SYNTHESIS OF 3,3-(^{14}C)-DIMETHYL-1-PHENYLTRIAZENE FROM ^{14}C-DIMETHYLAMINE HYDROCHLORIDE AND BENZENEDIAZONIUM FLUOROBORATE

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SUMMARY

3,3-(^{14}C)-Dimethyl-1-phenyltriazene was prepared from recrystallized benzenediazonium fluoroborate which was coupled with ^{14}C-dimethylamine in excess aqueous sodium carbonate at 0°. The crude product was extracted with ether and purified by distillation. Redistilled ^{14}C-labelled triazene was obtained in good yield (70.4 and 73.5%) and in high radiochemical purity (better than 99.9% by scan of thin-layer chromatograms). The specific activities of two preparations, determined by liquid scintillation counting and corrected for quenching, were 244 and 296 µCi/mmole. The determined activities were in close agreement with values computed from the specific activity of ^{14}C-dimethylamine hydrochloride used in the synthesis.

INTRODUCTION

The versatile biological activities of dialkaryltriazenes[1-4] depend to a large extent on biotransformations of the applied compound into reactive intermediates. Much interest therefore has been aroused in the molecular mechanisms and in the structure-activity dependence that govern the biological activity of this class of compounds. Because 3,3-dimethyl-1-phenyltriazene is the most widely investigated diazoamino compound, the chemi-

cal stabilities[5] and the biological activities[6,7] of related ring substituted or homologous triazene derivatives are frequently contrasted with those found in the parent compound.

Dialkaryltriazenes are readily formed in a reaction between arenediazonium cation and the nucleophilic nitrogen atom of a secondary aliphatic amine. In conventional preparations of triazenes, arenediazonium solutions are added to a solution of dialkylamine in the presence of excess inorganic base to consume the acid used in the diazotization[8,9]. However, diazotizations are often accompanied by side-reactions and triazenes which are prepared from these solutions are often contaminated with by-products from which they are difficult to separate.

This paper describes a convenient method for the synthesis of 3,3-(^{14}C)-dimethyl-1-phenyltriazene from recrystallized benzenediazonium fluoroborate and ^{14}C-dimethylamine hydrochloride as shown in Fig. I.

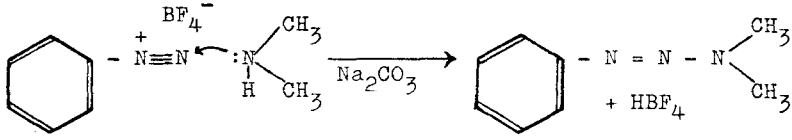

Fig. I. Formation of 3,3-dimethyl-1-phenyltriazene in the reaction between the nucleophilic nitrogen atom of dimethylamine and benzenediazonium fluoroborate.

The redistilled product was obtained in a good yield and its radiochemical purity has been shown to be better than 99.9 %.

RESULTS AND DISCUSSION

Although the dialkaryltriazenes arise by direct coupling of arenediazonium cations with secondary aliphatic amines, the preparation of small quantities of pure compounds is not easy. The diazotization of aromatic amines is often accompanied by side-reactions, such as coupling of the formed diazonium cation with the unreacted aromatic amine[10] or by nitrogen elimination from the diazonium cation and subsequent phenol formation. Moreover, the triazenes themselves are rather labile compounds which decompose on contact with acids and active surfaces. The impurities contained in the product of the reaction cannot be readily removed since dialkaryltriazenes decompose during column chromatography. Because of the inherent lability, syntheses of pure labelled triazenes are particularly difficult.

The condensation of recrystallized benzenediazonium fluoroborate with ^{14}C-dimethylamine hydrochloride in the presence of five equivalents of sodium carbonate yielded 3,3-(^{14}C)-dimethyl-1-phenyltriazene. The labelled compound, purified by repeated distillation under reduced pressure was isolated in good yield (70.4 and 73.4 %). The specific activities* of both preparations, determined by liquid scintillation counting as 244 μCi and and 296 μCi/mmole agreed very closely with computed values, 241.7 μCi and 286.2 μCi/mmole, respectively, in agreement with the postulated course of the reaction. The radiochemical purity of the triazene, determined by a scan of thin-layer chromatogram, was better than 99.9 %. A logarithmic plot of a typical trace is shown in Fig. II.

*The specific activities were determined by Dr. P. Kleihues, Max-Planck-Institut für Hirnforschung, 5 Köln 91, F.R.G., whose help is acknowledged.

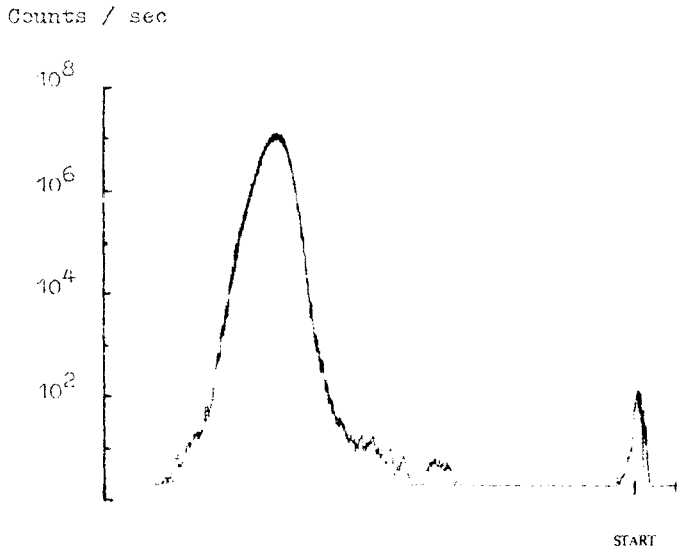

Fig. II. Thin-layer chromatographic scan of 3,3-(^{14}C)-dimethyl-1-phenyltriazene.

