SYNTHESIS OF THE 5-NITROFURAN 2,4-DIACETYLAMINO-6-(5-NITRO-2-FURYL)-1,3,5-TRIAZINE-6-^{14}C

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SUMMARY

The synthesis of the 5-nitrofuran 2,4-diacetylamino-6-(5-nitro-2-furyl)-1,3,5-triazine-6-^{14}C is described. Furyllithium in ether is carboxylated with radio-carbon dioxide generated from $Ba^{14}CO_3$. The furoic-carbonyl-^{14}C acid is then esterified with diazoethane and nitrated with nitronium acetate. The resulting ethyl 5-nitro-2-furoate-carbonyl-^{14}C is then condensed with biguanide to give 2,4-diamino-6-(5-nitro-2-furyl)-1,3,5-triazine-6-^{14}C. The final product is then obtained by refluxing the condensation product in acetic anhydride to give a yield of 18% based on the amount of $Ba^{14}CO_3$ at a specific activity of 43.2mCi/mmole.

Key Words: 5-Nitrofurans, Radio-carbon dioxide, Carcinogenic, Mutagenic.

INTRODUCTION

Various 5-nitrofurans are used clinically as antibiotics, in animal feeds as growth promoters and as pesticides (1). Many of these 5-nitrofurans have been shown to be carcinogens and/or mutagens (1). There is much circumstantial evidence that both the pharmacological and toxicological effects of these 5-nitrofurans are the consequence of the covalent binding of their activated reduced metabolites to DNA (1).

We have chosen to study the highly carcinogenic and mutagenic 2,4-diacetylamino-6-(5-nitro-2-furyl)-1,3,5-triazine (5). In order to study the interaction of this 5-nitrofuran with DNA it has been necessary to develop a carbon-14 synthesis. In deciding where in the molecule to place the label we were limited by several considerations. The first was that very high specific activity would be absolutely essential in order to facilitate the isolation of the nucleoside adducts. The second consideration was for a label that would not be lost during metabolic activation nor during any subsequent workup procedures. A third factor was our desire to synthesize a labeled compound that

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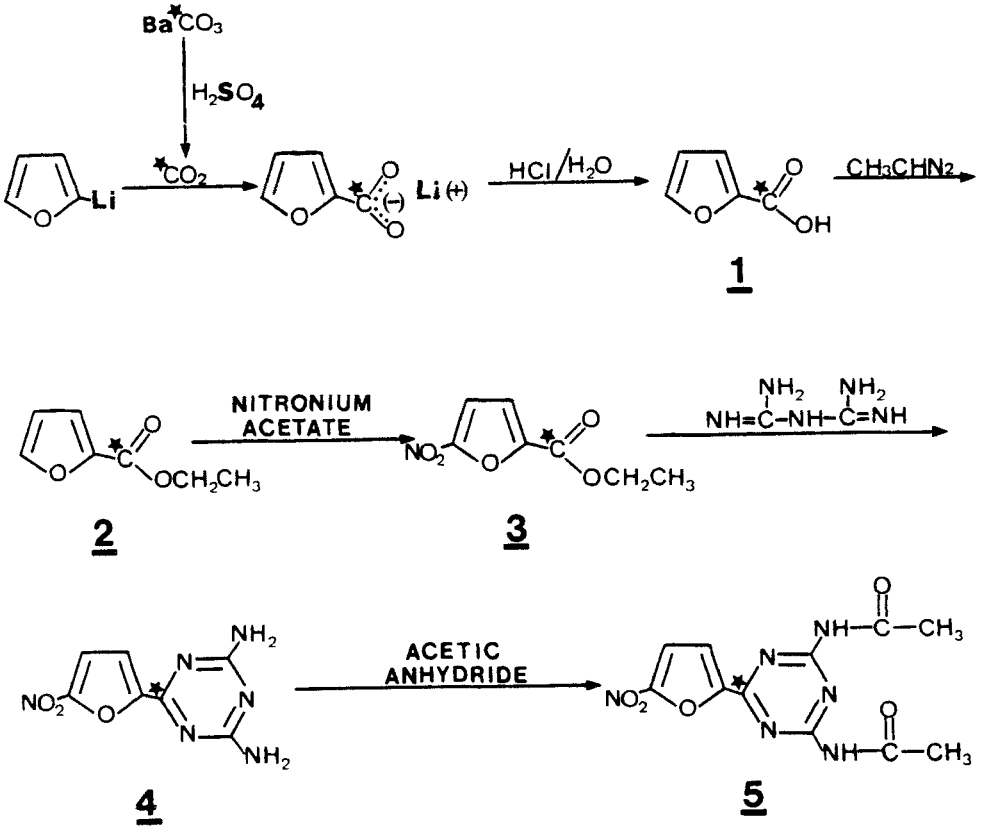
would be a common precursor for several of the 5-nitrofurans including those used in clinical (i.e., nitrofurantoin and nitrofurazone) and veterinary (i.e., furazolidone) medicine for use in future studies. Our final constraint was cost. The commercial procedures for making 5-nitrofurans all involve starting materials available only with low specific activity and/or at high cost (2). We had, therefore, to develop a new synthetic pathway. We decided to use $Ba^{14}CO_3$ and to synthesize ethyl 5-nitro-2-furoate-carbonyl-^{14}C in order to fulfill all of our criteria.

