Synthesis of (*R,S*)-[2,3-$^{13}C_2$]-1-(1'-Methyl-2'-pyrrolidinyl)propan-2-one; {(*R,S*)-[2',3'-$^{13}C_2$]Hygrine}

Timothy W. Abraham*,# and Edward Leete†

Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455, U. S. A.

SUMMARY

2-Ethoxy-1-methyl-5-pyrrolidinone (**1**) was reacted with ethyl [3,4-$^{13}C_2$]-acetoacetate (**2**) in the presence of $TiCl_4$ to give ethyl [3,4-$^{13}C_2$]-2-(1'-methyl-5'-oxo-2'-pyrrolidinyl)-3-oxobutanoate (**3**) in 85% yield. Decarboethoxylation of ethyl [3,4-$^{13}C_2$]-2-(1'-methyl-5'-oxo-2'-pyrrolidinyl)-3-oxobutanoate (**3**) was accomplished using NaCl and H_2O in DMSO to give (*R,S*)-[2,3-$^{13}C_2$]-1-(1'-methyl-5'-oxo-2'-pyrrolidinyl)propan-2-one (**4**) in 91% yield. Protection of the ketone as a ketal (ethylene glycol, H^+), followed by reduction of the amide to the amine using $LiAlH_4$ and subsequent deprotection of the ketal gave (*R,S*)-[2,3-$^{13}C_2$]-1-(1'-methyl-2'-pyrrolidinyl)propan-2-one ((*R,S*)-[2',3'-$^{13}C_2$]Hygrine) (**8**) in 78% yield. (61% overall yield from ethyl [3,4-$^{13}C_2$]acetoacetate).

Key Words : Biosynthesis, Tropane alkaloids, Hygrine.

INTRODUCTION

The biosynthesis of the tropane alkaloids, (-)-hyoscyamine and (-)-scopolamine have been extensively studied over the last several decades. 1-(1'-Methyl-2'-pyrrolidinyl)propan-2-one (Hygrine) has been shown to be a precursor of the tropine moiety of (-)-hyoscyamine and other tropane alkaloids.[1-2] O'Donovan and Keogh[1] fed (*R,S*)-[*N-methyl*-^{14}C,2'-^{14}C]hygrine to *Datura stramonium* plants and obtained an absolute incorporation of 2.1% into (-)-hyoscyamine. McGaw and Woolley[2] reported that the incorporation of (2*R*)-[2'-^{14}C]hygrine into the tropane alkaloids in *Datura innoxia* was 2.5 to 10 times higher than the incorporation of (2*S*)-[2'-^{14}C]hygrine.

Present address : Department of Medicinal Chemistry, University of Minnesota, Minneapolis.

† Deceased February 1992.

