AN IMPROVED SYNTHESIS OF N-ACETOXY-N-ACETYL-2-AMINOFLUORENE-9-^{14}C USING AN EXTRACTIVE ACYLATION TECHNIQUE

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SUMMARY

A rapid, high yield (90-100%) method for the preparation of N-acetoxy-N-acetyl-2-aminofluorene-9-^{14}C (N-AcO-AAF-9-^{14}C) from N-hydroxy-N-acetyl-2-aminofluorene-9-^{14}C (N-OH-AAF-9-^{14}C) is described. The procedure consists of the *in situ* formation of a quaternary ammonium salt of N-OH-AAF-9-^{14}C in a two-phase system and subsequent acetylation of this salt with acetic anhydride. The specific activity of the product was 40.6 mCi/mmole.

Key Words: Hydroxamic acid, Extractive acylation, Carbon-14 labeling

INTRODUCTION

N-Acetoxy-N-acetyl-2-aminofluorene (N-AcO-AAF) has been suggested as the ultimate carcinogen in 2-acetylaminofluorene (AAF)-induced mammary tumorigenesis [1]. It has also been implicated as being intimately involved in the mechanism of carcinogenesis by AAF in other tissues [2]. Thus, to facilitate metabolic and related studies concerning this labile ester we sought a rapid, high yield synthesis of N-AcO-AAF labeled with carbon-14 in the hydrocarbon moiety. Previously reported syntheses involved the reaction of the sodium salt of the hydroxamic acid with acetic anhydride [3] or the acylation of the free acid with acetic anhydride in pyridine [2b,c,4]. In general, the method utilizing the sodium salt results in low yields whereas the latter procedure gives higher yields but requires reaction times up to 24 hr. Further, purification of the product has been hindered by its tendency to decompose on silica gel [5] or in polar solvents [6].

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Since quaternary ammonium salts have been reported to enhance many types of organic reactions carried out in two-phase systems [7], we have investigated their possible utility in the preparation of the title compound. Herein, we report a rapid, high yield method for the extractive acylation of milligram quantities of N-hydroxy-N-acetyl-2-aminofluorene-9-^{14}C (N-OH-AAF-9-^{14}C) utilizing the in situ formation of quaternary ammonium salts of N-OH-AAF in a two-phase system and subsequent acetylation with acetic anhydride. Both benzyltriethylammonium (BTEA) and methyltricaprylammonium (MTCA) salts were used and gave comparable results. The procedure minimizes decomposition due to polar solvents [6] and allows convenient handling of this highly carcinogenic compound in solution.

EXPERIMENTAL

N-OH-AAF-9-^{14}C was prepared from 2-nitrofluorene-9-^{14}C by partial reduction [8] followed by acetylation with acetylchloride in ether. The 2-nitrofluorene-9-^{14}C was prepared as previously described by Heidelberger [9]. IR spectra were determined with a Beckman Acculab I, using Nujol. UV spectra were recorded with a Varian Super Scan UV/Vis Spectrophotometer. Radioactivity was determined in a Packard Model 3003 liquid scintillation counter using Liquifluor™ (New England Nuclear) as the counting medium. Radiochemical purity was determined in a Packard Model 7201 radiochromatogram scanner. High pressure liquid chromatography was performed with a Spectra-Physics 3500 B using 25% ethyl acetate/75% hexane on a 3 mm x 250 mm Spherisorb S5w column. All experimental operations were conducted under a nitrogen atmosphere.

N-Aco-AAF-9-^{14}C

N-Aco-AAF-9-^{14}C was prepared in 90% yield as outlined below using BTEA chloride as the phase-transfer reagent. N-OH-AAF-9-^{14}C, 1 mg (4.1 μmoles, 40.6 μCi/μmole) was dissolved in 0.25 ml of 0.8% NaOH, shielded from light under a nitrogen atmosphere. A layer of methylene chloride (0.5 ml) was added, followed by 10 mg

(50 µmoles) of BTEA chloride in 0.1 ml H_2O. The two-phase system was stirred for 10 min to allow the hydroxamate salt to migrate into the organic layer. The resulting mixture was treated with 5 µl (50 µmoles) of acetic anhydride resulting in immediate formation of N-AcO-AAF-9-^{14}C. After 5 to 10 min the organic layer was collected and the aqueous phase extracted with 4 x 1 ml of methylene chloride. The combined organic extracts were washed with water, dried (Na_2SO_4) and analyzed by HPLC [10] (Figure 1) indicating a minimum chemical and radiochemical purity of 93%. In addition, the radiochemical purity of the sample was determined to be ≥ 94% by thin-layer chromatography on silica gel, quantitated through use of autoradiography and radiochromatogram scans. Plates were developed in the dark in either chloroform:methanol (49:1) or hexane:ethyl acetate (1:2). The product co-migrated with N-AcO-AAF prepared by published procedures [3b].

