SYNTHESIS OF ^{14}C-LABELED INDELOXAZINE HYDROCHLORIDE (YM-08054), A CEREBRAL ACTIVATOR

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SUMMARY

Indeloxazine hydrochloride, (±)-2-[(inden-7-yloxy)methyl]morpholine hydrochloride (YM-08054), a new cerebral activator, was labeled with ^{14}C for metabolic studies. The labeled material with a specific activity of 14.9 mCi/mmol was synthesized in a 42.8% radiochemical yield from potassium [^{14}C]cyanide via a process of ten steps.

Keywords: Carbon-14, Indeloxazine hydrochloride,
(±)-2-[(Inden-7-yloxy)methyl]morpholine,
Dihydrocoumarin, Antidepressant,
Cerebral activator

INTRODUCTION

A number of morpholine derivatives having indenyloxymethyl moiety have been synthesized in our laboratories for the study of neuropharmacological activities. Among them (±)-2-[(inden-7-yloxy)methyl]morpholine hydrochloride (indeloxazine hydrochloride, YM-08054) not only showed antidepressive properties,[1)2)] but also had a protective effect on nitrogen-gas-induced amnesia and some other cerebral-activating properties in rats or mice.[3)] These kinds of pharmacological activities are important in connection with the treatment of organic brain diseases such as cerebral-vascular disorder.

As a matter of course, studies on the metabolic fate of this promising agent required preparation of the radioactive compound. The synthetic route

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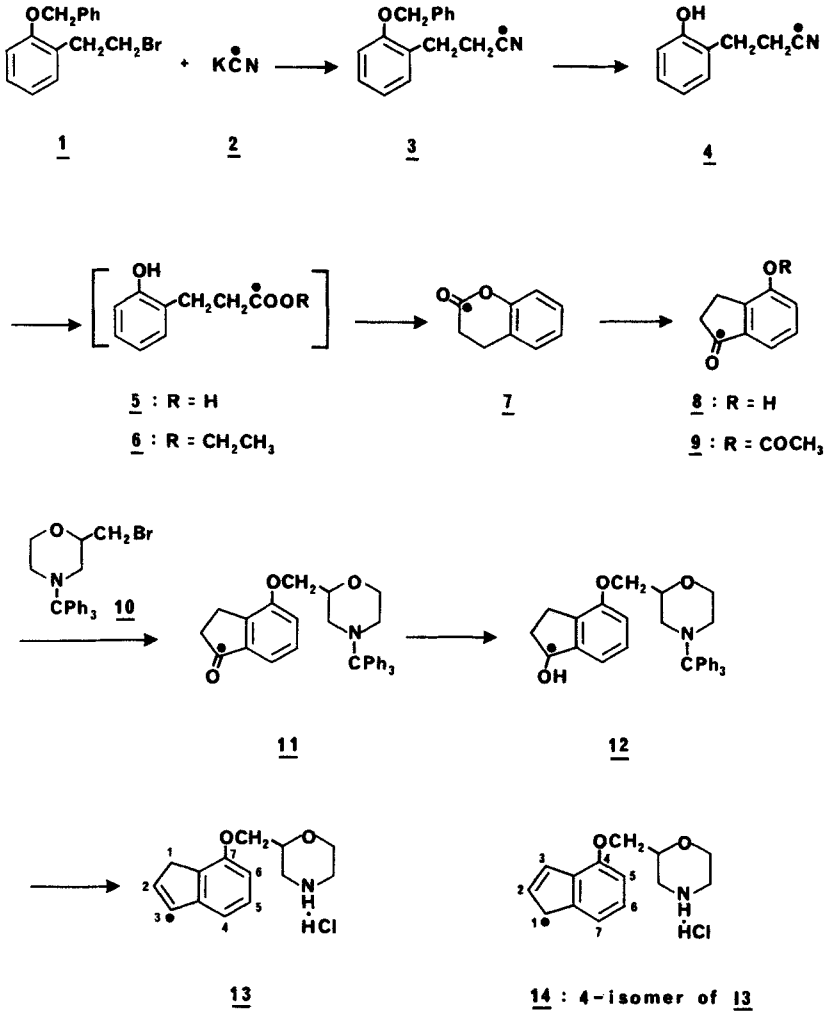
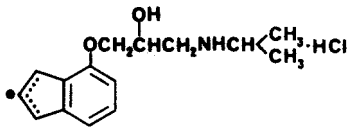


