

SYNTHESIS OF *p*-(Di-*n*-PROPYLSULFAMYL)BENZOIC-1-(RING)-¹⁴C ACID
AND *p*-(Di-*n*-PROPYLSULFAMYL)BENZOIC ACID-¹⁴C
(PROBENECID-¹⁴C)

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SUMMARY

The synthesis of carboxyl-¹⁴C and 1-(ring)-¹⁴C *p*-(di-*n*-propylsulfamyl)benzoic acid from carbon-¹⁴C dioxide and *p*-toluene-1-(ring)-¹⁴C-sulfonyl chloride, respectively, is described. *p*-Aminobenzoic-carboxyl-¹⁴C acid was prepared by an improved method from carbon-¹⁴C dioxide.

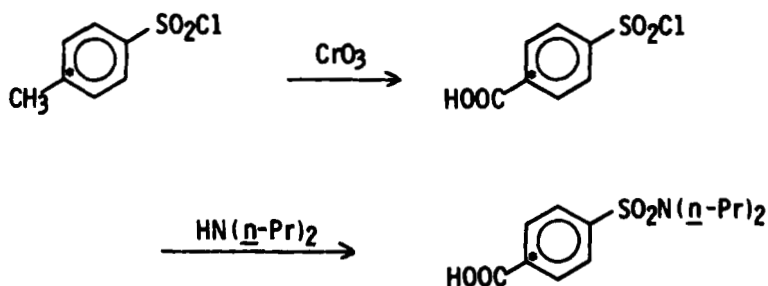
Key Words: *p*-(di-*n*-propylsulfamyl)benzoic acid-¹⁴C, *p*-(di-*n*-propylsulfamyl)benzoic-1-(ring)-¹⁴C acid, probenecid-¹⁴C, *p*-aminobenzoic-carboxyl-¹⁴C acid.

INTRODUCTION

The metabolic disposition of probenecid has been investigated in these laboratories as well as in others (1). A continuing and increasing demand for carbon-14 labeled probenecid for further studies prompted an investigation of more efficient radiochemical synthesis routes leading to this tracer. For the earlier studies probenecid-1-(ring)-¹⁴C was made by the classical chromic acid oxidation of *p*-toluene-1-(ring)-¹⁴C-sulfonyl chloride followed by reaction of the resulting 4-chlorosulfonyl-1-¹⁴C-benzoic acid with di-*n*-propylamine. The lack of ready availability of ¹⁴C-labeled *p*-toluenesulfonic acid made this process unattractive for the preparation of radioactive tracer. A synthesis of probenecid-carboxyl-¹⁴C has now been developed which uses readily available carbon-¹⁴C dioxide as starting material. The procedures are described below.

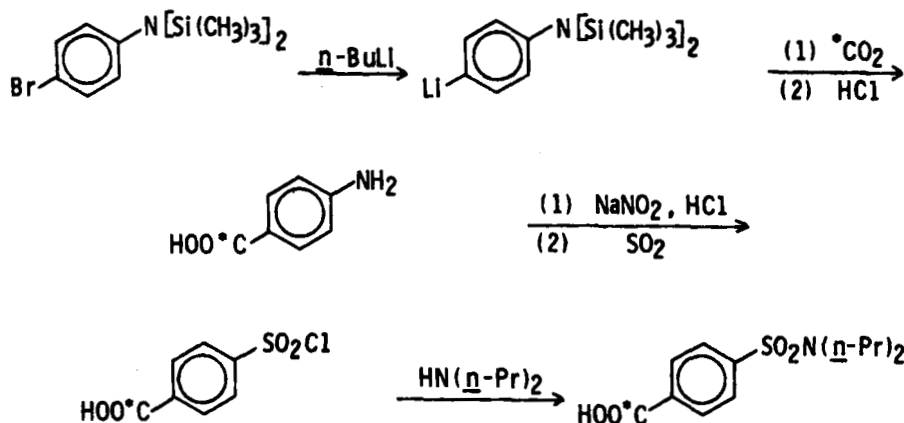
DISCUSSION

The route of synthesis of probenecid-1-(ring)-¹⁴C from *p*-toluene-1-(ring)-¹⁴C-sulfonyl chloride is based on a non-radioactive procedure described by De Jong (2) and is depicted by the following sequence:



