SYNTHESIS OF [$^2H_5$]ARECOLINE FOR USE AS INTERNAL STANDARD IN MASS SPECTROMETRIC ASSAY

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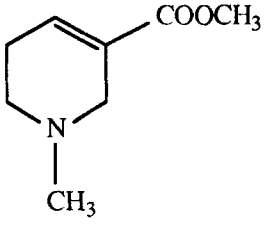
ABSTRACT

Five deuterium atoms, three in the *N*-methyl group and one each in positions 2 and 6, were incorporated into the cholinergic agonist, arecoline (1), methyl 1-methyl-1,2,5,6-tetrahydropyridine-3-carboxylate, with 96% efficiency. Trideuterated pyridinium iodide (2) was reduced with sodium borodeuteride or sodium cyanoborodeuteride in acidic medium to yield [$^2H_5$]arecoline (3). Significantly greater deuterium incorporation occurred when 2 was reduced with sodium cyanoborodeuteride. This reduction process is discussed. The synthesized labelled compound was determined to be a suitable internal standard for mass spectrometric assays.

Key words: arecoline, 1,2,5,6-tetrahydropyridines, deuterium labelling, reduction.

INTRODUCTION

Alzheimer's disease is accompanied by structural and functional impairments of forebrain cholinergic systems.[1-4] Because these abnormalities have been found to correlate with severity of dementia,[5] cholinergic supplementation with an appropriate agonist may improve cognitive function of Alzheimer's patients. Arecoline (1), the principal alkaloid of *Areca catechu*, is a



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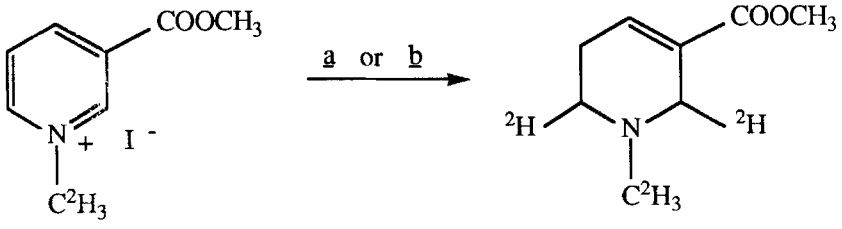
cholinergic agonist that readily crosses the blood-brain barrier. Although the alkaloid has been reported to enhance memory in normal healthy subjects,[6] its value in treating Alzheimer's patients remains uncertain,[7] due potentially to a failure to achieve and maintain a pharmacologically adequate concentration. Plasma and cerebrospinal fluid monitoring of cholinergic agents in treating Alzheimer's patients are integral parts of clinical studies initiated in our laboratory. This has prompted the development of an analytical procedure to determine arecoline, of sufficient sensitivity for detection in humans at clinical doses. A gas chromatography-mass spectrometric (GC-MS) assay procedure utilizing a deuterium labelled analog and a homolog of arecoline as internal standards, is currently under development. Two synthetic procedures to deuterium label arecoline and other 1,2,5,6-tetrahydropyridines are described in this paper.

RESULTS AND DISCUSSION

A deuterium labelled analog of arecoline, free of potentially interfering protio form and unchanged in chromatographic characteristics, would be a suitable internal standard to perform GC-MS quantitation of arecoline, especially at picogram levels. Among the few synthetic schemes available for arecoline, the method of reduction with potassium or sodium borohydride of 3-

Scheme I



2 3

Reagents: a NaB^2H_4, CH_3OH; b NaB^2H_3CN, CH_3COOH, CH_3OH.

methoxycarbonylpyridinium iodide[8,9] would be potentially useful for optimal incorporation of deuterium into the molecule. Thus, to deuterium label arecoline, methyl pyridine-3-carboxylate was first quaternized with [$^2H_3$]iodomethane and the intermediate pyridinium iodide, 2 (Scheme I), was then reduced with sodium borodeuteride in methanol. The labelled product,

[$^2H_5$]arecoline (3), was recovered by microdistillation, in a moderate yield (36%) similar to that obtained previously for the protio analog.[8,9]

