A LABELLED HYPOCHOLESTEREMIC AGENT. PREPARATION OF 2-(2-PYRIDINE)-1-(3-CHLOROPHENYL)-2-PHENYL-1-METHYLETHANOL UNIFORMLY TRITIATED ON THE 2-PHENYL RING.

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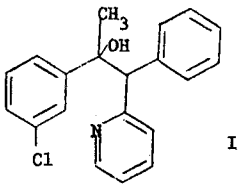
SUMMARY

2-(2-Pyridine)-1-(3-chlorophenyl)-2-phenyl-1-methylethanol (I), containing tritium uniformly distributed in the 2-phenyl ring, was synthesized in two steps with tritiated benzene as the labelled starting material.
An improved procedure was employed for the coupling of m-chloroacetophenone and 2-benzylpyridine. The higher melting of two enantiomeric pairs, known for its hypocholesteremic activity in rats, was isolated and found to have a chemical purity of >95% and a specific activity of 0.11 mCi/mmole.



I

INTRODUCTION

A program has been underway in this laboratory to realize the synthesis of an agent effective in the treatment of human atherosclerosis. The structure of the estrogenic drug chlorotrianisene (TACE®) was used as the basis for chemical modifications which might result in a compound which would

retain structural characteristics for cholesterol-lowering ability but would lack significant estrogenicity.[1] Compound I emerged as the most promising of 133 pyridyl modifications of chlorotrianisene.[2] Preclinical trials in rats established that I was highly potent, of low estrogenic character, and relatively nontoxic. However, additional trials in the dog and monkey and clinical trials in man failed to show significant antiatherogenic activity. It is hoped that finding the reason for the difference in effectiveness of I between man and rats will provide the key to the design and synthesis of new drugs with the desired activity.

Species variability in drug action often is related to species differences in the absorption, metabolism, distribution, or excretion of the drug. The use of radioisotopically-labelled drugs is one means of providing the necessary analytical methodology to conduct metabolic disposition studies. We report here the preparation of tritium-labelled compound I for use in such studies.

DISCUSSION

Compound I was labelled by a synthetic method (Scheme I) rather than an exchange procedure owing to the lability of I under tritium exchange conditions. Label was introduced into the 2-phenyl ring because of the availability of a two-step synthetic route beginning with labelled benzene which would yield a chemically and metabolically stable labelled compound. Although tritiated benzene was employed in this synthesis, the route should be equally applicable to the preparation of the carbon-14 labelled material.

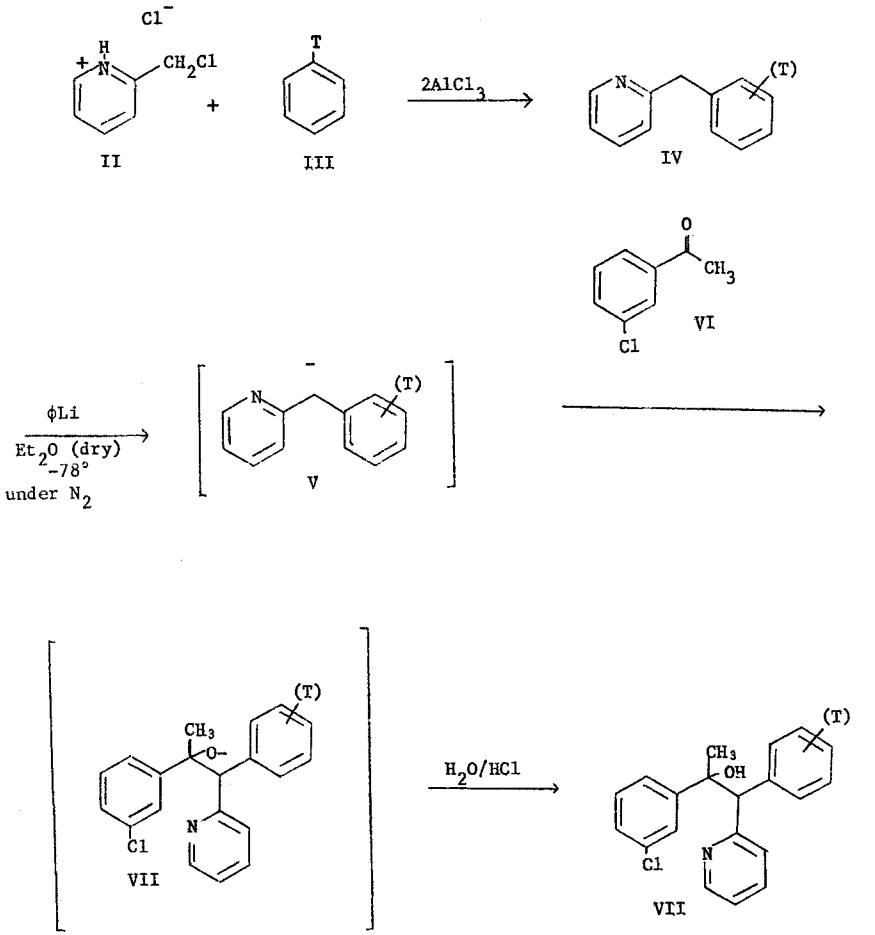
A nearly quantitative yield of tritiated 2-benzylpyridine (IV) was obtained [by a Friedel-Crafts type alkylation of tritiated benzene (III) with picolyl chloride hydrochloride (II)] when III was employed as solvent as well as reactant. It was also found that only enough benzene to give a stirrable mixture of IV and aluminum chloride was required for complete reaction. Excess benzene was recovered from the reaction mixture by distillation under reduced pressure.

