Synthesis of ^{14}C-Labeled Pramiracetam Hydrochloride, N-[2-(Bis[1-methylethyl]amino)ethyl]-2-oxo-1-pyrrolidine-acetamide-α-^{14}C Hydrochloride, CI-879 Hydrochloride[1]

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SUMMARY

Pramiracetam hydrochloride, N-[2-[bis(1-methylethyl)amino]ethyl]-2-oxo-1-pyrrolidineacetamide hydrochloride, CI-879, a new potential clinically useful cognition activator, was labeled with ^{14}C. Bromoacetic-2-^{14}C acid was converted to its ethyl ester which reacted with sodium 2-pyrrolidinone to yield ethyl 2-oxo-1-pyrrolidineacetate-α-^{14}C acid. Treatment of the latter with N,N-bis(1-methylethyl)-1,2-ethanediamine gave pramiracetam free base which was subsequently converted to the hydrochloride salt.

KEY WORDS: Pramiracetam hydrochloride, ^{14}C, N-[2-[bis(1-methylethyl)-amino]ethyl]-2-oxo-1-pyrrolidineacetamide hydrochloride, CI-879, senile dementia of the Alzheimer's type, cognition activator

INTRODUCTION

Cognition dysfunctions occur in persons of all ages as a result of many conditions including disease, accidents and injuries, developmental defects, and normal aging. An agent which would act favorably on learning and memory mechanisms would be beneficial in the treatment of learning disabilities, and

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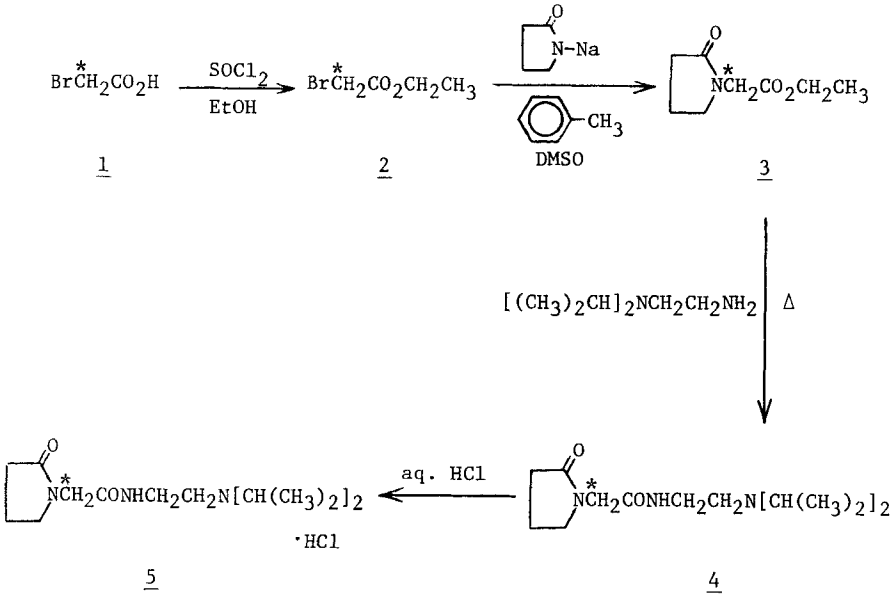
on the cognitive disorders and intellectual impairments which regularly occur in the elderly, particularly in senile dementia of the Alzheimer's type.

In a search for such an agent, our drug discovery program synthesized new N-(substituted aminoalkyl)-2-oxo-1-pyrrolidineacetamides and evaluated them in an electroconvulsive shock-induced amnesia reversal test procedure.[2,3,4]

Pramiracetam sulfate, CI-879 sulfate, N-[2-[bis(1-methylethyl)amino]-ethyl]-2-oxo-1-pyrrolidineacetamide sulfate, a highly active member of the series, was chosen for further testing. It demonstrated a wide margin of safety in animal species and in normal healthy volunteers. It has shown encouraging activity for "goal directed behavior" in a non-blinded trial in patients with primary degenerative dementia (senile dementia of the Alzheimer's type).[5]

CI-879 was labeled with carbon-14 for use in metabolic and pharmacokinetic studies. The carbon-14 was readily incorporated into the α-position of the acetamide using commercially available bromoacetic-2-^{14}C acid as the starting material as shown in Scheme I.