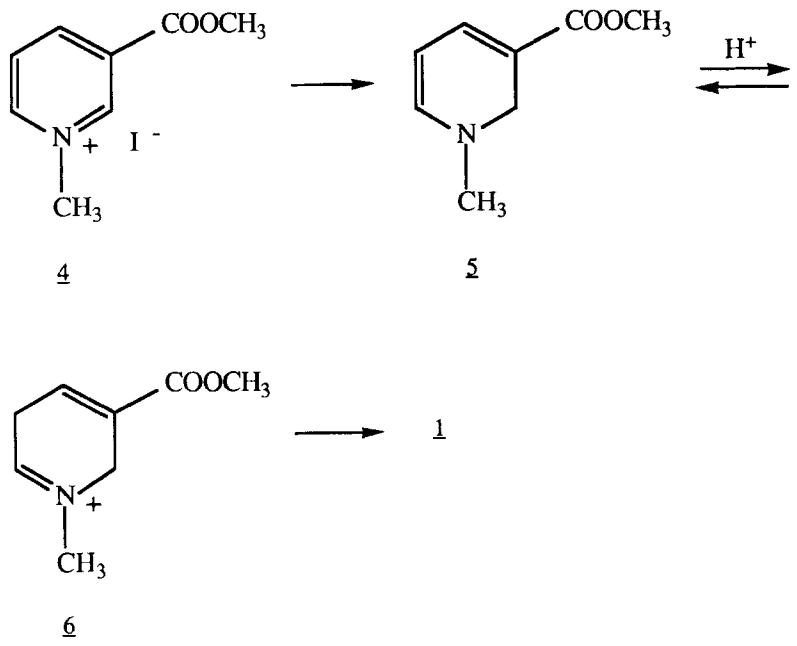
When the above reduction was carried out in CH_3O^2H for the synthesis of [$^2H_6$]arecoline, a significant amount of [$^2H_7$]- analog was produced. That this product was a result of simple deuterium exchange in alkaline reaction medium, was inferred by stirring the protio arecoline in 1M NaOH solution in CH_3O^2H. Mass spectrometric analysis of the recovered arecoline indicated 20-30% incorporation of one deuterium atom into the tetrahydropyridine ring, although the exact site of deuteration was not determined.

Studies on the mechanism of borohydride reduction of pyridinium ions in protic media suggest that 1,2-dihydropyridines are obligatory intermediates in the reaction leading to the formation of 1,2,5,6-tetrahydropyridines.[10-14] For instance, for 1,2-dihydropyridine 5 (Scheme II), having an 'enamine' character, protonation at position 5, either directly or through electrophilic borane activation, would generate immonium cation, 6. Borohydride reduction of this intermediate is easy, resulting 1,2,5,6-tetrahydropyridine (1). Because enamines are readily converted to more stable immonium ions in acidic medium,[15] reduction of pyridinium ions, quite possibly to 1,2,5,6-tetrahydropyridines *via* 1,2-dihydropyridine intermediates, can conveniently be accomplished in acidic medium as well, using sodium cyanoborohydride. Indeed, the pyridinium ion 4 (Scheme II) could be reduced to the tetrahydropyridine, arecoline (1), by sodium cyanoborohydride in acidic medium. Interestingly, the same reduction reaction did not proceed to the tetrahydropyridine stage when carried out in neutral protic medium, differing from the sodium borohydride reduction. The reaction product, probably a mixture of 1,2- and 1,6-dihydropyridines,[11,16] was unstable and was not characterized.

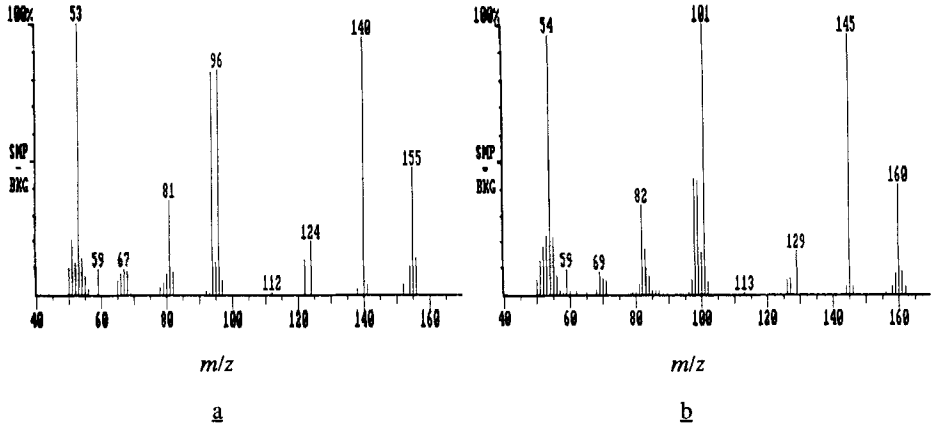
Arecoline also was labelled by this new reduction procedure. By analogous reduction of the pyridinium ion, 2 (Scheme I), with sodium cyanoborodeuteride in methanol acidified with acetic acid, [$^2H_5$]arecoline (3) with a superior isotopic purity was obtained. The product was found to be 96% [$^2H_5$]- analog, in contrast with 80% purity obtained by sodium borodeuteride reduction. Clearly, sodium cyanoborodeuteride is a preferred reducing agent to label arecoline and 1,2,5,6-tetrahydropyridines in general.

