THE PREPARATION OF CARBON-14 LABELED TOBACCO CONSTITUENTS! II. THE SYNTHESIS OF DL-NICOTINE (2'-^{14}C).

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SUMMARY

A 3-step synthesis of dl-nicotine (2'-^{14}C) is described. Commercially available nicotinamide (carbonyl-^{14}C) is dehydrated to 3-cyanopyridine (cyano-^{14}C). Reaction of the 3-cyanopyridine with cyclopropyl lithium yields 3-pyridyl cyclopropyl ketone (carbonyl-^{14}C) which is converted to dl-nicotine (2'-^{14}C) by refluxing in N-methylformamide. Gas-liquid radiochromatography is used for chemical and radiochemical purity determinations.

Discussion

Total cigarette smoke distribution studies of nicotine have been conducted using randomly labeled ^{14}C-nicotine.[2] The use of materials isolated from plants grown in a $^{14}CO_2$ atmosphere usually suffers from low specific activities. The use of a uniformly labeled tobacco constituent gives rise to all of the pyrolysis products that it produces during the smoking process. In orde to conduct the necessary mechanistic studies of a given component, it becomes necessary to investigate the pyrolytic behavior of each

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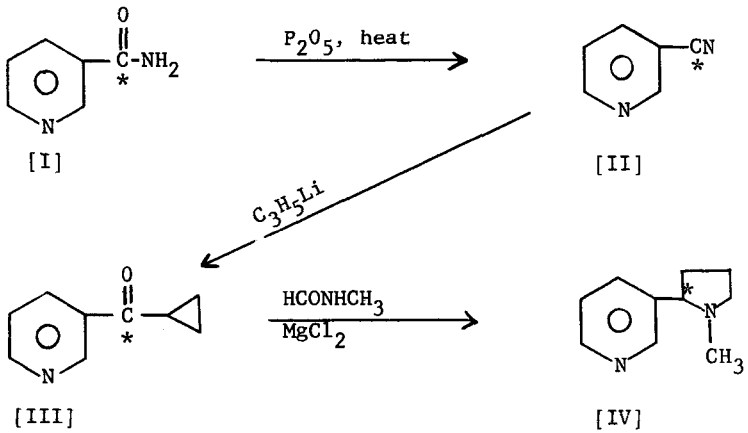
carbon atom. Ideally, these studies are best conducted in a series of separate experiments with the component specifically ^{14}C-labeled at each carbon atom. In practice, only those compounds which are commercially or synthetically available are used. A previously reported synthesis of specifically labeled ^{14}C-nicotine[3] proved long and produced low radiochemical yield. Commercially available ^{14}C-labeled nicotine is limited to that with the label at the N-methyl position.[4]

A novel 1-step synthesis of nicotine has been reported[5] in which 3-pyridyl cyclopropyl ketone is converted to dl-nicotine with N-methylformamide. We have utilized this reaction in a 3-step sequence for the synthesis of dl-nicotine (2'-^{14}C).



*-^{14}C-labeled position

Nicotinamide (carbonyl-^{14}C), I, was heated in vacuo in the presence of P_2O_5 to yield 3-cyanopyridine (cyano-^{14}C), II, in

60-80% yield.[6] The rate of heating was critical in that too rapid application of heat gave rise to a considerable amount of tarry residue and a lower yield. If the heating was applied too slowly, the product was not driven from the melt and the quantity of isolated material was small.

The preparation of 3-pyridyl cyclopropyl ketone (carbonyl-^{14}C), III, required a dry, inert atmosphere and freshly distilled solvents. Cyclopropyl lithium was prepared from lithium ribbon and cyclopropyl bromide in dry ether. Reaction of cyclopropyl lithium with II afforded III in 56% yield. All transfers and additions were conducted under N_2 with syringes through rubber septums.

Refluxing III with N-methylformamide and $MgCl_2$ under N_2 afforded crude dl-nicotine, IV, which was isolated by column chromatography on alumina.[7]

Experimental[8]

Gas chromatographic analyses were carried out on a F&M Model 720 Dual Column Gas Chromatograph. The columns (6 mm O.D. by 3 m copper) contained 5% Carbowax 20M on 80-100 mesh Chromasorb G, AW-DMCS. Injection port and detector temperatures were 245° and 230° respectively and He flow was 65 ml/min.

Liquid scintillation counting was accomplished on a Packard Model 3310 Tri-Carb Liquid Scintillation Spectrometer. The scintillation solution was prepared from Liquiflour (New England Nuclear Corp.) and spectral grade toluene (Packard). ^{14}C-Toluene (New England Nuclear Corp.) was used as an internal standard for applying proper quenching corrections.

Gas-liquid radiochromatography (GLRC) was accomplished by standard sample injection and detection by thermal conductivity on one column of the gas chromatograph. The exit port was coupled through a stainless steel tube (3 mm O.D. by 50 cm) maintained at 410° to a stainless steel combustion tube (9 mm O.D. by 32 cm, containing CuO at 710°) and a copper drying tube (6 mm O.D. by 25 cm, containing $MgClO_4$ followed by CoO). The oxidized sample was then mixed with the counting gas (propane) in the ratio 1.5:1 (propane:He) and passed into an 80 cc flow through counting tube (Lab. Prof. Dr. Berthold). A Berthold pre-amplifier was coupled to a Hewlett-Packard Model 5201L Scaler-Timer. Digitized data was printed on a Hewlett-Packard Model 562A Digital Recorder. Radioactivity measurements by scintillation counting and by GLRC were in agreement within experimental error. Efficiency determinations were conducted with toluene-^{14}C (New England Nuclear).

