SYNTHESIS OF N-(4-PYRIDYL[^{14}C]CARBONYLAMINO)-1,2,[^{3}H]3,6-TETRAHYDROPYRIDINE AND THE MONO-LABELLED [^{14}C]- and [^{3}H]-ANALOGUES

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SUMMARY

Reaction of isonicotinic [^{14}C] acid hydrazide (1) with the Zinke salt (2) afforded N-(4-pyridyl [^{14}C] carbonylimino)pyridinium ylide (3) in 81% chemical yield. Sodium borohydride reduction of 3 gave N-(4-pyridyl [^{14}C] carbonylamino) -1,2,3,6-tetrahydropyridine (4) in 60% chemical yield with a specific activity of 6.17 mCi mM^{-1}. Alternatively reduction of N-(4-pyridylcarbonylimino) pyridinium ylide (5) using absolute ethanol and [^{3}H]-water as solvent yielded N-(4-pyridylcarbonylamino)-1,2,[^{3}H]3,6-tetrahydropyridine (9) in 52.6% chemical yield with a specific activity of 1.14 mCi mM^{-1}. N-(4-Pyridyl[^{14}C]carbonylamino-1,2,[^{3}H]3,6-tetrahydropyridine (10) was similarly prepared by sodium borohydride reduction of 3 using absolute ethanol and [^{3}H]-water as solvent in 46% chemical yield with specific activities of 1.31 and 5.66 mCi mM^{-1} respectively for [^{3}H] and [^{14}C].

Key Words: Pyridinium ylide, Reduction, N-(Carbonylamino)-1,2,3,6-tetrahydropyridine.

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INTRODUCTION

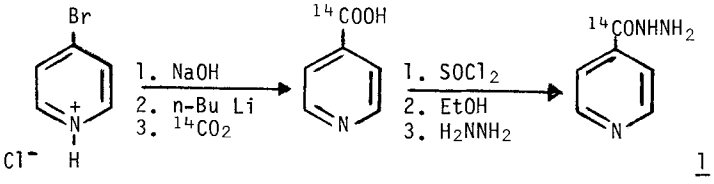
Recently we developed a facile procedure for the synthesis of N-(4-pyridylcarbonylamino)-1,2,3,6-tetrahydropyridine 4 (1). A broad spectrum pharmacological screen indicated that 4 exhibited analgesic and antiinflammatory activities comparable to Aspirin and Indomethacin respectively as well as a significant hyperglycemic effect (2). It was therefore of interest to prepare radiolabelled 4 for differential tissue distribution and metabolic studies. We now describe the synthesis of N-(4-pyridylcarbonylamino)-1,2,3,6-tetrahydropyridine labelled with [^{14}C] at the carbonyl carbon and [^{3}H] at the 3-position of the 1,2,3,6-tetrahydropyridine ring.

The rationale for incorporating [^{14}C] and [^{3}H] into the positions selected was: (i) The 3-position of the 1,2,3,6-tetrahydropyridine ring is expected to be a metabolically inactive site; (ii) Metabolic cleavage of the amide group in the dual labelled product would afford [^{14}C]-labelled isonicotinic acid and [^{3}H]-labelled 1,2,3,6-tetrahydropyridyl metabolites.

RESULTS AND DISCUSSION

Isonicotinic [^{14}C] acid hydrazide (1) was prepared from the reaction of 4-lithiopyridine with [^{14}C]-CO_2 using the reaction sequence reported by Murray and Langham (3) and the simplified apparatus described by Mayor and Wentrup (4) as illustrated by Scheme 1 in 46% chemical yield.

SCHEME 1

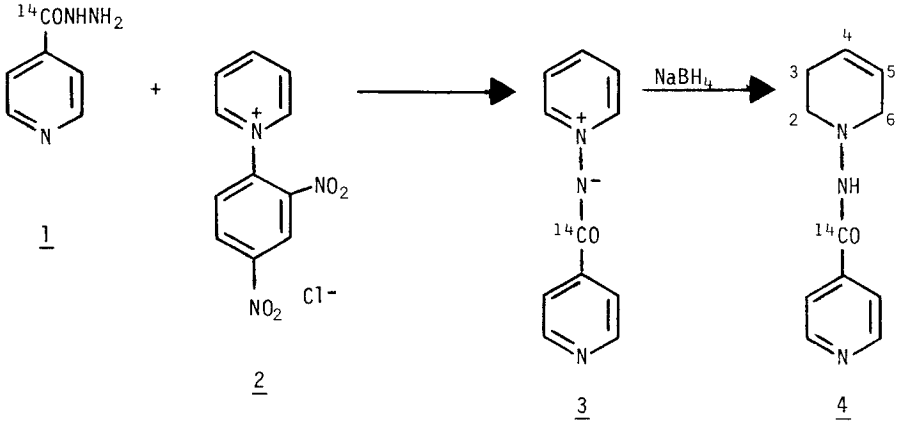


1

Reaction of isonicotinic [^{14}C] acid hydrazide (1) with 2,4-dinitrophenylpyridinium chloride (2) afforded N-(4-pyridyl[^{14}C]carbonylimino)pyridinium ylide

