 7.5 ml of water

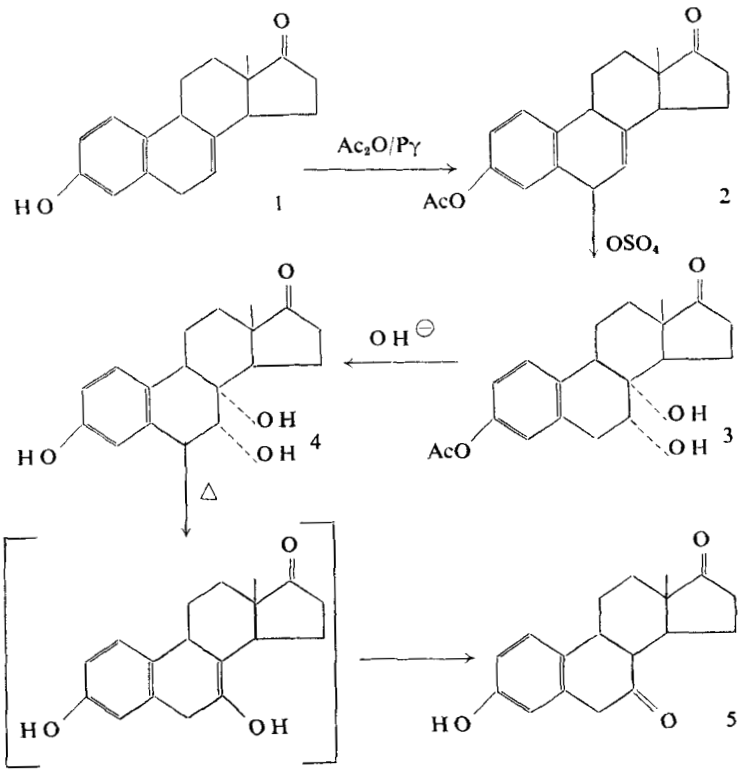

Fig. IV

was added and the mixture was stirred for one hour. The orange coloured solution was extracted with chloroform; chloroform extract was washed with water and was dried over sodium sulfate. Removal of solvent gave 325 mg of the 3-acetoxy-7α, 8α-dihydroxy-isoestrone, m.p. 198-200° C. Specific activity 325,105 dpm/mg. The specific activity remained constant after three crystallizations.

7α,8α-Dihydroxy-8-iso-estrone ^3H (4)

To a solution of 110 mg of 3-acetoxy-7α, 8α-dihydroxyisoestrone ^{3}H (S. A. 325,105 dpm/mg) in 10 ml methanol, 1 cc of 2N NaOH was added and solution kept at room temperature for one hour. It was acidified with cold 2N HCl and was extracted with methylene chloride. Methylene chloride solution was washed with water and dried over sodium sulfate. Removal of solvent gave 100 mg of 7α, 8α-dihydroxy-isoestrone ^{3}H, m.p. 247-248° C. S. A. 325,090 dpm/mg. [Lit. [5]m.p. 253-254 and 210-216° C].

7-Keto-estrone ^3H (5)

Twenty five milligrams of 7α, 8α-dihydroxyisoestrone ^3H (S. A. 325,090 dpm/mg) obtained above was introduced into a sublimation tube and sublimed at 200° C for 2 hours at a pressure of 0.002 mm Hg. The sublimate 20 mg of 7-keto estrone ^3H m.p. 209-211° C [Lit. [5]m.p. 209.5-210.5° C] had specific activity 325,000 dpm/mg.

Equilibration of 7-keto estrone ^3H

To a solution of 7-keto estrone ^3H (S. A. 325,000 dpm/mg) obtained above in 10 ml methanol, 1 ml of 2N NaOH was added and the solution was kept at room temperature under nitrogen atmosphere for 48 hours. After this, most of the methanol was removed under reduced pressure, diluted with water, acidified and extracted with methylene chloride. The methylene chloride solution was washed with water and dried over sodium sulfate. Removal of solvent gave a light yellow product which had the same mobility as the starting 7-keto estrone. This was crystallized with ether to give 8 mg of material identical with 7-keto estrone, and had specific activity of 82,345 dpm/mg. Thus there was a loss of 75 % of activity during equilibration of 7-keto estrone.

CONCLUSIONS

Equilin with high radioactivity has been made available for the first time. By the present method of preparation, the only impurity that is formed is equilenin. Equilenin was very easily separated from equilin by partition chromatography using aqueous sodium hydroxide as stationary and bezene as mobile phase. Under neutral condition, the exchange of tritium took place at C-9 and C-6 which are doubly activated being allylic as well as benzylic. Out of 90 % of radioactivity present in the B-ring of the molecule, 75 % were found to be at C-6. Abundance of tritium at C-6 can be explained on steric grounds; C-6 being less hindered than C-9.

ACKNOWLEDGEMENT

The authors are grateful to Dr. M. Gut of Worcester Foundation of Experimental Biology, Shrewsbury, Mass., for kindly providing facilities for carrying out part of the work.

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