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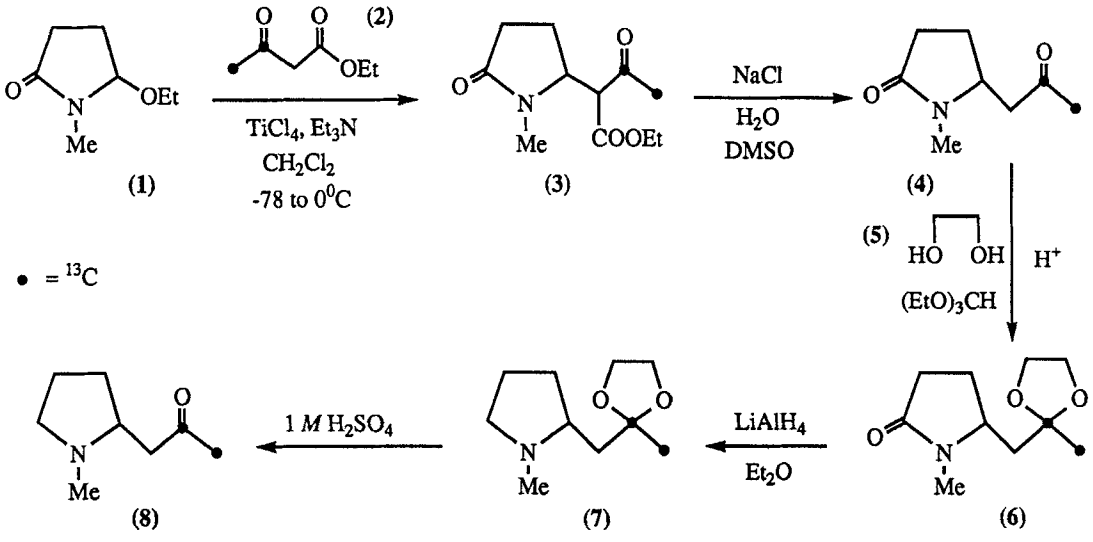
However, recent investigations have not only questioned the preference for the (*R*)-isomer of hygrine over the (*S*)-isomer, but have also cast doubts on the role of hygrine as a precursor of (-)-hyoscyamine and (-)-scopolamine.[3-5] In 1990, Sankawa *et al.*[3] reported the non-stereospecific incorporation of sodium [1,2-$^{13}C_2$]acetate into (-)-hyoscyamine and (-)-6β-hydroxyhyoscyamine in *Hyoscyamus albus*. This indicated that both enantiomers of hygrine and/or other intermediates were being utilized for formation of the tropane ring. Subsequently, in a similar study Hemscheidt and Spenser[4] demonstrated the non-regiospecific incorporation of sodium [1,2-$^{13}C_2$]acetate into 6β-hydroxytropine in *Datura stramonium*. Also, the results from feeding sodium [1,2,3,4-$^{13}C_4$]acetoacetate[4] indicated that acetoacetate was being cleaved to acetate before being incorporated into 6β-hydroxytropine. In addition to this, *N*-methyl-Δ^1-[2-^{2}H]pyrrolinium chloride labelled both C-1 and C-5 of 6β-hydroxytropine equally,[4] indicating that no chiral intermediate was involved in the formation of the tropane ring. Thus, it was suggested[4] that both enantiomers of *N*-methylpyrrolidineacetoacetate (or of hygrine) were being incorporated into the tropane ring. Also, numerous feeding experiments using labelled hygrine[5] did not show good incorporations into the tropane alkaloids.

RESULTS

Our interest[5] in attempting to resolve this discrepancy led us to synthesize hygrine which was doubly labelled with ^{13}C in the side chain. Initial attempts to synthesize labelled hygrine using the traditional method of reacting *N*-methyl-Δ^1-pyrrolinium salt with sodium acetoacetate[6] gave poor yields. The method that proved successful is based on methodology developed by Speckamp.[7-10]

2-Ethoxy-1-methyl-5-pyrrolidinone (**1**) was synthesized from *N*-methylsuccinimide as previously described.[7] 2-Ethoxy-1-methyl-5-pyrrolidinone (**1**) was then reacted with ethyl [3,4-$^{13}C_2$]acetoacetate (**2**) in the presence of $TiCl_4$ at -78 °C to give ethyl [3,4-$^{13}C_2$]-2-(1'-Methyl-5'-oxo-2'-pyrrolidinyl)-3-oxobutanoate (**3**) in 85% yield.[7-10] The carboethoxy group was cleaved using the Krapcho method[11] by heating ethyl [3,4-$^{13}C_2$]-2-(1'-Methyl-5'-oxo-2'-pyrrolidinyl)-3-oxobutanoate (**3**) with NaCl and H_2O in DMSO at 150 °C to give (*R,S*)-[2,3-$^{13}C_2$]-1-(1'-Methyl-5'-oxo-2'-pyrrolidinyl)propan-2-one (**4**) in 91% yield. (*R,S*)-[2,3-$^{13}C_2$]-1-(1'-Methyl-5'-oxo-2'-pyrrolidinyl)propan-2-one (**4**) was then converted to (*R,S*)-[2,3-$^{13}C_2$]-1-(1'-Methyl-2'-pyrrolidinyl)propan-2-one ((*R,S*)-[2',3'-$^{13}C_2$]Hygrine) (**8**) by a three step sequence[10] without