An investigation of in vivo methylation of RNA and DNA in rat liver by 3,3-(^{14}C)-dimethyl-1-phenyltriazene was reported by Krüger, Preussmann and Niepelt[11]. The labelled triazene was prepared from a solution of benzenediazonium chloride which was made alkaline before the coupling with dimethylamine. The product was isolated by steam distillation, extracted with ether, and the residue after the removal of the solvent was used for the tracer experiments.

The two-step synthesis of dialkaryltriazenes from isolable diazonium salts has been previously described[12]. In this work the method has been adapted for mmole-scale preparation of 3,3-(^{14}C)-dimethyl-1-phenyltriazene; it can be readily extended to the preparation of analogous ^{14}C-dimethyl-labelled aryltriazenes.

MATERIALS AND METHODS

General: ^{14}C-Dimethylamine hydrochloride, specific activity 17.9 mCi/g, was obtained from Farbwerke Hoechst, Frankfurt / M., F.R.G.

Specific activity of 3,3-(^{14}C)-dimethyl-1-phenyltriazene was determined in a Packard Tricarb 3375 Liquid Scintillation Spectrometer using toluene with 0.6 % "Permablend", containing PPO and POPOP organic scintillators (Packard Instrument, Frankfurt / M., F.R.G.). The counting efficiency in this mixture was 77 %. Pre-coated silica-gel plates (5 x 20 cm, F-254, E. Merck, Darmstadt, F.R.G.), developed in the solvent system toluene-acetone (3 : 1, v/v), were used for thin-layer chromatography. Radiochemical purity of the product was measured on thin-layer chromatograms by an LB 2723 Thin-layer Scanner II (Berthold, Wildbad, F.R.G.).

Preparation of benzenediazonium fluoroborate: Freshly distilled aniline (9.13 g, 0.1 mole) was dissolved in fluoroboric acid (0.2 mole, 45 ml, 35 %) and the solution was cooled in an ice-salt bath below 0°. Sodium nitrite (0.1 mole, 6.9 g) in water (15 ml) was added drop by drop over 30 min with mechanical stirring. The separated benzenediazonium fluoroborate was isolated by filtration, washed with ice-cold ether-methanol mixture (4 : 1) and pressed as dry as possible. After drying in a desiccator, the salt was dissolved in acetone and induced to crystallize by addition of n-pentane and cooling. The purified salt melted at 97°.

Pilot synthesis of inactive 3,3-dimethyl-1-phenyltriazene: A solution of dimethylamine hydrochloride (163.1 mg, 2.0 mmole) in water (15 ml) was placed in a three-necked 100 ml reaction flask

fitted with a thermometer, a dropping funnel and a gas inlet tube which was connected to a rubber balloon containing nitrogen. The flask, immersed in ice-salt bath, was mounted on a magnet stirrer. As soon as the temperature of the stirred solution fell below 0°, solid benzenediazonium fluoroborate (423.3 mg, 2.2 mmole, 10 % excess) was added in several portions through the wide neck so that the temperature remained close to zero. The stoppered flask was filled with nitrogen and, after the reaction mixture has become homogeneous, solution of sodium carbonate (529.9 mg, 5 mmoles in 5 ml of water) was added from the dropping funnel at such a rate that no increase in the temperature of the solution could be observed. The yellow mixture was allowed to react for 30 min after which the ice-bath was removed but the stirring was continued until the reaction reached room temperature. The formed triazene was extracted with ether (4 x 20 ml), and the combined extracts were dried over anhydrous sodium sulphate. The solvent was removed in a rotary film evaporator to a constant weight of the residue (290 mg). The crude product was distilled under reduced pressure in a cold-finger micro-distillation apparatus which was heated by a current of hot air (b.p. 80-85° at 2 mm). The yield of purified 3,3-dimethyl-1-phenyltriazene was 260 mg (87.3 % of the theoretical value).

Synthesis of 3,3-(^{14}C)-dimethyl-1-phenyltriazene:

a) ^{14}C-Dimethylamine hydrochloride (27 mg, 0.33 mmole, 483.3 μCi) was diluted with inactive compound (136.10 mg, 1.67 mmole) in water (15 ml) and reacted with benzenediazonium fluoroborate (423.3 mg, 2.2 mmole), as described above. The yield of twice distilled 3,3-(^{14}C)-dimethyl-1-phenyltriazene was 210 mg (70.4 % of the theoretical value).

b) ^{14}C-Dimethylamine hydrochloride (32 mg, 0.40 mmole, 572.8 μCi) was diluted with inactive compound (131.10 mg, 1.60 mmole)

in water (15 ml) and reacted with benzenediazonium fluoroborate (423.3 mg, 2.2 mmole). The yield of twice distilled 3,3-(^{14}C)-dimethyl-1-phenyltriazene was 219 mg (73.5 % of the theoretical value).

The determined specific activities, corrected for quenching, were a) 244 µCi/mmole and b) 296 µCi/mmole. These activities agreed closely with 241.7 µCi/mmole and 286.2 µCi/mmole, respectively, which were computed from the specific activity of ^{14}C-dimethylamine hydrochloride used in the synthesis.

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