SYNTHESIS OF [4-^{3}H]-2-PYRROLIDINONE, [4-^{3}H]-N-METHYL-2-PYRROLIDINONE AND N-[^{14}C-METHYL]-2-PYRROLIDINONE

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SUMMARY

An efficient synthesis of 2-pyrrolidinone and N-methyl-2-pyrrolidinone was developed in which the compounds can be labeled with tritium at either the 3- and 4-positions or the 4-position of the lactam ring. Cyclization of [2,3-^{3}H]-γ-aminobutyric acid (GABA) yielded [3,4-^{3}H]-2-pyrrolidinone and cyclization of [3-^{3}H]-GABA, prepared by acid-catalyzed exchange of tritium from [2,3-^{3}H]-GABA, yielded [4-^{3}H]-2-pyrrolidinone. [4-^{3}H]-N-Methyl-2-pyrrolidinone was synthesized by methylation of [3,4-^{3}H]-2-pyrrolidinone using phase transfer catalysis, followed by base-catalyzed tritium exchange. N-[^{14}C-Methyl]-2-pyrrolidinone was prepared from [^{14}C] methyl iodide and 2-pyrrolidinone, also using phase transfer catalysis.

Key Words: [^{3}H]-γ-aminobutyric acid, [^{3}H]-2-pyrrolidinone, [^{3}H]-N-methyl-2-pyrrolidinone, [^{14}C]-N-methyl-2-pyrrolidinone, double-labeled

INTRODUCTION

N-Methyl-2-pyrrolidinone (NMP) is an aprotic solvent widely used in chemical processing (1). Its complete miscibility in aqueous and organic systems, catalytic activity in ionic and polymerization reactions, and chemical stability make NMP an attractive choice as a chemical reaction medium. The

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petrochemical industry utilizes the selective affinity NMP has for unsaturated hydrocarbons, aromatics, and sulfur-containing gases in many of its large scale operations. These processes include the purification and recovery of acetylene, isoprene, and butadiene, the removal of sulfur compounds and carbon dioxide from natural gas, and the extraction of aromatics such as benzene, toluene and xylene from hydrocarbon mixtures. NMP is also used as a solvent for many agricultural chemicals and pharmaceuticals.

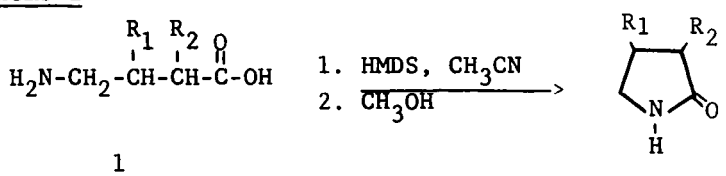
2-Pyrrolidinone (PYR) is a versatile industrial solvent, like NMP. PYR is important in the pharmaceutical industry as a chemical intermediate, a solubilizing agent for parenteral antibiotic preparations given to food-producing animals (2), and a vehicle system for topically applied drugs for veterinary use (3). Since PYR is the lactam of the neurotransmitter γ-aminobutyric acid (GABA), it has been investigated for GABA-like pharmacologic activity (4). Studies have suggested that PYR may serve as a precursor of GABA in the central nervous system (5,6). The potential importance of PYR derivatives having specific neuropharmacologic activity is exemplified by the long-standing interest in piracetam, a drug which enhances the cognitive functions of the brain (7).

The in vivo disposition of NMP and PYR is largely unknown. In consideration of their widespread use in the chemical and pharmaceutical industries it was decided to study their metabolic fate in animals. With this objective we describe the synthesis of [4-^{3}H]-2-pyrrolidinone, [4-^{3}H]-N-methyl-2-pyrrolidinone and N-[^{14}C-methyl]-2-pyrrolidinone.

RESULTS AND DISCUSSION

The cyclization of GABA (1a, Scheme 1) provides a one-step method for the preparation of PYR (2a) and is adaptable to small scale synthesis. GABA is commercially available as the tritium-labeled species on carbons-2 and -3 (1b) and upon cyclization results in the formation of [3,4-^{3}H]PYR (2b). For metabolic studies involving tritiated PYR or NMP the α-carbon (position-3) of the pyrrolidinone ring should not contain tritium since a precedent, shown by the metabolism of cotinine (the principal metabolite of nicotine), indicates that this position is metabolically unstable (8).

