[Chem. Pharm. Bull. 36(7)2679—2681(1988)]

Synthesis of [^{13}C]Phenacetin and Its Application to the Breath Test for the Diagnosis of Liver Disease

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(Received January 8, 1988)

N-(4-([1-^{13}C]Ethoxy)phenyl)acetamide (^{13}C-phenacetin) was prepared by two methods. In the first method, *p*-nitrophenol was alkylated with [1-^{13}C]iodoethane and reduced to give 4-([1-^{13}C]ethoxy)aniline (^{13}C-*p*-phenetidine), which was acetylated with acetic anhydride to give ^{13}C-phenacetin. In the second method, *N*-acetyl-*p*-aminophenol was alkylated with [1-^{13}C]iodoethane. By using an excess amount of the starting material, [1-^{13}C]iodoethane was converted to ^{13}C-phenacetin in high yield. The ^{13}C-phenacetin thus obtained was applied to the breath test, and could be detected by $^{13}CO_2$ analyzer. Thus, the ^{13}C-phenacetin breath test should be applicable to the diagnosis of liver disease.

Keywords——^{13}C-phenacetin; ^{13}C-*p*-nitrophenetole; [1-^{13}C]iodoethane; ^{13}C-*p*-phenetidine; breath test; $^{13}CO_2$ analyzer; liver disease

Introduction

Recently the use of stable isotope-labeled compounds for the study of drug metabolism in humans has increased in importance.[1] The breath test,[2,3] in which ^{13}C-labeled compounds are administered and the excretion of metabolized $^{13}CO_2$ in the breath is measured, is a valuable clinical test and is widely used in United States and Europe. By means of this test the detoxification ability of the liver can be estimated without taking a blood sample. This is valuable in the diagnosis of liver diseases such as hepatitis. The metabolism of phenacetin in man and other animals has been well studied[4,5]; the ethoxy group in phenacetin is oxidized in the liver and eliminated as CO_2. To utilize this ^{13}C-labeled phenacetin (**1**) (Fig. 1) in the breath test, two methods for ^{13}C-labeling of an ethoxy carbon of phenacetin were examined.

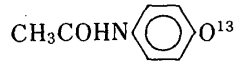

Fig. 1. [^{13}C]Phenacetin (**1**)

Results and Discussion

In the first method (method A), *p*-nitrophenol (**2**) was alkylated with [1-^{13}C]iodoethane (**3**) in the presence of potassium carbonate in acetone to form 4-([1-^{13}C]ethoxy)nitrobenzene (^{13}C-*p*-nitrophenetole) (**4**) in 89.9% yield. Compound **4** was then reduced with iron and hydrochloric acid to give 4-([1-^{13}C]ethoxy)aniline (^{13}C-*p*-phenetidine) (**5**) in 85.3% yield.[6] As **5** was very unstable, acetylation with acetic anhydride in benzene was carried out immediately to give *N*-(4-([1-^{13}C]ethoxy)phenyl)acetamide (^{13}C-phenacetin) (**1**) in 86.9% yield. The

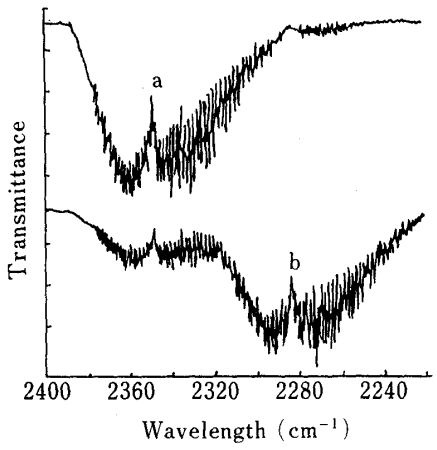

Fig. 2. IR Spectra of CO_2 and $^{13}CO_2$
a, $^{12}CO_2$, natural; b, $^{13}CO_2$, excess.

