Synthesis of ^{13}C-Labelled Compounds having a Urea Unit, and Observation of ^{13}C-Isotope Effect in Their Infrared Spectra

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Summary

Various drugs and nucleic acids containing a urea unit were synthesized from [^{13}C]urea. The ^{13}C-isotope effect in the infrared spectra of these compounds was examined.

Key words: [^{13}C]urea, [2-^{13}C]barbital, bromovaleryl[^{13}C]urea, [2-^{13}C]phenytoin, [2-^{13}C]trimethadione, [2-^{13}C]uracil, 6-amino[2-^{13}C]uracil, ^{13}C-isotope effect, infrared spectroscopy.

Introduction

We have already synthesized various ^{13}C-labelled compounds that are useful for metabolic studies and medical applications[1]. Recently, for example, we have synthesized [^{13}C]urea from [^{13}C]potassium cyanide at low cost[2], for use in a simple and non-invasive [^{13}C]urea breath test for infection with *Helicobacter pylori*, which is involved in the pathogenesis of gastritis and gastric ulcers. We have now used [^{13}C]urea to synthesize

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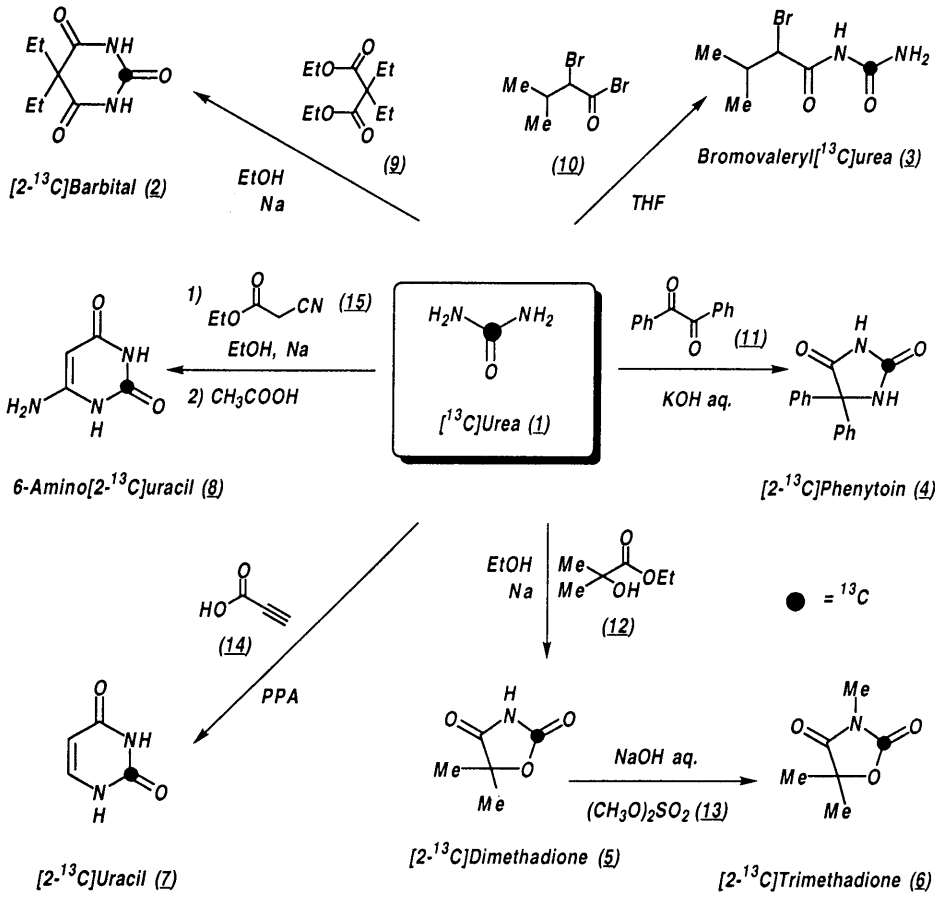
several drugs and nucleic acids, and we have observed the carbon-13 isotope effect in their infrared spectra.

Results and Discussion

[^{13}C]Urea (1) was utilized to synthesize [2-^{13}C]barbital (2)[3], bromovaleryl[^{13}C]urea (3)[4], [2-^{13}C]phenytoin (4)[5], [2-^{13}C]trimethadione (6)[6], [2-^{13}C]uracil (7)[7] and 6-amino[2-^{13}C]uracil (8)[8]. As shown in scheme 1, condensation of [^{13}C]urea (1) and diethylmalonic acid diethylester (9) in the presence of sodium in ethyl alcohol under reflux gave [2-^{13}C]barbital (2) in 57 % yield. Reaction of [^{13}C]urea (1) with bromoisovaleryl bromide (10) under reflux gave bromovaleryl[^{13}C]urea (3) in 48 % yield. For the synthesis of [2-^{13}C]phenytoin (4), rearrangement of benzil (11) in the presence of 40 % potassium hydroxide afforded benzilic acid, which reacted with [^{13}C]urea (1) to give 4 in 65 % yield. Condensation of [^{13}C]urea (1) and 2-hydroxyisobutyric acid ethylester (12) in the presence of sodium in ethyl alcohol under reflux gave [2-^{13}C]dimethadione (5) in 74 % yield. Methylation of [2-^{13}C]dimethadione (5) with dimethyl sulfate (13) gave [2-^{13}C]trimethadione (6) in 50 % yield. Condensation of [^{13}C]urea (1) and propiolic acid (14) in the presence of polyphosphoric acid gave [2-^{13}C]uracil (7) in 66 % yield. Reaction of [^{13}C]urea (1) and cyanoacetic acid ethylester (15) in the presence of sodium in ethyl alcohol under reflux, followed by treatment with acetic acid gave 6-amino[2-^{13}C]uracil (8) in 74 % yield.