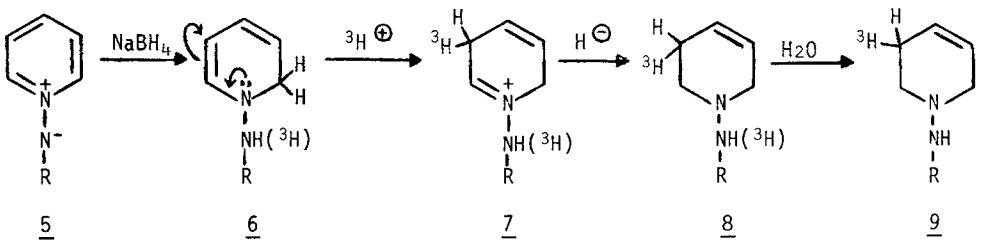
3 (81% chemical yield) which on subsequent reduction using sodium borohydride with ethanol as solvent at 0° gave rise to N-(4-pyridyl[^{14}C]carbonylamino)-1,2,3,6-tetrahydropyridine 4 in 60% chemical yield (Scheme 2) with a specific activity of 6.17 mCi mM^{-1}.

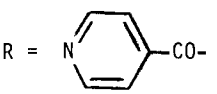
SCHEME 2



The mechanism for the sodium borohydride reduction of the pyridinium ylide 5 has not been investigated but it is expected to be analogous to that of pyridinium salts (5). Attack by hydride anion at carbon adjacent to the quaternary nitrogen would yield the dienamine 6 which on protonation ($^{3}H^{\oplus}$ or $^{1}H^{\oplus}$ from solvent) and subsequent reduction of the immonium species 7 would afford 9 (1) as illustrated by Scheme 3.

SCHEME 3

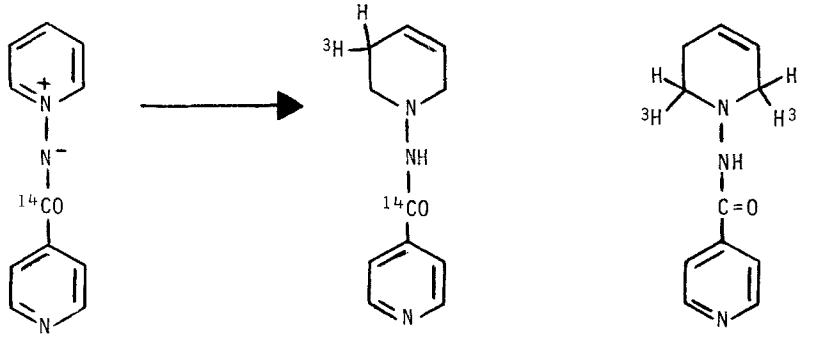


R = 

N-(4-Pyridylcarbonylamino)-1,2,[^{3}H]3,6-tetrahydropyridine 9 was therefore prepared by reduction of 5 (0.5 mmol) using sodium borohydride (1 mmol) with absolute ethanol (4.25 ml, 92.4 mmol) and tritiated water (5 Ci/ml, 0.25 ml, 13.88 mmol) as solvent in 62.5% chemical yield with a specific activity of 0.756 mCi mM^{-1}. Theoretically 9 should have a specific activity of 10.40 mCi mM^{-1} since the specific activity of the solvent is 10.40 mCi mM^{-1} available ^{1}H and ^{3}H. In an attempt to increase the specific activity of 9 the reaction was repeated as above using absolute ethanol (0.25 ml, 5.43 mmol) and tritiated water (5 Ci/ml, 0.25 ml, 13.88 mmol) as solvent. The specific activity of 9 should theoretically be 38.20 mCi mM^{-1} since the specific activity of the solvent is 38.20 mCi mM^{-1} available ^{1}H and ^{3}H. Radiolabelled 9 was obtained in 52.60% chemical yield with a specific activity of 1.14 mM^{-1}. The lower than expected specific activity may be due to a tritium isotope affect.

