Synthesis of Tritium Labelled Diphenhydramine Hydrochloride (Benadryl ®)

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SUMMARY

The synthesis and characterization of tritium-labelled diphenhydramine hydrochloride is described. An attempt to label diphenhydramine directly with tritium by an exchange reaction (CH_3COOH, 10 c ^3H_2O, Pt) resulted in a mixture of tritiated benzophenone, benzhydrol, diphenhydramine, and other products. A mixture of tritiated benzophenone and benzhydrol was isolated, reduced to benzhydrol-^3H, and then condensed with dimethylaminoethanol to yield diphenhydramine-^3H with the label in the benzhydryl group.

INTRODUCTION

Tritium-labelled diphenhydramine (I, Fig. 1) has been prepared for use in a detailed study of its metabolic disposition, extending the earlier studies of Glazko *et al.* [1-4]. A carbon-14 labelled preparation of diphenhydramine hydrochloride was synthesized by Fleming and Rieveschl [5] with the label in the alpha position of the benzhydryl group. A structurally similar compound, orphenadrine hydrochloride (II, Fig. 1), has recently been labelled by Hespe *et al.* with tritium at the alpha position of the benzhydryl group [6] as well as in the 2-(dimethylamino)-ethyl moiety [7]. The present report describes the synthesis of tritium-labelled diphenhydramine from a mixture of tritiated benzophenone and benzhydrol which was recovered following an unsuccessful attempt to label diphenhydramine directly with tritium by an exchange reaction. The sequence of reactions involved is outlined in Figure 2.

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I II

FIG. 1. Diphenhydramine (I) and orphenadrine (II).

$$\text{I·HCl} \xrightarrow[\text{CH}_3\text{COOH}]{10\text{ c }{}^3\text{H}_2\text{O, Pt}} \text{I-}^3\text{H·HCl} + \text{Benzophenone-}^3\text{H} + \text{Benzhydrol-}^3\text{H}$$

III-^{3}H IV-^{3}H

$$\text{III-}^3\text{H} + \text{IV-}^3\text{H} + \text{non-radioactive III} \xrightarrow{\text{NaBH}_4} \text{IV-}^3\text{H} \xrightarrow[2.\ \text{HCl}]{1.\ \text{HOCH}_2\text{CH}_2\text{N(CH}_3)_2} \text{I-}^3\text{H·HCl}$$

FIG. 2. Synthesis of tritium-labelled diphenhydramine hydrochloride.

EXPERIMENTAL

Benzophenone-^{3}H and benzhydrol-^{3}H.

A mixture of tritiated benzophenone and benzhydrol was recovered from the products of an exchange-labelling reaction originally designed to label diphenhydramine directly with tritium. The conditions for the tritium exchange reaction were as follows ** : 25 mg of diphenhydramine·HCl was dissolved in 0.3 ml glacial acetic acid containing 10 curies of tritiated water and 25 mg prereduced platinum catalyst. The reaction mixture was heated at 100° C overnight while being stirred magnetically. The reaction mixture was cooled and the reaction solvent was removed by vacuum distillation. The residue was mixed with 10 ml methanol and then vacuum distilled to dryness to remove labile tritium. The product was dissolved in 12.0 ml methanol. The total tritium incorporated into the product was 190 mc.

Chromatographic studies *** revealed that the product contained several tritium labelled compounds : diphenhydramine -^{3}H, 15-20 %; benzophenone-^{3}H and/or benzhydrol-^{3}H, approximately, 70 %; demethylated analogues of

** The exchange labelling procedure was performed by New England Nuclear Corporation, Boston, Massachusetts.

*** In all of the chromatographic studies, the chromatograms were scanned for tritium by analyzing 1 cm and/or 0.5 cm sections using liquid scintillation techniques.

diphenhydramine-^{3}H and other unidentified compounds, approximately 10 %. An attempt to isolate diphenhydramine-^{3}H by preparative TLC (Silica Gel G_F, 500μ thick; *n*-BuOH : H_2O : HOAc, 4 : 5 : 1, upper phase) yielded diphenhydramine-^{3}H with a radiochemical purity of approximately 50%. The predicted recovery from repetitive purifications by preparative TLC was estimated at 3 to 4 mc diphenhydramine-^{3}H. Since this quantity was considered inadequate for the metabolic studies planned, this approach was abandoned.