Gas chromatography-electron impact mass spectrometric analyses were performed on arecoline and on the synthesized [$^2H_5$]arecoline. Their recorded electron impact mass spectra are shown in Figure 1; molecular ions are *m/z* 155 and 160 for arecoline and [$^2H_5$]arecoline, respectively. Isotopic purity was determined by a selected ion monitoring technique, using *m/z*

Scheme II



140 ion in the mass spectrum of arecoline as the determinant ion. At the same time, the presence of $[^2H_0]$- analog was checked and none was found present. Further, as it coelutes with arecoline in a capillary GC setup, the synthesized $[^2H_5]$arecoline is a useful internal standard for a sensitive mass spectrometric assay of arecoline.



a b

Figure 1. Electron impact mass spectra: a, arecoline (1) and b, $[^2H_5]$arecoline (3).

EXPERIMENTAL SECTION

All reactions were carried out under a dry argon atmosphere. Sodium borodeuteride (>99% ^2H) and sodium cyanoborodeuteride (>98% ^2H) were purchased from Fluka Chemical Corporation and Aldrich Chemical Company Inc., respectively, and [$^2H_3$]iodomethane was obtained from MSD Isotopes, Canada. Analytical thin layer chromatography (TLC) was performed on Whatman PE SIL/UV (0.25 mm thickness) plates and spots were detected by a UV lamp or by iodine vapors. Flash chromatography[17] was carried out with Kieselgel 60 (230-400 mesh ASTM, EM Science). Melting points were determined on an Electrothermal Digital Melting Point Apparatus and are uncorrected. Microdistillation was carried out in a Kontes Single Collar Microdistillation column; the collar was cooled by a jacket of dry ice. High-field ^1H NMR was recorded at 300 MHz on an Varian VXR-300 spectrometer. Chemical shifts are expressed in terms of δ, relative to the position of the internal standard, tetramethylsilane (δ 0.0). Notations used to describe the splitting patterns are s = singlet, d = doublet and m = multiplet. Gas chromatography-electron impact mass spectrometric (GC-MS) analysis was performed on a Finnigan Mat 700 Ion Trap Detector (ITD^M) interfaced with a Perkin-Elmer Sigma 2000 gas chromatograph. The gas chromatograph consisted of a J & W DB-5 fused-silica capillary column of 30 m x 0.25 mm dimension and 0.25 μm film thickness. Helium, flowing at a linear velocity of 30 cm/sec, was the carrier gas. The injector temperature was maintained at 170 °C. An initial oven temperature was set at 50 °C, the injected sample was "cold trapped" for 30 sec, and thereafter the oven temperature was ramped at 20 °C/min to a final temperature of 160 °C. A split vent flow of 44 ml/min and a septum purge flow of 4 ml/min were allowed.

The reactions described below were also carried out with the protio compounds.

3-Methoxycarbonyl-1-[$^2H_3$]methylpyridinium iodide (2). To a solution of methyl pyridine-3-carboxylate (2.743 g, 20 mmol) in dry *n*-propanol (10 ml) was added [$^2H_3$]iodomethane (2.899 g, 20 mmol) dropwise at 4 °C, with continuous stirring. The resulting reaction mixture was heated to 60 °C for 30 min, and left at room temperature overnight. A yellow crystalline product was separated from the reaction mixture. Dry ether (10 ml) was added, and the mixture was stirred and filtered. The crude product was recrystallized from *n*-propanol:*n*-hexane (80:20) mixture and dried *in vacuo*, yielding yellow colored fine crystals, 4.160 g (74%), mp 134-136 °C (protio form 124.5-126.5 °C[18]): ^1H NMR ($C^2H_3O^2H$) δ 4.83 (s, 3, $COOCH_3$), 8.23 (m, 1, ArH-5), 9.04 (m, 1, ArH-4), 9.13 (d, J = 6.1 Hz, 1, ArH-6) and 9.51 (s, 1, ArH-2).