Scheme I

employed in the preparation of ^{14}C-labeled indeloxazine hydrochloride is illustrated in Scheme I. Details of the synthesis of the labeled compound are described in this paper.

RESULTS AND DISCUSSION

Previously we synthesized 3,4-dihydro[3-^{14}C]coumarin (carbon isotope isomer of 7)[4] by catalytic hydrogenation of [3-^{14}C]coumarin, which was



• denotes ^{14}C

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obtained from salicylaldehyde and [2-^{14}C]malonic acid according to the procedure of Kaighen and Williams.[5] The dihydrocoumarin was converted to 4-hydroxy-1-[2-^{14}C]indanone (carbon isotope isomer of 8), which was transformed by six steps into [indenyl-2-^{14}C]indenolol (15), a potent β-adrenergic blocking agent.[6] The radiochemical yield of 3,4-dihydro[3-^{14}C]-coumarin based on [2-^{14}C]malonic acid was 68%. However, this method was rather drastic and troublesome in the radiosynthesis. A noble route to prepare 3,4-dihydro[2-^{14}C]coumarin (7) which was a precursor of 4-hydroxy-1-[1-^{14}C]indanone (8), a key intermediate of [indenyl-3-^{14}C]indeloxazine hydrochloride (13), was therefore investigated.

By use of [^{14}C]KCN (2) as a starting material, bromide (1) was quantitatively converted into nitrile (3) in the presence of equimolar 18-crown-6 in dimethylformamide. Hydrogenolysis of 3 with palladium on charcoal gave nitrile (4) in a good yield. Acid hydrolysis of 3 into 3-(2-benzyloxyphenyl)-propionic acid was unsuccessful. On the other hand, when a solution of 4 in ethanol was saturated with hydrogen chloride followed by standing overnight, ester (6) with a small amount of acid (5) was obtained. Both of them were smoothly cyclized to afford 7 by azeotropic distillation using toluene in the presence of a catalytic amount of 4-toluenesulfonic acid. Yield of 7 based on 2 was 86.2%. This procedure was superior to that described above by use of [2-^{14}C]malonic acid from the viewpoint of yield, the cost of the starting material and manipulation. By fusion with NaCl-$AlCl_3$,[4] 7 was converted to 8. As purification of crude 8 was troublesome, 8 was transformed to acetate (9)

which was purified by column chromatography. Alkaline hydrolysis of 9 gave pure 8. Yield of pure 8 from 7 was 75.9%.

Kojima et al. obtained non-radioactive 1-oxo-indanyl compound (11) in a yield of 73.5% by the reaction of equimolar amount of the potassium salt of 8 and 4-triphenylmethyl-2-(4-toluenesulfonyloxymethyl)morpholine.[1] Higher conversion of 8 into 11 was observed by use of an excess of the tosylate above, but the separation of 11 from the excess reagent was tedious. The reaction was modified to improve the radiochemical yield. By use of bromo compound (10) instead of the tosylate, 8 was alkylated in the presence of anhydrous K_2CO_3 in dimethylformamide to provide 11 in a yield of 95.1% after the purification by column chromatography. An excess (2 to 3.5 mole equivalents) of 10 and K_2CO_3 was effective to complete the alkylation. Reduction of 11 with $NaBH_4$ followed by column chromatography gave the hydroxyindanyl compound (12) in 86.1% yield.