1 2 3 4 5

Scheme I

The carbon-14 label in CI-879-^{14}C·HCl (5) was presumably metabolically stable and, therefore, suitable for *in vivo* studies; studies using high doses of unlabeled drug had shown that the bulk of the material was recovered unchanged in the urine.[6]

RESULTS AND DISCUSSION

Bromoacetic-2-^{14}C acid (1) was converted to the corresponding acid chloride with thionyl chloride and subsequently, to the ethyl ester 2. Treatment of the ester 2 with the sodium salt of 2-pyrrolidinone, formed by reaction with sodium hydride in toluene containing DMSO, gave 3. Heating a mixture of the ester 3 and excess N,N-bis(1-methylethyl)-1,2-ethanediamine gave the amide 4 which was subsequently converted to its hydrochloride salt 5. The purification of 5 was accomplished by crystallization in toluene/acetonitrile. Although the synthesis was straightforward, the purification of the final product 5 proved difficult because of oiling-out problems, resulting in only a 19% radiochemical yield for that step; a subsequent purification of the mother liquor, producing the corresponding sulfate salt, resulted in an additional 28% radiochemical yield for that step.

EXPERIMENTAL

Bromoacetic-2-^{14}C acid with a specific activity of 25.2 mCi/mmol was purchased from the Amersham Corporation. N,N-Bis-(1-methylethyl)-1,2-ethanediamine was obtained from BASF, W. Germany; 2-pyrrolidinone, from Aldrich Chemical Company. The unlabeled intermediates and final product, used as references for comparison with the corresponding labeled compounds, were supplied by the Chemical Development Group of Warner-Lambert/Parke-Davis, Ann Arbor, Michigan.

Melting points were determined with a Thomas Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were obtained on a Nicolet MX-1 Spectrophotometer.

HPLC analyses were performed on a system consisting of an Alltech silica gel column (10 μ), an Altex 110A pump and a DuPont UV detector (215 nm) using a mobile phase of MeOH:H_2O (70:30) and 0.005M tetrabutylammonium hydroxide. A flow rate of 1 ml/min gave a t_R value for reference pramiracetam of five minutes; for ethyl 2-oxo-1-pyrrolidineacetate, 4.25 minutes.

GC analyses were done on 3% OV-17 gas chrom Q, 100-120 mesh, using nitrogen at a flow rate of 20 ml/min and a column program of 100-300°C at 10°C/min. The t_R value for reference pramiracetam was 16 minutes; for ethyl 2-oxo-1-pyrrolidineacetate, 8.5 minutes; for N,N-bis(1-methylethyl)-1,2-ethanediamine, 1.8 minutes.

Thin layer chromatography (TLC) was done using Analtech silica gel plates (250 μ); sections were scraped, and slurried in methanol and cocktail for liquid scintillation counting.

All chromatographic analyses (LC, GC, and TLC), used for determining radiochemical or chemical purity, included a comparison to reference standards.

Liquid scintillation counting was done with a Packard Model 3003 Liquid Scintillation Spectrometer using Beckman Ready-Solv MP liquid scintillation cocktail.