• denotes ^{14}C

The synthetic route used for the preparation of probenecid-carboxyl- ^{14}C from carbon- ^{14}C -dioxide is outlined in the following equations:



Several workers (3) have reported the preparation of carboxyl- ^{14}C labeled *p*-aminobenzoic acid by the carbonation of the organolithium species which was formed by a halogen-metal interconversion reaction between *p*-aminobromobenzene and an excess of *n*-butyllithium. The procedure at best gave about 30% of the radioactivity incorporated into the molecule. Better yields (52-67%) of *p*-aminobenzoic acid ^{14}C were obtained (4) by the carbonation of *p*-chlorophenyllithium or *p*-bromophenylmagnesium bromide and subsequent high temperature/pressure ammonolysis of the formed *p*-halobenzoic acid- ^{14}C . While this method gives an improved yield of product it has the obvious disadvantage of handling radioactivity under potentially hazardous conditions. A more efficient process for preparing labeled *p*-aminobenzoic acid was achieved by employing the sequence described by Broser (5). In this process the bis(trimethylsilyl) derivative of *p*-bromoaniline served as an intermediate for metal-halogen exchange and carbona-

tion. Adaptation of this procedure to the synthesis of carbon-14 labeled *p*-aminobenzoic acid gave the tracer in 59% yield (46% radiochemical conversion).

The resultant *p*-aminobenzoic acid-¹⁴C was converted to 4-chlorosulfonyl-7-¹⁴C-benzoic acid by treating the diazonium salt with sulfur dioxide and hydrochloric acid. By treatment of the sulfonyl chloride with di-*n*-propylamine, probenecid-carboxyl-¹⁴C was obtained in 97.5% yield.

EXPERIMENTAL

Analytical TLC was carried out on 5 x 20 cm glass plates precoated with silica gel 60 F-254 (E. Merck, Darmstadt, Germany). Radioactive zones were located with a Varian Aerograph / Berthold Model LB2722 scanner. Radioactivity was determined with a Packard Tri-Carb Model 3320 liquid scintillation spectrometer using 0.4% OMNIFLUOR in toluene-ethanol (7:3) as scintillator fluid. Purity and specific activity of the starting materials were taken as given by the supplier.

4-Chlorosulfonyl-1-¹⁴C-benzoic acid. - To a stirred solution of 82.7 mg (0.44 mmole, 1.0 mCi/mmole) of *p*-toluene-1-¹⁴C-sulfonyl chloride in 2 ml of acetic acid and 0.8 ml of acetic anhydride was added in portions 555 mg (5.55 mmole) of CrO₃ over 20 minutes at 45°C. After the addition was completed the mixture was stirred for an hour and then poured onto 25 g of ice. When all the ice was melted, the product was collected by filtration. The crude material was dissolved in 0.8 ml of acetone and centrifuged to remove sediment. The acetone supernatant was decanted into 3 ml of ice water and the product re-isolated after a 20 minute age period by centrifugation/decantation. After washing with a little cold water and drying in vacuo a yield of 54.5 mg (56%) of 4-chlorosulfonyl-1-¹⁴C-benzoic acid was obtained.