Attention was then focused on the isolation of benzophenone-^{3}H and/or benzhydrol-^{3}H for use in the synthesis of diphenhydramine-^{3}H. The fraction containing benzophenone-^{3}H and/or benzhydrol-^{3}H was eluted from the original preparative TLC plate with methanol. The total yield was 60.7 mc ^{3}H. Benzophenone and benzhydrol were found in approximately equal milligram quantities by GLC analysis (1% ECNSS-M on Gas Chrom P, 175° C, flame ionization detector). A TLC study (Silica Gel G_F; MeOH) of this fraction detected a major tritium spot having the same R_f as authentic benzophenone and benzhydrol; an apparent unidentified radiochemical impurity which was not clearly resolved from the benzophenone-benzhydrol spot was estimated as $<10\%$. A synthesis of diphenhydramine-^{3}H·HCl using a trace of the tritiated benzophenone-benzhydrol fraction as outlined in Figure 2 demonstrated that diphenhydramine-^{3}H·HCl with a radiochemical purity of $\geqslant 98\%$ could be obtained. Based on these characterization data, it was decided to proceed with the synthesis of the high specific activity diphenhydramine-^{3}H·HCl from the tritiated benzophenone-benzhydrol fraction.

To the tritiated benzophenone-benzhydrol fraction (60.2 mc ^{3}H in 61 ml methanol) was added 1.16 g (6.37 mmoles, non-radioactive) benzophenone and 40 ml methanol. This solution was stirred and cooled to $-10°$ C. To this solution, 0.24 g (6.34 mmoles) sodium borohydride was added, with stirring, over a period of about 30 minutes. The mixture was stirred magnetically overnight while warming gradually to room temperature. Methanol was removed by vacuum distillation. The residue was mixed with 30 ml 1 N NaOH and extracted with 30 ml and 15 ml portions of diethyl ether. The ether phases were combined and the ether was removed by distillation, leaving a white crystalline residue which was dried at 50° C at slightly reduced pressure to yield 1.1 g (94%) benzhydrol-^{3}H, m.p. = 62.5-64° C.

Diphenhydramine-^{3}H·HCl.

The above 1.1 g (5.97 mmoles) benzhydrol-^{3}H was mixed with 0.63 ml (6.27 mmoles) dimethylaminoethanol and 1.20 g (6.31 mmoles) *p*-toluenesulfonic acid monohydrate in 12 ml tetrachloroethane and 18 ml toluene. This mixture was refluxed overnight (pot temp. = 125° C) under a water trap. The reaction mixture was cooled to 60° C, diluted with 45 ml petroleum ether, and washed with 15 ml water. This mixture separated into three phases; the top organic phase was washed with 15 ml and 5 ml portions of water and the

washings were combined with the bottom two aqueous phases. To the combined aqueous phases (pH = 4) was added 3.5 ml 50% NaOH. This basic aqueous mixture which contained a large amount of precipitate was extracted with 30 ml and 15 ml portions of diethyl ether. The combined ether phases were washed first with 15 ml water and then with 5 ml water, dried two times with 2 g portions of $MgSO_4$, and filtered. The ether was removed by evaporation leaving an oil, diphenhydramine-^{3}H (free base). To the oil was added with stirring 1 ml isopropyl alcohol, then 1.3 ml isopropyl alcohol saturated with HCl, and finally 35 ml anhydrous diethyl ether. Crystallization of diphenhydramine-^{3}H·HCl began immediately. This ethereal mixture was stirred in an ice bath for one hour and filtered. The product was washed with cold diethyl ether and then dried under vacuum to yield 1.184 g diphenhydramine-^{3}H·HCl, m.p. 166-167.5° C, with a specific radioactivity of 6.0 mc/mmole. The characterization data are summarized in the Appendix.

Tritium label stability of diphenhydramine-^{3}H·HCl (in vitro).