Ethyl 2-Oxo-1-pyrrolidineacetate-α-^{14}C, (3). Sodium hydride (50% in mineral oil, 344 mg unwashed, 7.2 mmol) was added to a 50 mL flask, flushed with N_2; 5 mL toluene was added; a catalytic quantity of DMSO (75 μL) was added to the resulting slurry. 2-Pyrrolidinone (0.455 mL, 6 mmol) in 5 mL toluene was added dropwise to the slurry. The resulting reaction mixture was stirred under nitrogen at 60°C for four hours as hydrogen was evolved. Ethyl bromoacetate-2-^{14}C (9.7 mCi, 1.478 mCi/mmol, 6.6 mmol), which had been prepared from bromoacetic-2-^{14}C acid in 74% radiochemical yield by the acetyl chloride using thionyl chloride, was diluted with 5 ml toluene and added dropwise to the stirred slurry maintained at 45°C. Stirring was continued overnight. The reaction mixture was filtered. The yellow-orange

filtrate contained 7.9 mCi and was 91% radiochemically pure by LC analysis; no unreacted precursor was detected. The toluene was removed *in vacuo*. The product was distilled *in vacuo* with a heat assist to a short path distillation head using an electrical heating ribbon. The distillate product contained 7.5 mCi (77% radiochemical yield).

N-[2-[Bis(1-methylethyl)amino]ethyl]-2-oxo-1-pyrrolidineacetamide-α-^{14}C, (4). The ethyl 2-oxo-1-pyrrolidineacetate-2-^{14}C (3) (7.5 mCi, 5 mmol) from the previous step was transfered to a 10 mL flask flushed with N_2. N,N-Bis(1-methylethyl)-1,2-ethanediamine (1.32 mL, 7.6 mmol) was added. The reaction mixture was heated to 115°C with stirring while maintaining N_2 pressure; the ethanol distillate was collected. The reaction mixture was stirred overnight at 140°C. The unreacted precursors were distilled *in vacuo* at 125°C. The product was 78% radiochemically pure and contained 21% of the ^{14}C-labeled precursor by LC analysis. GC analysis confirmed the absence of the diamine. The product contained 5.4 mCi (72% radiochemical yield).

N-[2-[Bis(1-methylethyl)amino]ethyl]-2-oxo-1-pyrrolidineacetamide-α-^{14}C Hydrochloride, (5). The crude product of the previous step was dissolved in distilled water. The resulting deep amber solution was treated with Darco G-60, stirring the slurry at room temperature for two hours followed by filtration through a Celite bed. The Darco treatment was repeated twice. The pH of the resulting yellow filtrate was adjusted to pH 2 with 6N HCl and was washed twice with diethyl ether. The water was evaporated *in vacuo* to give an amber oil which was triturated with anhydrous diethyl ether. The oil was dissolved in 2-propanol maintained at 60°C; hot n-hexane was added to the haze point. The product oiled out and was stirred in an ice bath resulting in a tan solid (2.7 mCi). Dissolution in hot toluene with the subsequent addition of acetonitrile followed by cooling and seeding resulted in a crystalline, tan colored final product (1 mCi; 229 mg; 1.5 mCi/mmol) (19% radiochemical yield). The radiochemical purity was > 96% by TLC analysis (CH_2Cl_2:MeOH:NH_4OH:H_2O

[60:40:1:20] lower phase, R_f = 0.52; toluene:EtOAc:Et_2NH [7:2:1], R_f = 0.32; CH_2Cl_2:Acetone:MeOH:NH_4OH [70:20:10:1], R_f = 0.23), and 99% by HPLC analysis. The chemical purity was 100% (GC); the IR spectrum was identical to a reference standard of pramiracetam except for the presence of water. IR (KBr): 3435 and 3427 (H_2O), 3259 (N-H), 2400-2800 (multiple, N-H salt), 1690 (C = O), 1668 (C = O) cm^{-1}. Melting point: 141-146.5°C (Reference standard, 151-151.5°C). Analysis calc'd for $C_{14}H_{27}N_3O_2 \cdot 1/2H_2O \cdot HCl$: C, 53.40; H, 9.28; N, 13.35. Found: C, 53.68; H, 9.86; N, 13.50.

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