Scheme 1



1

1a, $R_1 = R_2 = H$

1b, $R_1 = R_2 = {}^3H$

1c, $R_1 = {}^3H$; $R_2 = H$

2

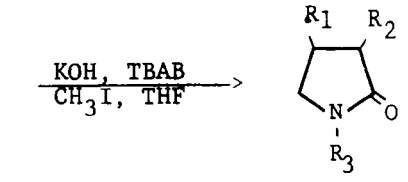
2a, $R_1 = R_2 = H$

2b, $R_1 = R_2 = {}^3H$

2c, $R_1 = {}^3H$; $R_2 = H$

One method to obtain the desired [4-^{3}H]-2-pyrrolidinone ring is to remove the tritium from carbon-2 of 1b before cyclization to its lactam. This removal was accomplished using acid catalysis and heat, and the resulting [3-^{3}H]GABA (1c) was then cyclized to yield [4-^{3}H]PYR (2c).

Scheme 2



3

3a, $R_1 = R_2 = H$; $R_3 = {}^{14}CH_3$

3b, $R_1 = R_2 = {}^{3}H$; $R_3 = CH_3$

3c, $R_1 = {}^{3}H$; $R_2 = H$; $R_3 = CH_3$

An alternate method to the removal of tritium from carbon-2 of 1b is to cyclize directly to 2b and then remove the tritium atom from the α-carbon of the lactam. Experiments involving deuterium have shown that the hydrogen atoms on the α-carbon of PYR can be exchanged using strong base ($NaOCH_3$ in CH_3OD) and heat but that a more rapid rate of exchange is achieved with an N-alkylated 2-pyrrolidinone (NMP). This observation can be explained as follows. In PYR the most acidic and easily exchanged proton is the amido proton. Subsequent abstraction of a proton from the α-carbon leads to a doubly charged intermediate which is energetically unfavorable. Abstraction of a proton from the α-carbon of NMP is easier in that no doubly charged carbanion is involved. In consideration of the above information the synthesis of [4-^{3}H]NMP (3c, Scheme 2) was accomplished through a synthetic sequence that involved cyclization of 1b to 2b, methylation to [3,4-^{3}H]NMP (3b) and then removal of the tritium from position-3 of the latter.

(This synthetic route eliminates the difficult conditions described for the exchange of GABA as the first step toward preparing the monolabeled tritiated lactam.)

The reaction involving N-methylation of 2b to 3b was found to require a purification step before proceeding with the synthesis. Radiomonitored HPLC analysis revealed the product to be radiochemically impure, consisting of 94% desired product 3b and 6% starting material 2b. Preparative thin-layer chromatography was used to isolate 3b but it was found that 56% of the radioactivity in the original sample applied to the TLC plates was lost in the process, probably due to the volatility of NMP in the elution solvent system. (A preparative HPLC solvent system was subsequently developed in order to avoid this significant loss of radioactivity from the sample in future syntheses.) An alternative to using preparative chromatography is to simply repeat the reaction in the same flask in which the product is isolated. This was found, in another synthesis of 3c, to successfully convert all the unreacted 2b to the desired 3b.

N-[^{14}C-Methyl]-2-pyrrolidinone (3a) was prepared from 2a and [^{14}C]methyl iodide using phase transfer catalysis. The synthesis is conveniently accomplished on a small scale at room temperature and the unreacted [^{14}C]methyl iodide can be recovered. Several modifications in the literature procedure reported by Takahata (9) for the N-alkylation of lactams were made in adapting the reaction to prepare 3a. The reaction mechanism involves phase transfer catalysis in a heterogeneous solid/liquid system. The catalyst is a quaternary