The final step of the synthesis involved several difficulties. In general, indenes are known to isomerize prototropically under basic conditions to afford an equilibrium mixture. Actually, isomerization of double bond between non-radioactive 7-indenyl isomer (13) and 4-indenyl isomer (14) in a methanol solution was observed in the presence of base giving a 2:1 mixture.[1] The [^{14}C]indenolol 15 mentioned above was also an equilibrium mixture with respect to double bond of the indene ring, since it was prepared under basic conditions.[4] Dehydration of 12 to give 7-indenyl isomer 13 without isomerization of the double bond was required for the preparation of [^{14}C]indeloxazine hydrochloride 13. It was reported that a practical synthetic method for indeloxazine hydrochloride was established by preferential crystallization from an equilibrium mixture of indeloxazine hydrochloride 13 and the 4-isomer 14 in the presence of a catalytic amount of base in methanol.[1] However, this method was not convenient for the small scale radiosynthesis in terms of radiochemical yield. The synthesis of 13 from 12 was therefore carried out by a modification of the reported method.[1]

Removal of the trityl group and dehydration of the 1-hydroxyindan moiety of 12 were accomplished in a dilute aqueous ethanolic hydrochloric acid

solution under reflux to furnish the desired 13. A large excess (about 17 mole equivalents) of HCl was essential to complete the reaction. Yields of the desired product under optimum conditions were 76-81%. Careful purification using column chromatography was carried out to isolate the desired 13 from the reaction mixture without isomerization of the double bond thus formed. A silica gel column was washed with chloroform-methanol (10:1 v/v) contained a small amount of ethanolic hydrogen chloride followed by elution with chloroform-methanol (10:1 v/v) until the eluates became neutral prior to charge of the reaction mixture on the silica gel column. The pre-treatment of the silica gel column was very useful for avoidance of isomerization owing to partial elimination of HCl from 13 during purification. After the chromatography, pure ^{14}C-labeled indeloxazine hydrochloride 13 crystallized from acetone was obtained in 80.2% yield based on 12. The tautomeric isomer 14 was not detected by reverse phase HPLC analysis. Overall radiochemical yield of 13 based on 2 was 42.8%.

EXPERIMENTAL

Potassium[^{14}C]cyanide was purchased from Amersham International plc, Amersham, England. The labeled products were characterized by co-chromatography (TLC and/or HPLC) with non-radioactive standards. TLC analyses were conducted on precoated plates of Merck Silica Gel 60F_{254}. HPLC analysis was performed on a Waters Associates, Inc., HPLC equipment (Model 6000A pump, Model 440 Absorbance Detector, 254 nm). For column chromatography, a Lobar^{R} column (LiChroprep^{R} Si 60, Size B, Merck) was used unless otherwise specified, and was connected in series with a pre-column (silica gel, 3 g). The column was eluted at a flow rate of 20 ml/min with UV monitoring at 280 nm (Oyo-bunko UV-detector Ubilog 5 III). All evaporations were carried out under reduced pressure. Radioactivity measurement was made with a Packard Tri Carb Liquid Scintillation Spectrometer, Model 3255. Radiochemical purity was determined by TLC with a Berthold Radio-TLC Scanner LB 2723.

3-(2-Benzyloxyphenyl)[1-^{14}C]propionitrile (3)

The non-radioactive starting material, 2-(2-benzyloxyphenyl)ethylbromide (1, $b_{0.37}$ 156°C), was prepared by the reaction of 2-(2-benzyloxyphenyl)ethanol with triphenylphosphine and N-bromosuccinimide in benzene according to the reported method.[7)]

A mixture of potassium[^{14}C]cyanide (2, 34.9 mg, 30 mCi), non-radioactive 2 (96.6 mg), 1 (646 mg) and 18-crown-6 (584 mg) in dimethylformamide (13 ml) was stirred for 23 hr at room temperature. The reaction mixture was then evaporated to dryness and the residue obtained was applied to a Lobar^{R} column using benzen (2 x 2 ml). The column was eluted with a mixture of n-hexane and ethyl acetate (10:1 v/v). The fraction (10-23 min) was collected and evaporated to afford 3 (478 mg; 100.3%) as an oil.