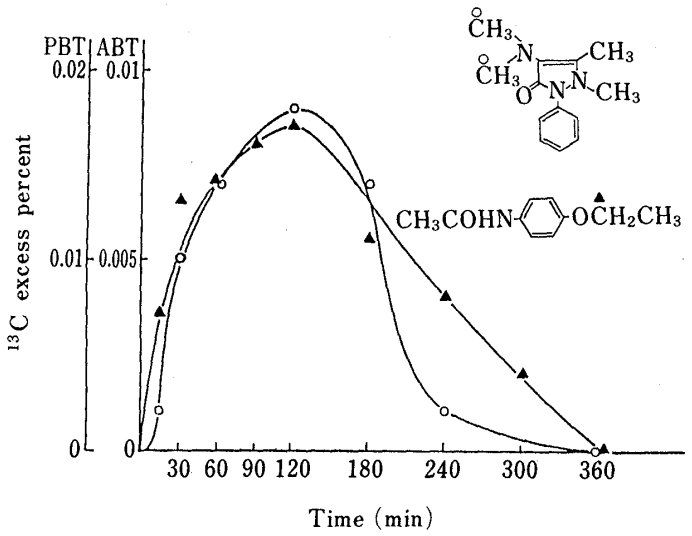

Fig. 3. The Breath Test with [^{13}C]Phenacetin (PBT) and [^{13}C]-Aminopyrine (ABT) (Control)
The plot shows $^{13}CO_2$ expired after oral administration of [^{13}C]PBT (▲) or [^{13}C]ABT (○); see the text for details. PBT, 3.5 mg/kg-body weight; ABT, 2.0 mg/kg-body weight.

overall yield of **1** from **2** was 66.6%. Compound **5** is important as an internal standard in the study of ^{13}C-phenacetin metabolism.

Another simple, one-step synthesis of **1** was achieved as follows (method B). *N*-Acetyl-*p*-aminophenol (**6**) was alkylated with [1-^{13}C]iodoethane in the presence of potassium carbonate in acetone. By using an excess (2.0 eq) amount of **6**, ^{13}C-phenacetin (**1**) was synthesized in 98.6% yield. The two-dimensional nuclear magnetic resonance (2D-NMR) spectrum (C-H shift correlation spectroscopy; C-H COSY) of **1** shows clearly that the methylene carbon of the ethoxy group was regioselectively labeled with ^{13}C to a high degree. From the mass spectrum and proton nuclear magnetic resonance (^{1}H-NMR) spectrum, **1** was confirmed to have 97% atom ^{13}C excess.

The breath test was conducted with **1** thus prepared. Up to now, mass spectroscopy (MS) has generally been utilized in the breath test. However, we used a $^{13}CO_2$ analyzer (JASCO EX-130), which was specially designed for the breath test. The ^{13}C excess percent was calculated from the absorption intensities of $^{13}C{=}O$ ($2280 \pm 10\,cm^{-1}$) and $^{12}C{=}O$ ($2380 \pm 10\,cm^{-1}$) (Fig. 2). Infrared spectrum (IR) measurement is simpler and more convenient, compared with MS measurement. The results of the phenacetin breath test (PBT) and the aminopyrine breath test (ABT) are illustrated in Fig. 3. In this control experiment PBT shows a similar curve of expired $^{13}CO_2$ to that of ABT. The latter has been widely used, but PBT is believed to be less toxic than ABT. With the aim of applying PBT to clinical diagnosis we are now examining its usefulness in various liver diseases.[7] We believe it should offer valuable information. In the case of hepatocirrhosis, which requires surgical treatment, determination of the liver function of the patient by PBT would provide essential information for reaching a clinical decision.

Experimental

All melting points are uncorrected. ^{1}H-, ^{13}C-, and 2D-CH-COSY-NMR spectra were recorded on a JEOL GSX-400 spectrometer using tetramethylsilane as an internal standard. IR spectra were measured with a JASCO IR-815

spectrometer. MS were obtained from a JEOL JMS-6ISG2 spectrometer.

4-([1-^{13}C]Ethoxy)nitrobenzene (^{13}C-*p*-Nitrophenetole) (4)——*p*-Nitrophenol (**2**) (0.886 g, 6.37 mmol) was dissolved in 30 ml of dry acetone, then potassium carbonate (0.881 g, 6.37 mmol) and [1-^{13}C]iodoethane (**3**) (1.00 g, 6.37 mmol, purchased from CIL, 1-^{13}C 99%) were added, and the mixture was refluxed. After 24 h the reaction mixture was concentrated to dryness. The residue was taken up in 20 ml of water and extracted with benzene (20 ml × 3). The extract was washed with water (20 ml), dried over anhydrous magnesium sulfate, filtered, and evaporated to give **4** as a pale yellow crystalline material (0.963 g, 89.9%). mp 56—58 °C. IR (KBr): 1595 (N=O), 1500, 1260 cm^{-1}. ^{1}H-NMR ($CDCl_3$, δ): 8.20, 6.94 (4H, AA′BB′, Ph), 4.13 (1.94H, dq, $J_{^{13}C-H}$=144.3 Hz, J=6.9 Hz, $^{13}CH_2$), 4.13 (0.06 H, q, J=6.9 Hz, CH_2), 1.47 (3H, dt, $^2J_{^{13}C-H}$=6.9 Hz, J=4.6 Hz, CH_3). ^{13}C-NMR ($CDCl_3$, δ): 164.1, 141.4, 125.9, 114.4 (Ph), 64.4 (enriched, $^{13}CH_2$), 14.5 (CH_3). MS *m/z*: 168 (M^+), 139 ($M^+ - {}^{13}CH_2 = CH_2$).