ammonium salt (tetrabutylammonium bromide, TBAB) which functions to exchange anions and bring the base (OH^-) into the organic solvent to abstract the labile amido hydrogen from PYR. Takahata's procedure used a base-to-catalyst molar ratio of 5.5:1 and a 92% yield was reported. This yield was not realized when the reaction conditions were scaled down to milligram quantities in order to prepare the radiolabeled NMP. In this study it was found that increasing the amount of catalyst, relative to the base, resulted in a higher yield of NMP and that a base-to-catalyst molar ratio of approximately 2:1 was optimum. Using this ratio a 78% yield for the preparation of NMP was achieved, as compared to previous yields of 54% for NMP when the 5.5:1 ratio was used. Additionally, the literature procedure was modified to exclude an aqueous/-dichloromethane phase separation of NMP from the catalyst after the removal of the solvent (tetrahydrofuran) since NMP is freely soluble in both phases. A single filtration step following ether extraction was found to selectively isolate the product.

The synthetic routes described in this communication permit the preparation of double-labeled (^3H and ^{14}C) N-methyl-2-pyrrolidinone and can be adapted to the synthesis of other N-substituted pyrrolidinones, e.g., double-labeled N-vinyl-2-pyrrolidinone. The latter is the monomer from which polyvinylpyrrolidinone is prepared. Additionally, the use of tritium-labeled 2-pyrrolidinone could provide a convenient means for incorporation of a radiolabel into many drugs which possess this lactam as an inherent part of the molecule.

EXPERIMENTAL

Reagents

[2,3-^{3}H]-γ-Aminobutyric acid and [^{14}C]methyl iodide were purchased from New England Nuclear. Hexamethyldisilazane and methyl iodide were obtained from Fluka Chemical Corporation, γ-aminobutyric acid and tetrabutylammonium bromide from Aldrich Chemical Company, N-methyl-2-pyrrolidinone from GAF Corporation and 2-pyrrolidinone from Fisher Scientific Company.

Instruments

Infrared spectra were determined on a Perkin-Elmer Model 567 spectrophotometer. NMR spectra were taken in $CDCl_3$, using tetramethylsilane as internal standard, and recorded on a Varian EM-360 spectrometer. Radioactive samples were counted in a Packard Model 3255 or 3375 Tri-carb liquid scintillation spectrometer using Fisher Scintiverse II liquid scintillation cocktail and were detected on TLC plates after elution by using a Berthold Model LB2760 or Packard Model 7201 thin-layer chromatogram scanner.

High-Performance Liquid Chromatography

Analyses of samples for identification and radiochemical purity were performed using a programmable HPLC system consisting of an Altex Model 110A solvent metering pump (Altex Scientific, Berkeley, CA), an Altex Model 420 system controller, and a Varian Vari-chrom analytical UV detector (Varian, Palo Alto, CA). Samples were introduced via a Rheodyne loop injector (Rheodyne, Cotati, CA) equipped with a 50 µl loop onto an Altex Ultrasil 10 µm ODS column, 25 cm x 4.6 mm ID, or a Whatman

Partisil PXS 10 μm ODS-2 column, 25 cm x 4.6 mm ID (Whatman, Clifton, NJ). The effluent was split by a Radiomatic Model ES streamsplitter (Radiomatic Instruments and Chemicals Co., Tampa, FL) after it had passed through the UV detector, with 50% going to a Radiomatic Model HP or Model HS Flo-One radioactive flow detector and 50% to waste. The UV output was recorded on a Linear Model 500 (Linear Instruments Corp., Reno, NV) or an Omniscribe Model 5000 dual-channel recorder (Houston Instruments, Austin, TX). The Model HP programmable radioactive flow detector mixed scintillation cocktail (Flo-Scint III, Radiomatic) with effluent in a ratio of 3:1, by volume, and the Model HS detector mixed cocktail (Flo-Scint II, Radiomatic) with effluent in a ratio of 4:1, by volume. The radioactivity output was recorded simultaneously on the second channel of the recorder. Preparative HPLC was performed using a Whatman Partisil Magnum 9 10 μm ODS-2 column, 50 cm x 9.4 mm ID, with methanol/water (30:70, v/v) as the mobile phase, a flow rate of 1.5 ml/min and UV detection at 230 nm.