Earlier work[2] suggested that the efficiency of coupling V with VI needed to be greatly increased. This was accomplished by the method shown in Scheme I. 2-Benzylpyridine dissolved in dry ether, under a nitrogen atmosphere, and cooled to -78° was treated with an ether-benzene solution of phenyllithium. The dark red solution of anion VII was kept at -78° and then titrated with an ether solution of VI. After slowly warming to room temperature, the reaction was stirred for six hours and was quenched with an equimolar hydrochloric acid solution. It was found by nmr that the reaction proceeded to the extent of 85% completion with starting materials as the only contaminants. To minimize the number of physical manipulations in this reaction, V was used without purification.

EXPERIMENTAL

Radioactivity was determined via liquid scintillation spectrometry in a cocktail composed of 3 g of 2-(4-tert-butyl-phenyl)-5-(4-biphenyl)-1,3,4-oxadiazole (butyl-PBD) per liter of toluene by use of a Packard Tri-Carb

SCHEME I



model 3320 liquid scintillation spectrometer equipped with automatic external standardization. NMR spectra were recorded on a Varian A60-A spectrometer.

2-Benzylpyridine(T) (IV) - An apparatus consisting of a 50-ml 3-necked flask containing a magnetic stirring bar, condenser topped with a drying tube, and a solid addition apparatus was oven dried, assembled warm and cooled to room temperature under a stream of N_2. The solid addition apparatus consisted of a standard tapered jointed straight tube fitted to the reaction flask and a 25-ml Erlenmeyer flask adapted for gas introduction at the side and toward the bottom of the flask. Both the tube and the Erlenmeyer flask were modified so they could be connected by a 5-inch length of tygon tubing with an inside diameter of a half inch. Vacuum desiccator dried 2-picolyl chloride hydrochloride (II) (Aldrich) (2.25 g, 0.014 mol) was combined with 4 ml of uniformly tritiated benzene (III) (ICN Pharmaceuticals, Inc., total activity of 5 mCi) in the reaction flask and dry aluminum chloride (4.4 g, 0.033 mol) was weighed into the solid addition flask. As the mixture of II and III was being stirred at a moderate rate, $AlCl_3$ was tapped into the reaction flask over a 5-min period. When the apparatus was assembled, nitrogen was passed through the system at a rate sufficient to exclude air, but during addition of $AlCl_3$ a rate was maintained that inhibited the benzene vapor from clogging the $AlCl_3$ in the addition tube and slow enough not to sweep benzene out of the condenser. After addition of the $AlCl_3$, the solid addition apparatus was replaced with a stopper and the reaction was refluxed for 16 hr. Unreacted tritiated benzene was then removed from the reaction system by short path aspirator pressure distillation, and it was collected in a Dry Ice-acetone cooled trap. The dark red residue was poured slowly into 50 ml of ice water. This water mixture was brought to pH 10 with conc. NaOH and then extracted three times with 100 ml portions of ether. The ether extracts were dried first over anhydrous sodium sulfate and finally over anhydrous magnesium sulfate. Removing the solvent by rotary evaporation gave 2.22 g (96%) of product whose nmr was identical to that of authentic cold 2-benzylpyridine (IV); nmr($CDCl_3$) δ 4.10(s, 2H), 6.8-7.6(m, 8H), 8.4-8.6(m, 1H).