Reduction of N-(4-pyridyl[^{14}C]carbonylimino)pyridinium ylide 3 (0.395 mmol) using sodium borohydride (0.79mmol) with absolute ethanol (0.25 ml, 5.43 mmol) and tritiated water (5 Ci/ml, 0.25 ml, 13.88 mmol) as solvent afforded N-(4-pyridyl [^{14}C]carbonylamino)-1,2,[^{3}H]3,6-tetrahydropyridine 10 in 46% chemical yield with specific activities of 1.31 and 5.66 mCi mM^{-1} respectively for [^{3}H] and [^{14}C].

It is expected that high specific activity N-(4-pyridylcarbonylamino)-1,[^{3}H] 2,3,[^{3}H]6-tetrahydropyridine 11 would be readily accessible. Reduction of 5



3 10 11

using [^{3}H]-sodium borohydride as illustrated by Scheme 3 would incorporate a ^{3}H-atom into both the 2- and 6-positions of the 1,2,3,6-tetrahydropyridine ring.

EXPERIMENTAL

[^{14}C]-Barium carbonate (53 mCi mM^{-1} and [^{3}H]-water (5 Ci/ml) were purchased from New England Nuclear. Radioactivity was determined using a Searle Mark III Liquid Scintillation counter. The radiolabelled compounds prepared were identical (tlc, mp) to authentic samples.

Isonicotinic [^{14}C] acid

The preparation followed was similar to the procedure of Murray et al (3) using the simplified apparatus of Mayor et al (4). A solution of 10% aqueous sodium hydroxide (25 ml) was added to a solution of 4-bromopyridine hydrochloride (1.4g, 7.19 mmol) in 25 ml water with stirring. 4-Bromopyridine, which separates as an oil, was extracted with diethyl ether (3 x 35 ml). The ether extract was dried for 1 h using sodium sulfate and then for 18 h using Linde 3A molecular sieves. This dry ether solution was placed in the reaction flask and cooled to -78°. n-Butyllithium (5 mmol as a 2.2 M solution in hexane) was added dropwise and the mixture was stirred gently for 5 min at -78°. The reaction mixture was degassed by cooling to -170°, evacuating to 2 x 10^{-2} mm and then warming to -55°. This solution of 4-lithiopyridine was carbonated by the dropwise addition of concentrated sulfuric acid (12 ml) onto 0.5 g [^{14}C]-barium carbonate (2.54 mmol, 25 mCi) over a period of 1 h and 20 min during which the contents of the reaction flask were stirred vigorously at -55° to -65°. The carbon dioxide generator flask was heated to 50° to complete the generation of [^{14}C]-carbon dioxide. The reaction mixture was hydrolyzed with 25 ml 2.5N nitric acid and continuously extracted with ether for 4 h. The remaining solution was then basified with 30 ml 3N sodium hydroxide and extraction continued for a further 24 h. These extracts were discarded. The alkaline solution was acidified to pH 3(±0.1) and extracted with ether for 5 days. Removal of the ether from the ether extract afforded isonicotinic [^{14}C]acid (250 mg, 20 mCi, 80% chemical and radiochemical yield, 9.85 mCi mM^{-1}) which was identical (tlc, mp) to an authentic sample.

Isonicotinic [^{14}C] acid hydrazide (1).

Isonicotinic [^{14}C]carbonyl chloride hydrochloride was prepared by treating the acid (250 mg, 2.03 mmol) with 2 ml purified thionyl chloride, heating under reflux for 15 min and removal of excess thionyl chloride under high vacuum (2 x 10^{-2} mm) at 25°. The acid chloride hydrochloride was converted to the ester hydrochloride by reaction with 4 ml absolute ethanol, heating under reflux for 1 h and removal of the solvent under reduced pressure. The ester was isolated by treatment with 500 mg sodium bicarbonate in 25 ml water followed by continuous ether extraction. Evaporation of the ether afforded the ester which was heated under reflux with 85% hydrazine hydrate (0.23 g) in 10 ml absolute ethanol for 4 days. Removal of the solvent and drying *in vacuo* for 48 h gave isonicotinic [^{14}C] acid hydrazide (0.16 g, 57.6% chemical yield) identical (tlc and mp) with an authentic sample.

N-(4-Pyridyl[^{14}C]carbonylimino)pyridinium ylide (3).