Methods

[3-^{3}H]-γ-Aminobutyric acid hydrochloride (1c). Tritium on the α-carbon of [2,3-^{3}H]-γ-aminobutyric acid was exchanged according to a procedure adapted from Kumarev (10). A solution containing 1.29 ml of [2,3-^{3}H]-γ-aminobutyric acid (1.29 mCi, 43.3 Ci/mmol) in 0.01 N HCl was mixed in a 5 ml beaker with a solution of unlabeled GABA (124 mg) in 1 ml of water. The resulting solution was transferred via a pipette to a constricted glass reaction tube (15 cm in length) open at one end. Rinsing was accomplished using

1 ml of water, then 2.5 ml of concentrated HCl and 0.4 ml of water were added to make a final HCl concentration of 5N. The tube was then sealed using an acetylene torch and was secured inside an iron cylinder with glass wool. The cylinder was closed with screw-on caps and it was heated at 120° for 64 h. After heating, the tube was opened and the contents were mixed with 13 ml of an aqueous solution of unlabeled GABA (110 mg) in a beaker. The solution was transferred to a 250 ml round bottom flask equipped with a Dean-Stark trap apparatus. The water and HCl were removed by azeotroping with toluene. (Caution: severe bumping resulting in a loss of solution can occur.) The toluene was decanted to leave solid 1c which was dissolved in 3 ml of water and vacuum-filtered into a 25 ml round bottom flask. The filtrate was then freeze-dried to yield the hydrochloride salt of 1c: 312 mg, 2.24 mmol (99% yield); 0.623 mCi, 0.278 mCi/mmol (48% radiochemical yield). (Caution: the product is hygroscopic.)

An NMR spectrum of a similar exchange reaction done using 5N DCl in D_2O containing approximately 10% GABA showed quantitative exchange of the protons adjacent to the carbonyl group by 48 h at 120°. Also, using unlabeled GABA it had been demonstrated by NMR that aqueous hydrochloric acid could be removed from GABA by azeotropic distillation using toluene which left GABA HCl intact.

[3,4-^{3}H]-2-Pyrrolidinone (2b). Conversion of 1b to its lactam was adapted for radiochemical synthesis from the method reported by Pellegata (11). An aqueous solution containing 0.35 ml of [2,3-^{3}H]GABA (0.350 mCi, 80.8 Ci/mmol) in 0.01 N HCl was added to unlabeled GABA-

HCl (252 mg, 1.81 mmol) in a 25 ml round bottom flask resulting in a specific activity of 0.193 mCi/mmol. The solution was freeze-dried and then acetonitrile (7.0 ml), hexamethyldisilazane (HMDS, 4.0 ml, 19.2 mmol) and a magnetic stirring bar were added to the flask. The resulting suspension was refluxed and stirred for 72 h under a nitrogen atmosphere maintained at a slight positive pressure (complete solution occurs after 3-5 h). The solution was then cooled to room temperature, poured into cold methanol (15 ml) in a 50 ml round bottom flask, and the volatile components were evaporated under vacuum at room temperature. Chloroform was sparingly added to the oily residue and the resulting suspension was filtered under vacuum into a 10 ml round bottom flask, using a bell jar with side tubulation and a 15 ml fritted disc (4-5.5 μm) Buchner funnel. The chloroform was evaporated under a gentle stream of nitrogen gas to give 2b: 124 mg, 1.46 mmol (81% yield); 0.268 mCi, 0.184 mCi/mmol (77% radiochemical yield).

Radiochemical purity of the product as determined by HPLC was >99% using methanol/water (10:90, v/v) as the mobile phase with a flow rate of 1.0 ml/min and UV detection at 210 nm (retention time 6.5 min). Thin-layer radiochromatography (co-chromatographed with authentic material) was done on silica gel G plates (250 μm, 5 x 20 cm; Analtech, Newark, DE) which were eluted with 95% ethanol/-water (70:30) and detected in an iodine chamber (R_f 0.66). Synthesis of unlabeled 2-pyrrolidinone was carried out under exactly the same conditions and scale as described for 2b.

NMR and IR were identical to that of authentic unlabeled material.