Rutschmann *et al.* found that a portion of the tritium can be removed from some ring-labelled compounds when exposed to hot aqueous HCl for several hours [8]. To meet the needs of anticipated metabolic experiments with diphenhydramine-^{3}H·HCl, it was necessary to explore the *in vitro* tritium stability of this preparation in aqueous media.

The following experiments were conducted at room temperature : 5 ml aliquots of a chloroform solution containing diphenhydramine-^{3}H·HCl (14 μg/ml) were analyzed for tritium content and then extracted with 5 ml of aqueous HCl for 5 minutes. Without separating the phases, the aqueous phase was made alkaline with NaOH, and the diphenhydramine-^{3}H (free base) was extracted back into the chloroform layer. The two liquid phases were separated and the tritium content of each was measured. In two experiments, the pH of the aqueous phases ranged from pH 2 to pH 11.5; in another experiment, 1 N HCl and 1 N NaOH were used.

The analyses showed that the tritium content in the chloroform phases was unchanged after the double extraction procedure; conversely, no tritium was detected in the aqueous phases. The analytical sensitivity permitted the detection of $\geqslant$0.3% ^{3}H. These observations indicated that $\geqslant$99.7% of the tritium in diphenhydramine-^{3}H·HCl was stable under the conditions employed.

Discussion

Diphenhydramine-^{3}H·HCl, with a specific activity of 6.0 mc/mmole, was synthesized from benzophenone-^{3}H having an approximate specific

activity of 9.5 mc/mmole****. The specific activity of the product was 37% lower than expected from the specific activity of benzophenone-^{3}H. Similarly, in the preliminary synthesis, the specific activity of diphenhydramine-^{3}H·HCl was 28% lower than expected. There may be several reasons for this loss of tritium. One possible explanation is that the mixture of tritiated benzophenone and benzhydrol contained a major radiochemical impurity which was eliminated in the synthesis and isolation of diphenhydramine-^{3}H·HCl, thus yielding a product of lower specific radioactivity. Another possible explanation for the loss of tritium can be found in a review of the reaction conditions. Diphenhydramine-^{3}H was prepared by condensing benzhydrol-^{3}H with dimethylaminoethanol in the presence of *p*-toluenesulfonic acid catalyst in toluene, tetrachloroethane, and traces of water. The reaction mixture was hot and strongly acidic. These conditions may have promoted an exchange of tritium from the benzhydryl group to yield a product of lower specific radioactivity. However, as demonstrated in the label stability tests with diphenhydramine-^{3}H·HCl, no apparent instability of the tritium label could be detected at room temperature in the presence of aqueous acid or base.

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APPENDIX

CHARACTERIZATION DATA FOR DIPHENHYDRAMINE-^{3}H·HCl.

Ultraviolet Anal. in pH 3 HCl. Authentic diphenhydramine·HCl : λ 257, ε 476; λ252, ε406; Found : λ257, ε470; λ 252, ε 403. Chromatography : a major ^{3}H spot having the same R_f as authentic diphenhydramine·HCl was detected in four TLC (Silica Gel G_F) solvent systems (*n*-BuOH : H_2O : HOAc, 4 : 5 : 1 (upper phase), R_f 0.39; MeOH : NH_4OH (3%), 1 : 1, R_f 0.56; Benzene : MeOH : HOAc, 45 : 8 : 4, R_f 0.12; MeOH, R_f 0.30); a radiochemical impurity of $\leqslant$1.8% was detected; nearly all of the impurity behaved chromatographically the same as benzophenone and benzhydrol in the estimated ratio of 4 : 1. A major mass peak having the same retention time as authentic diphenhydramine·HCl was detected by

**** Since benzhydrol-^{3}H was present only in milligram quantities and the molecular weights of benzophenone and benzhydrol are approximately equal, the specific activity was estimated to be (60.2 mc ^{3}H) $\div$ (6.37 mmoles non-radioactive benzophenone) = 9.5 mc/mmole.

GLC analysis (1% ECNSS-M on Gas Chrom P, 175° C, flame ionization detector); a trace of benzhydrol was also detected; poor resolution of diphenhydramine·HCl and benzophenone prevented the possible detection of any benzophenone.

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