3-Cyanopyridine (cyano-^{14}C) [II]

Nicotinamide (carbonyl-^{14}C)[9], I, (122 mg, 1 mM, 2 mCi) was placed in a 10 ml round bottom flask and P_2O_5 (142 mg, 1 mM) added. The flask was shaken to mix the solids and connected to a distilling head and short downward air condenser. The system was evacuated to 14 mm and the receiver chilled in an ice-water bath. Rapid heating of the reaction flask was accomplished with a Bunsen burner at which time the solids melted and distillation was observed. Heating was continued until distillation ceased. When the system had cooled to room temperature the condensed solids were washed from the condenser and receiver with CH_2Cl_2 and the solution brought to a total volume of 50 ml. Liquid scintillation counting of this solution gave a total activity of 1250 μCi (62%). GLRC at 150° showed a solvent peak and one other mass peak with a retention

time of 24 min., II. This peak contained 100% of the activity. Concentration of the CH_2Cl_2 solution in vacuo yielded 60 mg of an off-white solid which was used in the next step without further purification.

3-Pyridyl cyclopropyl ketone (carbonyl-^{14}C) [III]

Lithium ribbon (67.7 mg, 9.8 mM) was wiped thoroughly with a dry towel to remove mineral oil and cut in small pieces and placed into a dry flask which was then placed under positive nitrogen pressure. Ether (3 ml) freshly distilled from $LiAlH_4$ was transferred (syringe) under N_2 to the flask. A solution of 300 µl (471 mg, 3.9 mM) of cyclopropyl bromide in 2 ml distilled (LAH) ether was added dropwise with stirring to the flask over 30 min. and the reaction was allowed to proceed for 24 hours at room temperature (slow reflux usually occurred during cyclopropyl bromide addition).

3-Cyanopyridine (cyano-^{14}C, II, 60 mg, 0.58 mM, 1250 µCi) was transferred to a dry flask and the system sealed under a positive pressure of dry N_2. Distilled (LAH) ether (5 ml) was added (syringe) to the flask under N_2. The cyclopropyl lithium (3.9 mM) was transferred under N_2 and added dropwise with stirring (syringe) at -20° over 2-3 min. The cold bath was removed immediately following addition and the mixture allowed to stir at room temperature for 1.5 hours. A solution of NH_4Cl (424 mg, 8 mM) in 5 ml water was added and the reaction mixture acidified (pH 1-2) with 10% HCl. The layers were separated and the aqueous layer was extracted with two 25 ml portions of ether for removal of non-basic impurities. The combined ether layers were extracted twice with 50 ml portions of 10% HCl. The combined aqueous layers (125 ml) were basified (pH 11) with 50% NaOH and extracted with three 125 ml portions of ether. The combined ether extracts were washed twice with 10 ml

portions of distilled water and dried (anh. Na_2SO_4). The solution was filtered (N_2 blanket), the solvent removed in vacuo below room temperature, and the residue redissolved in 100 ml of CH_2Cl_2 and redried (anh. Na_2SO_4). Filtration (N_2 blanket) and removal of solvent (in vacuo below room temperature) afforded a clear, orange colored, viscous oil. This oil was taken up in 10 ml of CH_2Cl_2 for activity determinations and storage.

GLRC of the CH_2Cl_2 solution at an oven temperature of 150° for 5 min. followed by temperature programming to 200° at 5°/min. yielded a solvent peak and one other mass peak at a retention time of 26 min., III. Total activity was found to be 705 μCi (56% based on the starting activity of the 3-cyanopyridine). Of this activity, 96% (679 μCi) was in the 3-pyridyl cyclopropyl ketone activity peak, 2.5% (17.5 μCi) in an activity peak at 37 min. and 1.2% (8.2 μCi) at 54 min. (retention times from activity tape).

The 3-pyridyl cyclopropyl ketone was of adequate purity for the next step and was used without further purification.

dl-Nicotine (2'-^{14}C) [IV]

A solution containing 3.9 mg (0.041 mM) of anhyd. $MgCl_2$ in 295 mg (5 mM) N-methylformamide was added to 60 mg (0.41 mM) of 3-pyridyl cyclopropyl ketone (carbonyl-^{14}C) [705 μCi] under N_2. The mixture was heated at reflux for 24 hours under N_2 and allowed to cool to room temperature. The total reaction mixture was dissolved in a minimum of CH_2Cl_2 and transferred to a 1.2 cm I.D. chromatography column containing 40 g. of Al_2O_3.[7] Elution with CH_2Cl_2 yielded 36.5 μCi in fractions 1-6 (70 ml) - no nicotine by GLRC. Fractions 7-12 (30 ml) contained pure nicotine (204 μCi). Fractions 13-20 (40 ml) contained nicotine (63 μCi) contaminated with N-methylformamide. Additional elution with CH_2Cl_2 (50 ml) gave 8.5

μCi of an impurity with a shorter GC retention time than that of nicotine.

Fractions 13-20 were combined, concentrated and rechromatographed on another 40 g Al_2O_3 column from which 29.1 μCi of pure nicotine were isolated.

Combination of all purified nicotine fractions, concentration and dilution to 10 ml with CH_2Cl_2 yielded 215 μCi (32% yield based on activity of III) of dl-nicotine (2'-^{14}C) at a retention time of 22 min. at 170°C. Activity determinations by GLRC and liquid scintillation counting were in excellent agreement.

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8. In all cases, reactions were first carried out on non-radioactive materials and products identified by retention time (GC), and IR and mass spectral data run against authentic samples.
9. Purchased from Amersham/Searle Corp. This material had a specific activity of 60 mCi/mMole and was diluted with sufficient cold nicotinamide to make to 1 mMole.