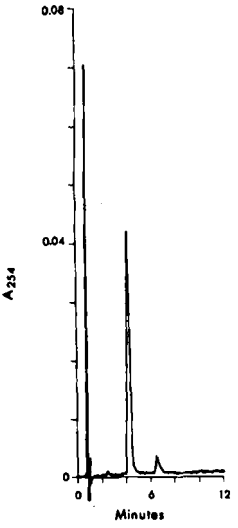

Figure 1. N-AcO-AAF-9-^{14}C was injected in CH_2Cl_2 onto a Spherisorb S5w column in 25% ethyl acetate/75% hexane at 1.6 ml/min. A minimum of 93% of the eluted radiolabel was associated with the product peak at 4.4 min.

In a second reaction, N-AcO-AAF-9-^{14}C was prepared in quantitative yield from the MTCA salt of N-OH-AAF-9-^{14}C. Since this highly aliphatic salt is a strong transfer catalyst, equimolar quantities of MTCA chloride and the hydroxamic acid were

added. The procedure was the same as described above, except that the amounts of N-OH-AAF-9-^{14}C, sodium hydroxide and acetic anhydride were doubled. MTCA chloride, 3.3 mg (8.1 μmoles), was added in 65 μl 50% aqueous acetone, and the reaction was worked up and analyzed as before (≥ 94% purity). The radiochromatogram scan of the product obtained from this reaction is shown in Figure 2.

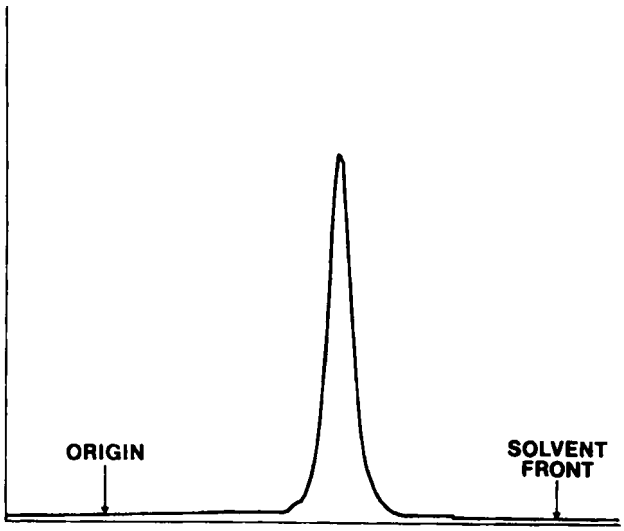

Figure 2. Radiochromatogram scan of N-AcO-AAF-9-^{14}C prepared using MTCA chloride as the phase transfer agent, TLC:silica gel/chloroform:methanol (49:1).

A larger scale preparation of unlabeled N-AcO-AAF demonstrated that the use of a catalytic amount of quaternary ammonium salt gives a reaction directly analogous to that observed under the ion-pair conditions described above. Thus, N-OH-AAF, 50 mg (0.2 mmoles, m.p. 147-148°) was dissolved in 10 ml of 1.0% NaOH, shielded from light under a nitrogen atmosphere, and BTEA chloride, 1 mg (5 μmoles) in 10 μl H_2O, was added along with 25 ml methylene chloride and 0.5 ml (5 mmoles) acetic anhydride. The mixture was stirred for 5 min after which the organic layer was removed. The

aqueous layer was washed with 2 x 25 ml methylene chloride, and the combined organic extracts were washed with water. After drying (Na_2SO_4), the methylene chloride solution was evaporated, yielding 60 mg (100%) of tan crystals, m.p. 107-109°. Analysis by thin-layer chromatography showed a trace (≤ 5%) of a lower R_f impurity, presumably an o-acetoxy-N-acetyl-2-aminofluorene [10].

DISCUSSION

Because the N-AcO-AAF and N-AcO-AAF-9-^{14}C obtained from these reactions are sufficiently pure for most uses, many of the problems normally encountered in preparing N-acyloxyarylacetamides are avoided. The hydroxamic acid remains in aqueous base for a minimal time, and the inert atmosphere and absence of light prevent radical decomposition [11]. Use of an equimolar or greater amount of quaternary ammonium salt insures total transfer to the organic phase, where the acetic anhydride can rapidly react with the hydroxamate ion.

As indicated in the preparation of unlabeled N-AcO-AAF, the use of a catalytic amount of quaternary ammonium salt improves the yield and reaction time compared to previous methods, but exposes the hydroxamic acid to aqueous base during the reaction. Although risk of decomposition of the hydroxamic acid is higher than under ion-pair extraction conditions, with careful handling, a ≥ 95% pure product is obtained. Since contact of the product with water is minimized in both procedures, disproportionation and Bamberger rearrangement [10] are avoided. The method should prove applicable to the preparation of unlabeled and radiolabeled N-acetoxy derivatives of other carcinogenic aromatic hydroxylamines and hydroxamic acids. Flexibility in the position of possible radiolabels is manifest; e.g., labeled acetic anhydride could be used if N-acetoxy labeled derivatives were desired. Extension of this work to the preparation of other unlabeled, mass- and radiolabeled compounds is in progress.

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