Isonicotinic [^{14}C] acid hydrazide 1 (0.16 g, 1.17 mmol) in 10 ml methanol was added dropwise to an ice-cooled solution of 2,4-dinitrophenylpyridinium chloride 2 (0.335 g, 1.19 mmol) in 5 ml methanol, after which triethylamine (0.12 g, 1.19 mmol) was added. The reaction mixture was allowed to stand at 25° for 16 h prior to centrifugation to remove the solid which had precipitated. The solid product was washed with 5 ml each of methanol, water, methanol and ether. A suspension of the solid obtained above in dioxane-water (4:1 v/v)(10 ml) was heated under reflux for 4.5 h to afford a clear solution. Removal of the solvent *in vacuo* gave a dark red solid which was purified on a 2.5 x 30 cm silica gel column. The column was eluted starting with 5% methanol in methylene chloride and gradually increasing the composition to 15% methanol in methylene chloride. Fractions having a volume of 25 ml were collected and analyzed, using pure reference standards, by micro tlc on silica gel G plates with 15% methanol in methylene chloride as development solvent. Fractions containing only ylide 3 were combined and the solvent removed *in vacuo* to yield 3 (0.164 g). Those fractions containing some 3 in addition to other components were rechromatographed on three 20 x 20 cm

preparative silica gel G plates, 0.75 mm in thickness, with 15% methanol in methylene chloride as development solvent. The band having a Rf identical to authentic ylide was removed and extracted with 20 ml warm methanol to give 24.2 mg 3. The combined products which were identical (tlc, mp) to an authentic sample represented an 81% chemical yield of 3 (188.2 mg, 0.946 mmol).

N-(4-Pyridyl[^{14}C]carbonylamino)-1,2,3,6-tetrahydropyridine (4).

A solution of N-(4-pyridyl[^{14}C]carbonylimino)pyridinium ylide 3 (83.2 mg, 0.418 mmol) in 95% ethanol (1.2 ml) was added dropwise to a solution of sodium borohydride (34 mg, 0.9 mmol) in 95% ethanol (2.5 ml) at 0° over 2 min. The reaction was allowed to proceed for 4 h at 0° before pouring onto 50 ml crushed ice. The ice was allowed to melt, after which the aqueous solution was extracted with chloroform (3 x 20 ml). The chloroform extract was dried (Na_2SO_4) and the solvent removed in vacuo to give impure 4 (57.3 mg) which was purified on three 20 x 20 cm silica gel G plates, 0.75 mm in thickness, with 15% methanol in chloroform as development solvent. Elution of the major band, having a Rf identical to authentic 4, gave pure 4 (51.1 mg, 0.2514 mmol, 60% chemical yield, 6.17 mCi mM^{-1}). This sample of 4 was diluted with unlabelled 4 to give 1.351 g of product with a specific activity of 1.15 μCi/mg.

N-(4-Pyridylcarbonylamino)-1,2,[^{3}H]3,6-tetrahydropyridine (9).

Sodium borohydride (38 mg, 1 mmol) was added to a solution of 5 (100 mg, 0.5 mmol) in 4.25 ml absolute ethanol (92.4 mmol) and [^{3}H]-water (5 Ci/ml, 0.25 ml, 13.88 mmol) at 0°. The reaction was allowed to proceed at 0° for 4 h, and completed as described above for the preparation of 4, to yield 9 (63.4 mg, 0.3123 mmol, 62.5% chemical yield, 0.756 mCi mM^{-1}).

Repetition of the procedure used for the preparation of 9 above using absolute ethanol (0.25 ml, 5.43 mmol) and [^{3}H]-water (5 Ci/ml, 0.25 ml, 13.88 mmol) as solvent afforded 9 (53.4 mg, 0.2631 mmol, 52.6% chemical yield, 1.14 mCi mM^{-1}).

N-(4-Pyridyl[^{14}C]carbonylamino)-1,2[^{3}H]3,6-tetrahydropyridine (10).

Sodium borohydride (30 mg, 0.79 mmol) was added to a solution of N-(4-pyridyl[^{14}C]carbonylimino)pyridinium ylide 3 (78.6 mg, 0.395 mmol) in absolute ethanol (0.25 ml, 5.43 mmol) and [^{3}H]-water (5 Ci/ml, 0.25 ml, 13.88 mmol) at 0°. The reaction was allowed to proceed at 0° for 4 h, and completed, as described for the preparation of 4, to yield 10 (36.8 mg, 0.1813 mmol, 46% chemical yield). The dual labelled product 10 was diluted with 300 mg unlabelled N-(4-pyridylcarbonylamino)-1,2,3,6-tetrahydropyridine to afford 10 (336.8 mg) with specific activities of 0.709 and 3.05 μCi mM^{-1} respectively for [^{3}H] and [^{14}C].

ACKNOWLEDGEMENTS

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