Synthesis of (*R,S*)-[2',3'-$^{13}C_2$]Hygrine.

purification of the intermediates. First, the ketone of (*R,S*)-[2,3-$^{13}C_2$]-1-(1'-Methyl-5'-oxo-2'-pyrrolidinyl)propan-2-one (**4**) was protected as a ketal using ethylene glycol (**5**) and *para*-toluenesulfonic acid. This was followed by reduction of the amide (**6**) to the amine (**7**) using $LiAlH_4$ in Et_2O. Subsequently, deprotection of the ketal with 1 *M* H_2SO_4 gave (*R,S*)-[2,3-$^{13}C_2$]-1-(1'-Methyl-2'-pyrrolidinyl)propan-2-one ((*R,S*)-[2',3'-$^{13}C_2$]Hygrine) (**8**) in 78% yield for the three steps. (61% overall yield from ethyl [3,4-$^{13}C_2$]acetoacetate). This synthetic scheme can also be used for the synthesis of radiolabelled (^{14}C) hygrine.

EXPERIMENTAL

Materials & Methods :

Ethyl [3,4-$^{13}C_2$]acetoacetate (99 atom % ^{13}C) was purchased from Isotec Inc. Elemental Analyses were performed by M-H-W Laboratories. Electron impact (EI) mass spectra were obtained on an AEI MS-30 direct inlet mass spectrometer. NMR spectra were obtained on Brüker NR-300 and Varian VXR-300 spectrometers. All ^1H-NMR spectra were obtained at 300 MHz and all ^{13}C-NMR spectra were obtained at 75 MHz. Chemical shifts are referenced internally relative to the solvent used. Infrared spectra were obtained on a Beckman Acculab 1, Perkin Elmer 1600 series FTIR or a Mattson Sirius 100 spectrophotometer. Gas-Liquid Chromatography (GLC) was

performed on a Hewlett-Packard 5890A gas chromatograph equipped with a Hewlett-Packard 3392A integrator, a flame ionization detector and a 25 m, 0.31 mm internal diameter fused silica capillary column internally coated with a 0.52 μm layer of HP-1 (100% dimethyl-polysiloxane). A helium flow rate of 1.3 mL/min was used along with the following temperature gradient program. Initial temperature of 50 °C was held for 4 minutes, followed by a ramp of 30 °C/min to 250 °C. The final temperature was held for 19.34 minutes to give a total run time of 30 minutes. Analytical TLC was performed on Machery-Nagel Polygram Sil G/UV254 0.2 mm silica gel coated plastic plates. Column chromatography was performed using 60-200 mesh Baker silica gel. Flash chromatography was performed as described by Still et al. (J. Org. Chem. 43: 2923-2925 (1978)) using Silica gel 60 (230-400 mesh) purchased from EM Reagents. CH_2Cl_2 and Et_2O were distilled under N_2 from CaH_2. Triethylamine was distilled from calcium hydride and stored over 3Å molecular sieves. Concentration under reduced pressure refers to solvent removal on a Büchi rotary evaporator.

Synthesis :

Ethyl [3,4-$^{13}C_2$]-2-(1'-Methyl-5'-oxo-2'-pyrrolidinyl)-3-oxobutanoate (3).