2-(2-Pyridine)-1-(3-Chlorophenyl)-2-phenyl(T)-1-methylethanol (VII) - Tritiated 2-benzylpyridine (IV) (2.22 g) and 15 ml of dry ether (freshly distilled from $LiAlH_4$) were added to a 50-ml 3-necked reaction flask. The flask, containing a magnetic stirring bar, was equipped with a pressure equalizing dropping funnel (Teflon stopcock required), stopper and nitrogen inlet adapter. The entire apparatus was oven dried, assembled warm and cooled under a nitrogen atmosphere. After immersion of the reaction flask in a Dry Ice-acetone bath, phenyllithium (Foote) (2.05 M, 7.0 ml) was transferred by syringe to the dropping funnel and then was added dropwise (10 min) giving a deep red solution. The dropping funnel was rinsed with ether and the reaction mixture was allowed to stir at -78° for 10 min. A solution of m-chloroacetophenone (Eastman) (2.09 g, 0.0135 mole) dissolved in 5 ml of dry ether was placed in the dropping funnel and added over a period of 10 min. As addition neared completion, the color of the solution lightened noticeably.

After the dropping funnel was rinsed with ether, the reaction was allowed to warm slowly to room temperature and to stir for 6 hr. Concentrated HCl (37%, 1.1 ml) was dissolved in 10 ml of water and added (2 min) to the ice bath cooled reaction mixture. After separation of the water (pH 7) from the organic layers, the water layer was extracted three times with 50-ml portions of ether. The combined organic solution was dried first over Na_2SO_4 and then over $MgSO_4$, and was finally treated with charcoal. Solvent was removed by rotary evaporation to give 3.85 g (89% recovery) of material which by nmr was composed of the desired product (both isomers in a ratio of 1:1), 2-benzylpyridine, and m-chloroacetophenone. The crude mixture was dissolved in boiling n-heptane and the solution was treated with charcoal. After two further repetitions of this procedure, crystallization of the higher melting,

somewhat less soluble enantiomeric pair was accomplished by seeding. The first crop (629 mg, mp 120-128°) was further recrystallized to give 0.3005 g (14%)* (mp 129-132°, Lit.[2] 134-136°) of product (VII) with a specific activity of 0.11 μCi/mmole. Radiochemical purity was examined by thin layer chromatography (TLC) on silica gel GF in acidic (n-hexane, chloroform, acetic acid 60:30:10) and basic (n-hexane, chloroform, diethylamine 50:40:10) solvent systems. The preparation contained >95% of the higher melting racemate with the lower melting racemate as the only radioactive contaminant. Reverse isotope dilution with the higher melting racemate indicated a radiochemical purity of >92%:nmr($CDCl_3$); high melting racemic mixture (mp 134-136°), δ 1.30(s, 3H), 4.40(s, 1H), 6.6-7.6(m, 12H), 7.72(s, OH, 1H), 8.31 (crude t, 1H); low melting racemic mixture (mp 108-110°), δ 1.57(s, 3H), 4.16(s. 1H), 6.8-7.6(m, 12H), 7.52(s, OH, 1H), 8.55 (crude t, 1H).

It should be noted that both racemic mixtures of I are readily decomposed by acid, silica gel and especially a combination of the two. In fact, the low melting pair can be completely and selectively destroyed by use of active or inactive silica gel preparative TLC plates. Yet, the high melting pair is resistant and can be recovered from the silica gel when the contact time between I and silica gel and acid is minimal. By seeding the recrystallization solution successively with the pure higher melting racemate and then the lower melting racemate, both isomers can be obtained in relatively pure state.

ACKNOWLEDGEMENTS

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* James R. Bradley, New England Nuclear Corporation, has prepared this material from 3 Ci of tritiated benzene following our procedure. A similar yield was obtained yielding VII with a specific activity of 53 mCi/mmole.