Methyl 1-[$^2H_3$]methyl-1,2,5,6-[2,6-$^2H_2$]tetrahydropyridine-3-carboxylate [[$^2H_5$]Arecoline] (3). (a) Sodium borodeuteride reduction. A solution of 2 (2.821 g, 10 mmol) in dry methanol (50 ml) was cooled to 0 °C and then sodium borodeuteride (628 mg, 15 mmol) was added in small portions with vigorous stirring. When evolution of gas subsided, the reaction mixture was allowed to reach room temperature and stirred for a further 15 min. It then was poured into a cooled (4 °C) solution of HCl (5% w/v, 20 ml) with stirring. The acidified mixture was rotoevaporated to approximately one third of its volume. Following addition of solid Na_2CO_3 and adjusting pH to 9.0, the mixture was extracted with ether (50 ml). The organic layer was separated and the aqueous layer was saturated with NaCl and extracted again with ether (25 ml x 4). The ether layers were combined, dried (Na_2SO_4) and filtered. Upon removal of ether, a product was obtained as yellow oil, which was purified by microdistillation (3 mm Hg pressure, 90 °C bath temperature). A colorless oil, 589 mg (36%), TLC (ethyl acetate:methanol:triethylamine::70:30:1 v/v) showed a single spot which co-eluted with arecoline, Rf 0.42: ^1H NMR (C^2HCl_3) δ 2.37 (m, 2, tetrahydropyridine H-5), 2.46 (m, 1, tetrahydropyridine H-6), 3.13 (m, 1, tetrahydropyridine H-2), 3.74 (s, 3, OCH_3) and 7.01 (m, 1, tetrahydropyridine H-4). The free base was stored under argon at 0 °C.

(b) Sodium cyanoborodeuteride reduction. Pyridinium iodide, 2 (564 mg, 2 mmol), was dissolved in methanol (10 ml) and cooled in an ice bath. Sodium cyanoborodeuteride (119 mg, 1.8 mmol) was added in small portions to this solution and stirred for 15 min. Then, acetic acid (0.5 ml) was gradually added and the reaction mixture was stirred overnight at room temperature. Most of the solvent was removed by rotoevaporation, and the residue was dissolved in water (10 ml). The aqueous solution was made basic (Na_2CO_3), saturated with NaCl and extracted with ether (20 ml x 4). Ether layers were combined, dried (Na_2SO_4), filtered and rotoevaporated. The crude product was purified by flash chromatography: 2 x 30 cm column, eluting with methylene chloride:methanol:triethylamine::97:3:0.1 v/v. Approximately 4 ml fractions were collected and were checked by TLC (methyene chloride:methanol:triethylamine::90:10:0.5 v/v, Rf 0.56). Fractions containing the labelled arecoline were pooled and rotoevaporated. A pale yellow oil, 210 mg (38 %) was obtained: ^1H NMR, as above.

[$^2H_5$]Arecoline hydrobromide. The base 3 (500 mg, 3.12 mmol) was dissolved in dry ether (10 ml), cooled (0 °C) and ethereal HBr (10% w/v, freshly prepared) was added dropwise with stirring until the complete salt was precipitated. The solvent and excess HBr were removed

by evaporation *in vacuo*, leaving a white powder. Recrystallization from *n*-propanol:*n*-hexane (80:20) mixture yielded fine needles, filtered and dried under vacuum, 594 mg (79% recovery), mp 175-176 °C (protio 172-173 °C[19]).

Mass spectral analyses. Solutions (5 ng/µl) of arecoline (1) and of [$^2H_5$]arecoline (3) were prepared in *n*-hexane containing 1% triethylamine. A 1 µl sample of each solution was injected into the GC-MS setup. A retention time of 8 min 7 sec was noted for the compounds. For the determination of isotopic purity, a range of ions, *m/z* 140 to 146, was monitored in the multiple ion detection mode of the mass spectrometer. The labelled product obtained by sodium borodeuteride reduction procedure was found to be of [$^2H_5$]- (80%), [$^2H_4$]- (18%) and [$^2H_3$]- (1.4%) analogs and in those proportions, respectively. Similar determination of the product of sodium cyanoborodeuteride reduction was found to contain 96% of [$^2H_5$]- and 4% of [$^2H_4$]- analogs.

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