A solution of 2-ethoxy-1-methyl-5-pyrrolidinone (**1**) (1.668 g, 11.7 mmol) in dry CH_2Cl_2 (100 mL), under nitrogen, was cooled to -78 °C in a dry-ice/acetone bath. Titanium tetrachloride (1.3 mL, 11.8 mmol) was added slowly via syringe and the mixture stirred for 30 minutes. The solution turned bright yellow in color. Then a mixture of ethyl [3,4-$^{13}C_2$]acetoacetate (**2**) (1 g, 7.7 mmol), triethylamine (1.1 mL, 7.9 mmol) and dry CH_2Cl_2 (3 mL) was added in two portions via syringe. The solution turned dark orange to red in color. The resulting mixture was stirred at -78°C for 45 minutes, then allowed to warm to ~0 °C and stirred in an ice-bath for an additional 4 hours. Brine (50 mL) was then added, the ice-bath removed and the reaction mixture stirred for 15 mins. The two layers were separated and the aqueous phase extracted with CH_2Cl_2 (3 x 25 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the crude product. Column chromatography on silica gel, starting with 100% $CHCl_3$, then using $CHCl_3$:MeOH:NH_4OH (99:1:0.1), and finally $CHCl_3$:MeOH:NH_4OH (98:2:0.1) gave the product as a colorless liquid (**3**) (1.502 g, 85% yield, mixture of diastereoisomers not separated). GLC-r.t. = 12.25 and 12.36 mins. ^{13}C-NMR ($CDCl_3$, labelled): δ 201.98 (d, C-3, $^1J_{3-4}$ = 42.7 Hz), 30.13 (2 overlapping d, C-4, $^1J_{4-3}$ = 42.6 Hz). ^{13}C-NMR ($CDCl_3$, unlabelled, diastereoisomers): δ 200.9/200.8 (C-3), 175.2/175.1 (C-1), 167.7/167.6 (C-5'), 62.3/60.7 (C-2), 62.0/61.9 ($O\underline{C}H_2CH_3$), 58.9/58.8 (C-2'), 30.4/29.9 (C-4), 29.4/29.3 (C-4'), 28.9/28.3 (N-Me), 22.7/21.2 (C-3'), 14.0 ($OCH_2\underline{C}H_3$). ^{1}H-NMR ($CDCl_3$,

diastereoisomers): δ 4.1 (m, 3H, $OC\underline{H}_2CH_3$ and H-2'), 3.6 (two t, 1H, H-2, $^2J_{1H\text{-}13C}$ ≈5.7 Hz), 2.6 (s, 3H, N-Me), 2.15 (m, 3H, H-4, $^1J_{1H\text{-}13C}$ = 132 Hz), 2.6-1.5 (m, 4H, H-3' and H-4'), 1.25 (dt, 3H, $OCH_2C\underline{H}_3$). IR: cm^{-1} 1734 (ester C=O), 1711 (ketone C=O), 1690 (amide C=O). MS-EI (unlabelled) m/e (relative intensity): 227 (0.07), 184 (0.01), 154 (26.5), 138 (7.7), 112 (12.6), 98 (100). Elemental analysis (unlabelled) Found: 57.86% C, 7.47% H, 6.33% N. $C_{11}H_{17}NO_4$ requires: 58.14% C, 7.54% H, 6.16% N.

(*R,S*)-[2,3-$^{13}C_2$]-1-(1'-Methyl-5'-oxo-2'-pyrrolidinyl)propan-2-one (4).

In a flask equipped with a stir bar and a reflux condenser was placed ethyl [3,4-$^{13}C_2$]-2-(1'-methyl-5'-oxo-2'-pyrrolidinyl)-3-oxobutanoate (**3**) (1.502 g, 6.6 mmol), NaCl (423 mg, 7.2 mmol), deionized H_2O (0.36 mL, 20 mmol) and DMSO (50 mL). This mixture was heated at 150 °C, under N_2, for 5 hours. After cooling to room temperature, 2.5 *N* aqueous NaOH (25 mL) was added and the mixture extracted with CH_2Cl_2 (4 x 30 mL). The combined organic extracts were washed with deionized water (50 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the crude product containing traces of DMSO. Column chromatography on silica gel eluting with a gradient from 100% $CHCl_3$ to $CHCl_3$:MeOH:NH_4OH (90:10:0.25) gave the product as a colorless liquid (**4**) (0.933 g, 91% yield). GLC-r.t. = 10.76 mins. ^{13}C-NMR ($CDCl_3$, labelled): δ 205.9 (d, C-2, $^1J_{13C\text{-}13C}$ = 40.9 Hz), 30.8 (d, C-3, $^1J_{13C\text{-}13C}$ = 41 Hz). ^{13}C-NMR ($CDCl_3$, unlabelled): δ 205.8 (C-2), 174.7 (C-5'), 55.6 (C-2'), 47.1 (C-1), 30.6 (C-3), 29.6 (C-4'), 27.7 (NMe), 24.8 (C-3'). ^1H-NMR ($CDCl_3$)[10]: δ 3.9 (m, 1H, H-2'), 2.9 (m, 1H, H-1), 2.7 (s, 3H, N-Me), 2.2 (dd, 3H, H-3, $^1J_{1H\text{-}13C}$ = 126 Hz, $^2J_{1H\text{-}13C}$ = 5.9 Hz), 2.6-2.1 (m, 4H, H-1, H-3', H-4'), 1.5 (m, 1H, H-3'). IR: cm^{-1} 1708 (ketone C=O), 1682 (amide C=O). MS-EI (unlabelled) m/e (relative intensity): 155 (5.1) 140 (2.6), 127 (2.1), 112 (6.9), 99 (5.7), 98 (100). Elemental analysis (unlabelled) Found: 61.93% C, 8.33% H, 8.88% N. $C_8H_{13}NO_2$ requires: 61.91% C, 8.44% H, 9.03% N.