EXPERIMENTAL

Materials and Methods. All reagents and chemicals were of reagent grade unless otherwise stated. The $Ba^{14}CO_3$, 45mCi/mmol, was obtained from New England Nuclear. Radioactivity measurements were done using a Beckman 7800 liquid scintillation counter with PCS (Amersham) liquid scintillation cocktail and quench correction by sample channels ratio method. Specific activities and radiochemical purity were determined by HPLC with fraction collection for radioactivity measurement using a reverse phase C_{18}μ-Bondapak (10μ, 3.9mm x 30cm) column and 40% MeOH/H_2O eluent at 2.0 ml/min.

Synthesis. The overall synthetic scheme is shown in Scheme 1. Only intermediates 2 and 3 were isolated in the synthetic procedure and therefore only these two intermediates and the final product were characterized.

Ethyl 2-furoate-carbonyl-^{14}C (2). Furyllithium in ether was prepared from n-butyllithium in ether according to the method of Ramanathan and Levine (3). The furyllithium solution was diluted to a concentration of 0.7mmol/10ml and a 10ml aliquot charged into a dry 25 ml pear flask. The contents of the flask were frozen with liquid nitrogen and 25mCi of radio-carbon dioxide 45.0mCi/mmol (0.55 mmol) generated from $Ba^{14}CO_3$ and H_2SO_4 was transferred under high vacuum. The carboxylation was allowed to continue overnight at -80°C.



SCHEME 1

The resulting suspension was treated with H_2O, magnetically stirred to dissolve all solids and transferred to a 125 ml separatory funnel with ethyl ether and H_2O rinsings. In the separatory funnel the two phase mixture was shaken for about 5 min to insure complete aqueous extraction of the lithium 2-furoate-carbonyl-^{14}C. The aqueous extract was transferred to a continuous liquid/liquid extractor, acidified with concentrated HCl and extracted for 2 hr with ethyl ether. The volume of the ethyl ether extract was reduced to about 5 ml by removing the condenser on the liquid/liquid extractor and the

remaining extract cooled to room temperature. The extract was then treated with excess diazoethane generated from N-ethyl-N'-nitro-N-nitrosoguanidine (Aldrich) according to the method of McKay et al. (4). The solvent was then removed by roto-evaporation, the residue reconstituted with 3.0 ml hexanes/ethyl acetate (98/2, V/V), and (2) was purified by chromatographing on a 1.5 cm x 3.0 cm Bio-Sil A/Bio-Rad (100-200 mesh) column using the above hexanes/ethyl acetate mixture as the eluent. The eluent fractions containing (2), retention volumes 17 ml to 43 ml, were roto-evaporated to dryness yielding 52.4 mg (0.368 mmol, 16.8 mCi), 44.5 mCi/mmol, 67% radiochemical yield, m.p. 35°-36°C.

Ethyl 5-nitro-2-furoate-carbonyl-^{14}C (3). The purified ethyl 2-furoate-carbonyl-^{14}C (2) (52.4 mg, 0.368 mmol, 16.8 mCi) was reconstituted with 135 µl acetic anhydride cooled to -20°C and nitrated by a modified procedure of Prousek et al. (5). The nitrating reagent was prepared by adding 575 µl HNO_3 (90%) to 1.295 ml acetic anhydride at -20°C. It was magnetically stirred for about 5 min and an aliquot of 530 µl was transferred into the flask containing (2) at -20°C. After 1.0 hr the reaction was stopped by the addition of 1.41 ml H_2O and 310 µl pyridine. The mixture was heated to 50°C with stirring for 15 min, transferred to a 25 ml microfiltration flask, and (3) was allowed to crystallize after adding 10 ml of H_2O and placing the flask in an ice bath. The crystals were filtered and washed with ice cold H_2O followed by drying in a vacuum dessicator yielding 31.0 mg (0.166 mmol, 7.56 mCi), 44.1 mCi/mmol, 45% radiochemical yield, mp 100-101°C.

2,4-Diacetylamino-6-(5-nitro-2-furyl)-1,3,5-triazine-6-^{14}C (5). The biguanide condensation with (3) was done by a modification of a patented Abbott process (6). A solution of biguanide (7) (32 mg, 0.316 mmol) in 1.0 ml absolute methanol was added to the 31.0 mg (0.166 mmol, 7.56 mCi) (3) in 1.0 ml absolute methanol and stirred at ambient temperature overnight in the

absence of light. DMSO (3 ml) was added to the alcoholic suspension of (4) and stirred until it was completely dissolved. Then 20 ml H_2O was added and the purified (4) allowed to crystallize out at -10°C. The crystals were then refluxed in acetic anhydride for 2.0 hrs and the resulting (5) stored as a solution in acetic anhydride at -10°C. Identity of (5) was confirmed by UV spectral comparison (λ_{max} 324 nm and 231 nm) with an authentic unlabeled sample obtained from Abbott Laboratories. A yield of 30.7 mg (0.0996 mmol, 4.54 mCi), 60% radiochemical yield, (5) was obtained. The final product had a specific activity of 43.2 mCi/mmol and a radiochemical purity of 94.7%.

RESULTS

Based on the amount of $Ba^{14}CO_3$ (0.55 mmol, 25mCi) at 45mCi/mmol yields of 67% ethyl 2-furoate-carbonyl-^{14}C (2), 30% ethyl 5-nitro-2-furoate-carbonyl-^{14}C (3), and 18% 2,4-diacetylamino-6-(5-nitro-2-furyl)-1,3,5-triazine-6-^{14}C (5) were obtained. As suggested above (3) can be used to synthesize several other 5-nitrofurons including those used in clinical and veterinary medecine.

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