[4-^{3}H]-2-Pyrrolidinone (2c). Into a 50 ml round bottom flask containing 1c (312 mg, 2.24 mmol; 0.623 mCi, 0.278 mCi/mmol) were added acetonitrile (12.0 ml), hexamethyldisilazane (HMDS, 7.0 ml, 33.6 mmol) and a magnetic stirring bar. The resulting suspension was then cyclized by the method described for 2b to give 2c: 158 mg, 1.85 mmol (83% yield); 0.204 mCi, 0.110 mCi/mmol (32% radiochemical yield).

Radiochemical purity of the product as determined by HPLC was >99% using methanol/water (30:70, v/v) as the mobile phase with a flow rate of 1.0 ml/min and UV detection at 220 nm (retention time 4.5 min). Thin-layer radiochromatography was done on silica gel plates (250 μm, 5 x 20 cm; Whatman, LK5) which were eluted with ethyl acetate and detected in an iodine chamber (R_f 0.23). The NMR spectrum of 2c corresponded to that of authentic 2-pyrrolidinone.

N-[^{14}C-Methyl]-2-pyrrolidinone (3a). A procedure using phase transfer catalysis was modified from that of Takahata (9). Into a 10 ml round bottom flask containing 2-pyrrolidinone (32 mg, 0.38 mmol) in anhydrous tetrahydrofuran (1 ml) were added tetrabutylammonium bromide (172 mg, 0.53 mmol) and potassium hydroxide (77 mg, 1.4 mmol) which had been pulverized in tetrahydrofuran. A 1.25 ml aliquot of [^{14}C]methyl iodide (0.218 mCi, 10.0 mCi/mmol) was withdrawn from a previously prepared stock solution (0.245 mCi/1.40 ml tetrahydrofuran) cooled in dry ice and was added to the above mixture, which was then magnetically stirred. Unlabeled methyl iodide (71 mg, 0.50 mmol) was

immediately added, the flask was sealed and stirring continued for 7 h (under a nitrogen atmosphere). The solvent and unreacted methyl iodide were distilled from the flask using a microdistillation system and were collected in a round bottom flask cooled in dry ice. Anhydrous ether and molecular sieves (3Å) were added to the distillation residue and the suspension was filtered under vacuum into a 5 ml round bottom flask, using a bell jar with side tubulation and a 2 ml fritted disc (4-5.5 μm) Buchner funnel. Evaporation of the ether under a gentle stream of nitrogen gas gave 3a: 30 mg, 0.30 mmol (78% yield); 0.164 mCi, 0.55 mCi/mmol (75% radiochemical yield).

Radiochemical purity of the product as determined by HPLC was >99% using acetonitrile/water (25:75, v/v) as the mobile phase with a flow rate of 1.0 ml/min and UV detection at 210 nm (retention time 5.2 min). Thin-layer radiochromatography (co-chromatographed with authentic material) was done on Analtech silica gel G plates which were eluted with chloroform/methanol/cyclohexane (18:2:1) and detected in an iodine chamber (R_f 0.54). Synthesis of unlabeled N-methyl-2-pyrrolidinone was carried out under exactly the same conditions and scale as described for 3a. NMR and IR were identical to that of authentic unlabeled material.

[3,4-^{3}H]-N-Methyl-2-pyrrolidinone (3b). Into a 10 ml round bottom flask containing 2b (124 mg, 1.46 mmol; 0.268 mCi, 0.184 mCi/mmol) in anhydrous tetrahydrofuran (2 ml) were added tetrabutylammonium bromide (322 mg, 1.0 mmol) and potassium hydroxide (112 mg, 2.0 mmol) which

had been pulverized in tetrahydrofuran. Methyl iodide (285 mg, 2.01 mmol) and a magnetic stirring bar were added to the mixture, the flask was sealed and stirring was begun at room temperature (under a nitrogen atmosphere). After 7 h the solvent was evaporated under a gentle stream of nitrogen gas. Anhydrous ether and molecular sieves (3Å) were added to the residue remaining in the flask and the mixture was filtered under vacuum into a 10 ml round bottom flask, using a bell jar with side tubulation and a 15 ml fritted disc (4-5.5 μm) Buchner funnel. Evaporation of the ether under a gentle stream of nitrogen gas gave a product which was subsequently found by HPLC to contain a small amount (6%) of the starting material as a radiochemical impurity.