3-(2-Hydroxyphenyl)[1-^{14}C]propionitrile (4)

To a solution of 3 (478 mg) in methanol (20 ml) was added 10% palladium on charcoal (90 mg). The mixture was subjected to hydrogenation at room temperature until the uptake of hydrogen had ceased (1 hr). The reaction mixture was filtered and the filtrate was evaporated to obtain an oil of 4 (317 mg; 107%).

3,4-Dihydro[2-^{14}C]coumarin (7)

A solution of 4 (317 mg) in ethanol (10 ml) was staturated with dry hydrogen chloride at 0°C with stirring protected from moisture. The reaction mixture was then allowed to stand at room temperature for 15 hr. The mixture was evaporated to dryness carefully, and to the redidue obtained were added 4-toluenesulfonic acid (10 mg) and toluene (30 ml). The resulting mixture was refluxed for 6 hr using an azeotropic distillation apparatus. During the reaction ethanol and water produced were removed from the reaction system as the toluene azeotrope (25 ml), and fresh toluene (25 ml) was added to the reaction mixture. This operation was repeated three more times at an interval

of 1 hr. After the completion of the cyclization checked by TLC ($CHCl_3$; Rf value of 5, 6 and 7 = 0.02, 0.16 and 0.50, respectively), the reaction mixture was concentrated to about 2 ml and the residue was subjected to column chromatography. The column was eluted with a mixture of n-hexane and ethyl acetate (3:1 v/v) and the fraction (9-15 min) was collected and evaporated to obtain an oil of 7 (257 mg; 80.3%).

4-Hydroxy-1-[1-^{14}C]indanone (8)

Sodium chloride-aluminum chloride complex (2.4 g), obtained by melting 1 mole of NaCl with 1 mole of anhydrous $AlCl_3$ on a hot plate, was added to 7 (257 mg) and the mixture was heated at 140°C with stirring. To the mixture obtained anhydrous $AlCl_3$ (1.5 g) was added in several portions followed by stir at 188-191°C for 2 hr. After ice-cooling, ice-water (10 g) was added to the reaction mixture. The crude crystals of 8 which were deposited by scratching in ice-water bath were collected by filtration, washed with chilled water (3 x 1 ml) and dried over P_2O_5 *in vacuo*.

A mixture of crude 8 (270 mg) obtained above, anhydrous 1,2-dichloromethane (4.5 ml), triethylamine (0.35 ml) and acetic anhydride (0.24 ml) was stirred at room temperature for 1 hr. The mixture was then applied to a Lobar^R column and the column was eluted with a mixture of n-hexane and ethyl acetate (3:1 v/v). The fraction (24-34 min) was collected and evaporated to obtain crystals of 4-acetoxy-1-[1-^{14}C]indanone (9). Yield: 270 mg (81.9% based on 7).

To a solution of 9 (270 mg) in methanol (6 ml), 1M-NaOH (3.7 ml) was added at room temperature and the mixture was stirred for 45 minutes. After ice-cooling the reaction mixture was acidified to pH 2 by dropwise addition of 1M-HCl (3.8 ml). The crystals of pure 8 precipitated were collected by filtration, washed with water (6 x 1 ml) and dried over P_2O_5 *in vacuo*. Yield: 195 mg (92.7% based on 9).

2-[(1-Oxo[1-^{14}C]indan-4-yloxy)methyl]-4-triphenylmethylmorpholine (11)

The alkylating reagent, 2-bromomethyl-4-triphenylmethylmorpholine (10), was prepared from 2-hydroxymethyl-4-triphenylmethylmorpholine by the same method as the preparation of 1. The crude product was purified by silica gel column chromatography using a mixture of n-hexane and ethyl acetate (10:1 v/v) as eluent; mp. 72-82°C (powder from aqueous ethanol).