p-Aminobenzoic Acid-¹⁴C. - To 1580 mg (5.0 mmole) of *p*-bromo-N,N-bis-(trimethylsilyl)aniline dissolved in 40 ml of dry ether was added 2.5 ml (4.0 mmole) of (1.6M in hexane) *n*-butyllithium. The resulting mixture was heated under an atmosphere of nitrogen at reflux for 1/4 hour and then cooled to liquid nitrogen temperature. Radioactive carbon dioxide (37.2 mg, 0.85 mmole, 59.1 mCi/mmole) was added by vacuum transfer, followed by 124.1 mg (2.82 mmole) of carrier carbon dioxide. The mixture was warmed to -78°C and aged at that temperature for 1/2 hour. Water (5.0 ml) was added and after warming to 0° - 5°C, the aqueous layer was acidified by the addition of 12N HCl to a final pH of 1.0. Removal of the trimethylsilyl blocking groups was achieved by warming the two phase mixture at 35°C for 1/2 hour. The pH of the aqueous phase was then raised to 9.5 using 2.5N NaOH, and the ether layer was removed. After readjusting the pH of the aqueous phase to 2.5 - 3.0, the product was extracted into three 20 ml portions of ether. The combined ether

extracts gave after evaporation 297 mg (59.1%) of p-aminobenzoic acid- ^{14}C at a specific activity of 10.7 mCi/mole (23.2 mCi, 46.4% radiochemical yield). Analysis of this material by radioscan of a TLC plate developed with chloroform-methanol (4:1) indicated a product of about 98% radiochemical purity.

4-Chlorosulfonyl-7- ^{14}C -benzoic acid. - To 898.5 mg (6.56 mmole, 45.3 mCi) of p-aminobenzoic acid- ^{14}C was added 4.0 ml each of glacial acetic acid and 12N HCl. The resulting slurry was cooled to $0^\circ - 5^\circ\text{C}$ and then a solution of 580 mg (8.15 mmole) of NaNO_2 (97%) in 1.0 ml of water was added dropwise with continued cooling so as to maintain the reaction temperature at $0^\circ - 5^\circ\text{C}$. After the addition was completed the reaction mixture was aged for 1/2 hour and then added, cold, to a $0^\circ - 5^\circ\text{C}$ solution of 5.0 g of SO_2 in 5.0 ml of glacial acetic acid to which was added a solution of 400 mg of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ in 1.0 ml of water. The reaction mixture was allowed to warm to 25°C over 1 hour and maintained at 25°C for an additional 2 hours. Water (100 ml) was added and the precipitated solid collected and dried to constant weight, giving 1356.5 mg (93.7%) of product with a specific activity of 6.4 mCi/mole (39.4 mCi, 86.9% radiochemical yield). Analysis of this material by radioscan of a TLC plate developed in chloroform-methanol (4:1) indicated essentially pure product.

p-(Di-n-propylsulfamyl)benzoic acid- ^{14}C . (Probenecid-carboxyl- ^{14}C). - To a rapidly stirred mixture of 5 ml of di-n-propylamine and 5 ml of water at 25°C was added a solution of 1350 mg (6.12 mmole, 6.4 mCi/mole) of 4-chlorosulfonyl-7- ^{14}C -benzoic acid in 20 ml of acetone over 1/4 hour. The resulting solution was stirred at 25°C for 3 hours and then concentrated under vacuum to a residual oil. This residue was dissolved in 10 ml of warm water and the solution was added dropwise to 100 ml of rapidly stirred 2.5 N HCl. The slurry which formed was stirred at 25°C for 1/2 hour and then filtered. The collected solid, washed well with water and dried at $56^\circ\text{C}/0.5\text{ mm}$, weighed 1700 mg (97.5%). TLC radioscan indicated a purity of about 99%. A single crystallization from ether gave 1484 mg (89.4% recovery) of product, probenecid-carboxyl- ^{14}C , with a specific activity of 6.7 mCi/mole (31.6 mCi, 80.6% radiochemical yield). Analysis of the material by scintillation counting of sections scraped from a TLC plate developed in either chloroform-methanol (4:1) or ethyl acetate-methanol-ammonium hydroxide (10:10:1) indicated a purity of 99.6%.

Probenecid-1-(ring)- ^{14}C was made in an analogous manner from 4-chlorosulfonyl-1- ^{14}C -benzoic acid.

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