Preparative thin-layer chromatography was accomplished on Whatman PLK5 1000 μm silica gel plates, 20 x 20 cm, in the following manner. The crude product was dissolved in 0.8 ml of methanol and 0.2 ml portions were applied to each of four TLC plates which were then developed in chloroform/methanol/cyclohexane (18:2:1). A thin-layer radiochromatogram scanner was used to identify the location of the desired radioactive species which was then scraped from the plate and extracted with methanol. Subsequent vacuum filtration into a 10 ml round bottom flask (using a bell jar with side tubulation and a 15 ml fritted disc Buchner funnel), followed by evaporation of the methanol under vacuum at room temperature, gave 3b: 105 mg, 1.06 mmol (73% yield); 0.109 mCi, 0.103 mCi/mmol (41% radiochemical yield).

Radiochemical purity of the product as determined by HPLC was >99% using methanol/water (20:80, v/v) as the mobile phase with a flow rate of 1.0 ml/min and UV detection at 210 nm (retention time 7.0 min). Thin-layer radiochromatography (co-chromatographed with authentic material) was done on Analtech silica gel G plates which were eluted with chloroform/methanol/cyclohexane (18:2:1) and detected in an iodine chamber (R_f 0.54).

[4-^{3}H]-N-Methyl-2-pyrrolidinone (3c). Tritium on the α-carbon of the lactam 3b was exchanged according to a procedure adapted from Matsuo (12). Into a 10 ml round bottom flask containing 3b (105 mg, 1.06 mmol; 0.109 mCi, 0.103 mCi/mmol) were added 1.25 ml of a freshly prepared 1.0 M solution of sodium methoxide in methanol. The solution was refluxed for 36 h at 65° and then allowed to cool to room temperature. Methanol (now tritiated) was distilled from the flask using a microdistillation system and collected in a round bottom flask cooled in dry ice. Anhydrous ether was added to the reaction flask and the resulting mixture was filtered under vacuum into a 10 ml round bottom flask, using a bell jar with side tubulation and a 15 ml fritted disc (4-5.5 μm) Buchner funnel. Evaporation of the ether under a gentle stream of nitrogen gas gave 3c: 74 mg, 0.75 mmol (71% yield); 0.033 mCi, 0.044 mCi/mmol (30% radiochemical yield).

Radiochemical purity of the product as determined by HPLC was >99% using the same conditions as described for 3b. An NMR spectrum of a similar exchange reaction done using sodium methoxide in methanol-d (CH_3OD) under exactly

the same conditions as described for 3c showed quantitative exchange of the protons adjacent to the carbonyl group of NMP by 36 h at 65°.

REFERENCES

1. GAF Corporation - M-Pyrol (N-Methyl-2-pyrrolidone) Handbook, New York, (1972)

2. Armstrong W. W. - U. S. Patent 4,086,332 (1978) to Pfizer, Inc.

3. Stoughton R. B., Harris R. L. and Dea F. J. - German Patent 2,615,140 (1976) to National Research Development Corporation

4. Hawkins, Jr. J. and Sarett L. H. - Clin. Chim. Acta 2: 481 (1957).

5. Callery P. S., Geelhaar L. A. and Stogniew M. - Biochem. Pharmacol. 27: 2061 (1978)

6. Callery P. S., Stogniew M. and Geelhaar L. A. - Biomed. Mass Spectrom. 6: 23 (1979)

7. Diurgea C. - In: Current Developments in Psychopharmacology (Essman W. B. and Valzelli L., eds.), Spectrum Publications, Inc., New York, Vol. 3, 221 (1976).

8. Dagne E. and Castagnoli, Jr. N. - J. Med. Chem. 15: 356 (1972).

9. Takahata H., Hashizume T. and Yamazaki T. - Heterocycles 12: 1449 (1979)

10. Kumarev V. P. and Almanov G. A. - Zh. Fiz. Khim. 49: 1361 (1975); Chem Abstr. 83: 206502n (1975)

11. Pellegata R., Pinza M. and Pifferi G. - Synthesis 8: 614 (1978)

12. Matsuo H., Kawazoe Y., Sato M., Ohnishi M. and Tatsuno T. - Chem. Pharm. Bull. 15: 391 (1967)