A Concise Synthesis of [*carbonyl*-^{11}C]Melatonin and *N*-[*carbonyl*-^{11}C]Acetylserotonin

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SUMMARY

Rapid chemical syntheses of [*carbonyl*-^{11}C]melatonin and *N*-[*carbonyl*-^{11}C]acetylserotonin starting from [^{11}C]carbon dioxide are described. The radiochemical yield (based on [*carbonyl*-^{11}C]-acetic acid), purity, and the specific activity (end of bombardment) of the former are 12%, >98%, and 4.2 Ci/μmol, respectively. The latter is also synthesized with the same radiochemical yield, purity, and specific activity. The total times required for their syntheses are *ca.* 45 min, respectively.

Key Words: [*carbonyl*-^{11}C]Melatonin, *N*-[*carbonyl*-^{11}C]acetyl-5-methoxytryptamine, *N*-[*carbonyl*-^{11}C]acetylserotonin, *N*-[*carbonyl*-^{11}C]acetyl-5-hydroxytryptamine, [^{11}C]carbon dioxide.

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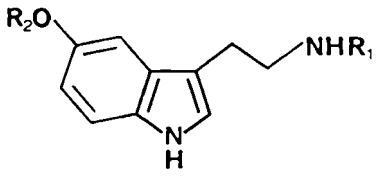
INTRODUCTION

The pineal gland hormone melatonin (*N*-acetyl-5-methoxytryptamine) (1) is now considered an important neuroendocrine component of animal physiology (1) and great progress has been made in the identification of its binding sites in recent years (2). The serotonin (5-hydroxytryptamine) (2) system in the human brain is sensitive to aging (3). Melatonin (1), a metabolite of (2), is produced *in vivo* by *N*-acetylation and *O*-methylation. We are interested in studying such biologically active substances (1 and 2) labelled with positron emitting radionuclide, Carbon-11 ($t_{\frac{1}{2}}$=20.4 min).

As a part of the synthetic study of biologically active compounds labelled with Carbon-11 for positron emission tomography (4, 5), the concise syntheses of [*carbonyl*-^{11}C]melatonin (3) and *N*-[*carbonyl*-^{11}C]acetylserotonin (4) as potential diagnostic imaging agents will be reported here.

RESULTS AND DISCUSSION

The chemical synthesis of [*carbonyl*-^{11}C]melatonin (3) and its 6-fluoro-derivative from 5-methoxytryptamine (5) and 6-fluoro-5-methoxytryptamine, respectively, with [*carbonyl*-^{11}C]acetyl chloride have been reported by Bars *et al.* (6, 7). Recently, Mannens *et al.* reported the enzymatic synthesis of (4) from (2) using a specific enzyme catalyzed reaction (8). We established the rapid method for the introduction of an [*carbonyl*-^{11}C]acetyl group into aminosugar with [*carbonyl*-^{11}C]acetic acid using dicyclohexylcarbodiimide (DCC) (5). The method was applied to the syntheses of title compounds (3 and 4) from (5) and (2), respectively, with some modifications. [*carbonyl*-^{11}C]Acetic acid was prepared from [^{11}C]carbon dioxide with a Grignard reagent by the ordinary procedure.



(1): R_1=$COCH_3$, R_2=CH_3
(2): R_1=R_2=H
(3): R_1=$^{11}COCH_3$, R_2=CH_3
(4): R_1=$^{11}COCH_3$, R_2=H
(5): R_1=H, R_2=CH_3
(6): R_1=$COCH_3$, R_2=H

[^{11}C]Carbon dioxide was produced from the proton bombardment of nitrogen gas by the ^{14}N (p, α) ^{11}C nuclear reaction at the Tohoku University Cyclotron (9). The carbon dioxide thereby formed was bubbled into a solution of methylmagnesium bromide in tetrahydrofuran (THF), and diluted hydrochloric acid was then added to destroy an excess of the Grignard reagent. To the resulting mixture a mixture of (5) and DCC was added. The mixture was then heated for 10 min at 80°C. After removal of an excess of DCC, the desired compound (3) was obtained by high performance liquid chromatography (HPLC) technique. The total synthesis time, the radiochemical yield, and purity of (3) were *ca.* 45 min, 12% (based on [*carbonyl*-^{11}C]acetic acid), and >98%, respectively. The specific activity (EOB) of (3) is 4.2 Ci/μmol.

The treatment of (2) in an analogous fashion gave a 12% radiochemical yield (based on [*carbonyl*-^{11}C]acetic acid) of the desired compound (4). The synthesis time and the radiochemical purity of (4) are the same as that of (3), and its specific activity (EOB) is 4.2 Ci/μmol.