A suspension of 8 (195 mg), anhydrous K_2CO_3 (362 mg) and 10 (1.126 g) in anhydrous dimethylformamide (5.5 ml) was heated at 80°C with strring. The reaction mixture was checked by radio-TLC using the solvent system of n-hexane/ethyl acetate (3:1 v/v). After 7 hr the ratio of 8 to 11 was 44:56. After a further addition of K_2CO_3(180 mg) and 10 (560 mg) to the reaction mixture, heating was continued for more 15 hr. At this time (22 hr after the initial heating) the ratio of 8 to 11 was 97:3 by TLC. After cooling, the solid materials were removed by filtration and the filtrate was concentrated. The residual oil was charged to the top of a silica gel column using CH_2Cl_2 (2 x 2 ml). The column was eluted with n-hexane/ethyl acetate (3:1 v/v). The fraction (11-22 min) was concentrated to dryness to give crystals of 11 (611 mg; 95.1%).

2-[(1-Hydroxy[1-^{14}C]indan-4-yloxy)methyl]-4-triphenylmethylmorpholine (12)

To a cooled solution of 11 (611 mg) in 1,2-dichloromethane (14 ml) and methanol (7 ml) at 0°C was added by portions 80 mg of $NaBH_4$. The mixture was stirred for 30 minutes at 0°C and further 1 hr at room temperature. To the cooled reaction mixture at 0°C, was added acetic acid (0.14 ml) and the resulting mixture was stirred at room temperature for 2.5 hr. After evaporation of the solvent the crude crystals obtained were subjected to column chromatography using CH_2Cl_2 (5 x 2 ml). The column was eluted with a mixture of CH_2Cl_2 and ethyl acetate (10:1 v/v). The fraction (12-24 min) was collected and concentrated to dryness to afford pure crystals of 12 (528 mg; 86.1%).

(±)-2-[([3-^{14}C]Inden-7-yloxy)methyl]morpholine hydrochloride (13)

A mixture of 12 (528 mg) and 0.5M-HCl (36.5 ml) in ethanol (15.6 ml) was heated under reflux for 2 hr. After cooling the solid precipitated was removed by filtration and washed with a chilled mixture of ethanol and water (3:7 v/v, 4 x 2 ml). The combined filtrates and washings were concentrated to about 20 ml and saturated with NaCl (6 g). The mixture was extracted with chloroform (3 x 20 ml) and the combined extracts were dried ($MgSO_4$) and concentrated. The residual oil obtained was purified by column chromatography in a manner described below. A Lobar^R column (Si 60, Size A) was washed with a mixture of chloroform/methanol/1M-HCl in ethanol (100:9:1 v/v) until the washings became acidic. The column was then eluted with chloroform/methanol (10:1 v/v) until the eluates became neutral. The oil obtained above was charged to the pre-treated column using chloroform (2 x 2 ml) followed by elution with chloroform/methanol (10:1 v/v) at a flow rate of 10 ml/min. The fraction (5-18 min) was collected and concentrated to dryness. To the residue obtained was added acetone (2 ml) and the resulting mixture was allowed to stand at 5°C overnight to complete crystallization. The crystals were collected by filtration to obtain pure 13. Yield: 231.2 mg (80.2%); 12.83 mCi. Specific activity: 55.5 μCi/mg, 14.9 mCi/mmol; radiochemical purity: greater than 99% by TLC analysis (Rf value =0.37 in benzen/ethyl acetate/methanol/conc. NH_4OH (40:40:15:2 v/v)). The tautomeric isomer 14 was not detected by HPLC on reverse phase column (TSK-Gel^R LS-410, Toyosoda, 4 x 300 mm) using 0.05M ammonium acetate/tetrahydrofuran/acetic acid (83:15:2 v/v) at a flow rate of 1.5 ml/min; retention time of 13 and 14 ≈13.7 mintute and 11.2 minute, respectively.

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