(*R,S*)-[2,3-$^{13}C_2$]-1-(1'-Methyl-2'-pyrrolidinyl)propan-2-one; {(*R,S*)-[2',3'-$^{13}C_2$]Hygrine} (8).

A mixture of (*R,S*)-[2,3-$^{13}C_2$]-1-(1'-methyl-5'-oxo-2'-pyrrolidinyl)propan-2-one (**4**) (0.933 g, 5.9 mmol), *para*-toluenesulfonic acid (30.2 mg, 0.18 mmol), ethylene glycol (**5**) (2.2 mL) and triethylorthoformate (4.4 mL) was refluxed, under N_2, for 1 hour. The solution was cooled to R. T., 2 *N* NaOH (50 mL) added and the mixture extracted with CH_2Cl_2 (4 x 30 mL). The organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure.

The excess ethylene glycol in the product was removed by Kugelrohr distillation (110°C/1 mm Hg) to give the crude ketal, **6**.

The above ketal (**6**) was dissolved in dry Et_2O (50 mL), under N_2. A 1 *M* solution of $LiAlH_4$ in Et_2O (10.5 mL, 10.5 mmol) was added via syringe and the mixture refluxed for 7 hours. The reaction mixture was then cooled to room temperature and a cold solution of 1 *M* H_2SO_4 (50 mL) added and stirred for 4 hours. The Et_2O was removed under reduced pressure and the remaining aqueous solution stirred for another 1 hour. It was then made basic with 25% aqueous NaOH and extracted with CH_2Cl_2 (4 x 30 mL). The combined organic extracts were washed with saturated brine (50 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the crude product. Flash chromatography on silica gel with $CHCl_3$:MeOH:NH_4OH (185:15:1) as the solvent gave the pure product as a pale yellow oil (**8**) (0.664 g, 78% yield from **4**, 61% overall yield from ethyl [3,4-$^{13}C_2$]acetoacetate (**2**)). GLC-r.t. = 8.57 mins. ^{13}C-NMR ($CDCl_3$): δ 208.1 (d, C-2, $^1J_{2\text{-}3}$ = 40.3 Hz), 61.7 (C-2'), 56.8 (C-5'), 48.5 (d, C-1, $^1J_{1\text{-}2}$ = 52.7 Hz), 30.9 (d, C-3, $^1J_{3\text{-}2}$ = 40.3 Hz), 22.1 (C-4'). ^{1}H-NMR ($CDCl_3$): δ 3.0 (m, 1H, H-2'), 2.7 (m, 1H, H-1), 2.6-1.9 (m, 4H, H-1, H-3', H-5'), 2.3 (s, 3H, N-Me), 2.15 (dd, 3H, H-3, $^1J_{1H\text{-}13C}$ = 128 Hz, $^2J_{1H\text{-}13C}$ = 6 Hz), 1.7 (m, 2H, H-4'), 1.35 (m, 1H, H-3'). MS-EI m/e (relative intensity): 143 (3.2), 98 (3.2), 85 (5.5), 84 (95.0), 82 (12.9), 70.5 (5.6), 45 (100).

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