Additionally, this method for the preparations of the title compounds (3 and 4) is suitable for automated synthesis because the simple apparatus and easy operation have been used. Their

Table 1: Retention Times of Melatonin (1) and *N*-Acetylserotonin (6) in Some HPLC Systems

Run	Compd.	Column Size (mm)	Flow Rate (ml/min)	Retention Time (min)
1	(1)	μ-Bondapak C_{18} (3.9 x 300)	1.0	3.16
2	(6)		1.0	2.76
3	(1)	YMC-Pack A-303 (4.6 x 250)	1.0	3.34
4	(6)		1.0	2.68
5	(1)	YMC-Pack A-324 (10.0 x 300)	2.5	7.40
6	(6)		2.5	5.98

Mobile phase (Ratio) is methanol/water (70/30, v/v).

medical uses are being investigated and the results will be reported elsewhere.

EXPERIMENTAL

Methylmagnesium bromide (1*M* solution in THF) was purchased from Kanto Chemical Co. Inc. Jpn. and the other reagents were from Wako Chemical Ltd. Jpn. These reagents were used without further purification. The purity of each compound was always checked by thin-layer chromatography. HPLC analyses were carried out either with a Waters Assoc. model 6000 equipped with a UV (254 nm) detector or with a Waters Assoc. model 4500 equipped with a radioactivity monitor. The packed columns [μ-Bondapak C_{18} (Waters Assoc. USA), YMC-Pack A-303, and YMC-Pack A-324 (Yamamura Chem. Lab. Co. Jpn.)] were used in HPLC. The retention times of (1) and *N*-acetylserotonin (6) in some HPLC systems are shown in Table 1.

[*carbonyl*-^{11}C]Melatonin (3).

[^{11}C]Carbon dioxide was produced by irradiation of nitrogen gas

with 18 MeV protons at 10 μA for 15 min. The irradiated target gas was released to a hot cell where [^{11}C]carbon dioxide is frozen out into a copper coil immersed in liquid argon (flow rate, 1 l/min). The coil with the trapped [^{11}C]carbon dioxide was then heated with a hot air blower. The [^{11}C]carbon dioxide was swept out by a dry argon flow (30 ml/min) and into a reaction vessel containing 1*M* solution of methylmagnesium bromide in THF (0.5 mmol, 0.5 ml). After the trapping of [^{11}C]carbon dioxide, 3 N hydrochloric acid (0.3 ml) was added to destroy an excess of the Grignard reagent.

To the resulting mixture, (5) (0.03 mmol, 5.7 mg) and a solution of DCC (0.4 mmol, 82 mg) in THF (0.4 ml) were added, heated at 80°C for 10 min with stirring, diluted with water (2 ml) to decompose an excess of DCC, and then filtered. The filtrate was made basic with 1N potassium hydroxide and extracted with ethyl acetate. The organic layer was passed through a Sep-Pak silica cartridge (Waters Assoc. USA), and eluted with dichloromethane/ethanol (25/1, v/v). The effluent was evaporated to dryness under a reduced pressure and the residue was dissolved in methanol/water (70/30, v/v) (0.5 ml). The solution was then subjected to preparative HPLC. The radiochromatogram is shown in Fig 1-A. A radioactivity peak corresponding to (3) was then

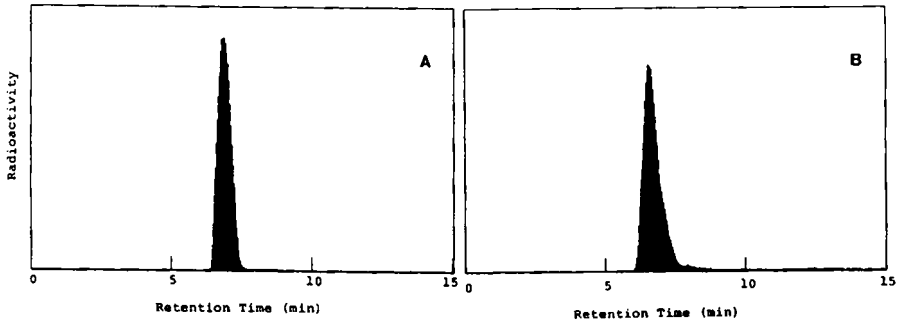


Fig. 1 Preparative HPLC Chromatograms of Reaction Mixtures. The peaks in Chromatograms A and B are (3) and (4), respectively. The HPLC were carried out under the similar conditions of Run 5 shown in Table 1.

collected and the identity of the peak was confirmed by analytical HPLC (Run 3 shown in Table 1).

N-[*carbonyl*-^{11}C]Acetylserotonin (4).

Hydrochloride of (2) (0.03 mmol, 6.4 mg) was treated in a similar manner as in (3) to afford (4) in a 12% radiochemical yield (based on [*carbonyl*-^{11}C]acetic acid), except the extraction of ethylacetate was carried out under acidic condition, and purified with a Sep-Pak C_{18} cartridge (Waters) instead of using a Sep-Pak silica cartridge. The radio-chromatogram is shown in Fig 1-B.

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