4-([1-^{13}C]Ethoxy)aniline (^{13}C-Phenetidine) (5)——In a 50 ml two-necked flask, 1.146 g (20.5 mmol) of iron was placed, then 6 ml of water and 0.6 ml of concentrated hydrochloric acid were added. After 10 min, 0.908 g (5.40 mmol) of **4** was added slowly and the mixture was stirred at 80 °C for 3 h. After cooling of the mixture, 6 ml of 5% aqueous NaOH was added and the whole was extracted with benzene (4 × 30 ml). The extract was washed with water, dried over $MgSO_4$, and evaporated to give **5** as a light brown oil (0.636 g, 85.3%). IR (neat): 3410, 3360 (NH), 1512, 1230 cm^{-1}. ^{1}H-NMR ($CDCl_3$, δ): 6.74, 6.63 (4H, AA′BB′, Ph), 3.95 (1.94H, dq, $J_{^{13}C-H}$=142.7 Hz, J=6.9 Hz, $^{13}CH_2$), 3.95 (0.06 H, q, J=6.9 Hz, CH_2), 1.37 (3H, dt, $^2J_{^{13}C-H}$=4.4 Hz, J=6.9 Hz, CH_3). ^{13}C-NMR ($CDCl_3$, δ): 151.7, 139.9, 116.4, 115.6 (Ph), 63.7 (enriched, $^{13}CH_2$), 14.9 (CH_3).

***N*-(4-([1-^{13}C]Ethoxy)phenylacetamide (^{13}C-Phenacetin) (1)**——Method A: In a 50 ml flask, 0.626 g (4.53 mmol) of **5** was dissolved in 30 ml of benzene, then 0.51 ml (5.44 mmol) of freshly distilled acetic anhydride was added and the mixture was stirred at 80 °C for 1.5 h. It was evaporated to give a brown solid, which was recrystallized from ethanol to give **1** as colorless crystals (0.606 g, 74.2%).

Method B: In a 100 ml of flask, *N*-acetyl-*p*-aminophenol (**6**) (3.876 g, 25.6 mmol) and potassium carbonate (3.548 g, 25.6 mmol) were dissolved in 80 ml of dry acetone, then 2.0 g (12.7 mmol) of [1-^{13}C]iodoethane (**3**) was added and the mixture was refluxed for 60 h. The reaction mixture was filtered and the filtrate was evaporated to give a white solid. This was partitioned between chloroform and saturated sodium bicarbonate. The water layer was reextracted with chloroform. The combined organic layer was washed with water, dried over magnesium sulfate, and evaporated to give **1** as a white solid (2.250 g, 98.6%). mp 131—132 °C. IR (KBr): 3280 (NH), 1660 (C=O), 1248 cm^{-1}. ^{1}H-NMR ($CDCl_3$, δ): 7.37, 6.83 (4H, AA′BB′, Ph), 4.00 (1.94H, dq, $J_{^{13}C-H}$=143.3 Hz, J=6.9 Hz, $^{13}CH_2$), 4.00 (0.06 H, q, J=6.9 Hz, CH_2), 1.72 (1H, br, NH), 1.39 (3H, t, J=6.9 Hz, CH_3). ^{13}C-NMR ($CDCl_3$, δ): 168.6 (s, C=O), 155.7, 131.0, 121.9, 114.6 (Ph), 63.6 (enriched, $^{13}CH_2$), 24.1 ($NHCOC\underline{H}_3$), 14.7 ($CH_2C\underline{H}_3$). MS *m/z*: 180 (M^+), 138 ($M^+ - CH_2CO$).

References

1) P. D. Klein, "Stable Isotopes," ed. by T. A. Baillie, MacMillan, 1978, p. 3.
2) T. Sasaki, "Progress in Nuclear Medicine," Vol. 5, S. Karger, 1978, p. 205.
3) D. A. Schoeller, A. N. Kotake, G. H. Lambert, P. S. Krager, and A. L. Baker, *Hepatology*, **5**, 276 (1985).
4) B. B. Broidie and J. Axelrod, *J. Pharmacol. Exp. Ther.*, **97**, 58 (1949).
5) S. D. Nelson, "Stable Isotopes," ed. by T. A. Baillie, MacMillan, 1978, p. 139.
6) R. W. West, *J. Chem. Soc.*, **127**, 494 (1925).
7) The 108th Annual Meeting of the Pharmaceutical Society of Japan, Hiroshima, April 1988.