The IR spectra of the ^{13}C-labelled and the unlabelled compounds were measured. For example, the spectra of [2-^{13}C]dimethadione (5) and unlabelled dimethadione are shown in figure 1. The [^{13}C]carbonyl absorption of [2-^{13}C]dimethadione (5) appeared at 1720 cm^{-1}, while that of unlabelled dimethadione was seen at 1744 cm^{-1}, so that the shift in the carbonyl absorption owing to the isotope effect was 24 cm^{-1}. The carbonyl absorptions of the other ^{13}C-labelled compounds were similarly shifted by 18~35 cm^{-1} (table 1).



Scheme 1; Synthesis of ^{13}C-Labelled Drugs and Nucleic Acids Having a ^{13}C-Urea Unit

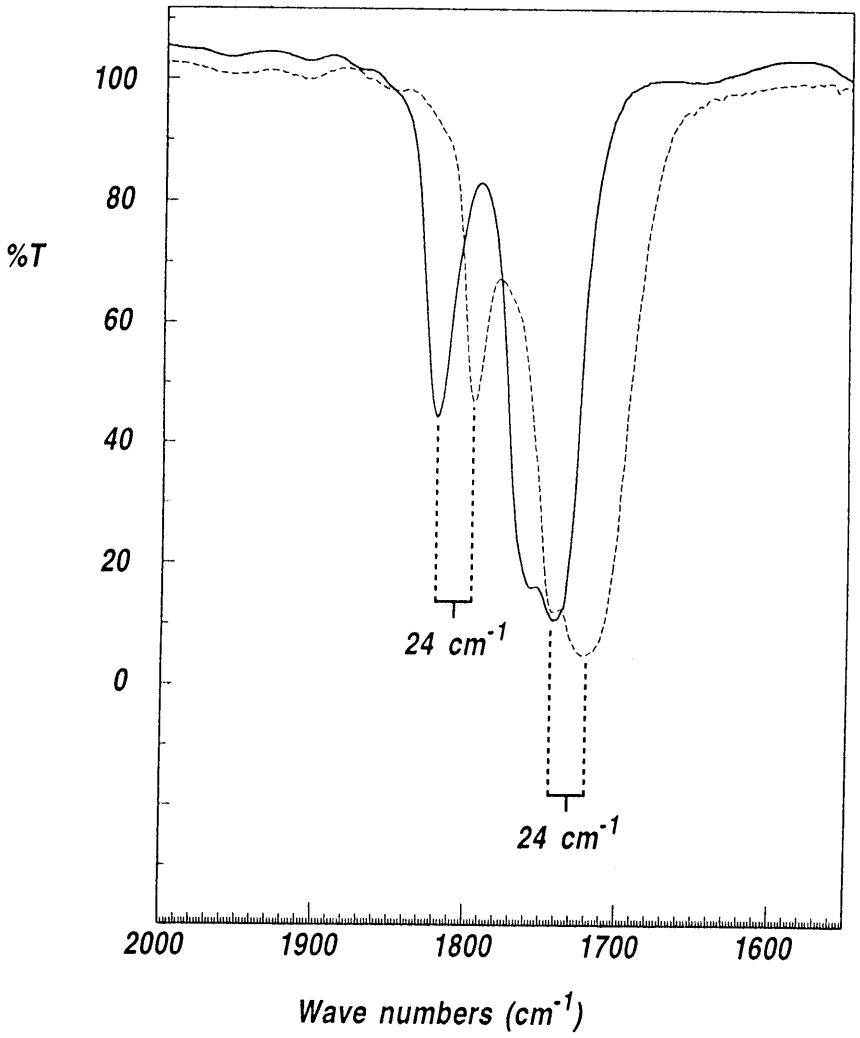


Figure 1; Comparison of IR Spectra of [2-^{13}C]Dimethadione (5) (-----) and Unlabelled Dimethadione (——).

Table 1; Carbonyl Absorptions in the Infrared Spectra of ^{13}C-Labelled and Unlabelled Compounds

Compound	$^{13}C=O$ (cm^{-1})	$C=O$ (cm^{-1})	Δ (cm^{-1})	concentration (w/w or w/v %)
[^{13}C]Urea (1)	1667	1685	18	0.62 (KBr)
[2-^{13}C]Barbital (2)	1737	1765	28	0.66 (KBr)
Bromovalery[^{13}C]urea (3)	1706	1733	27	0.75 (KBr)
[2-^{13}C]Phenytoin (4)	1691	1717	26	0.52 (KBr)
[2-^{13}C]Dimethadione (5)	1720	1744	24	0.28 ($CHCl_3$)
[2-^{13}C]Trimethadione (6)	1711	1736	25	0.10 ($CHCl_3$)
[2-^{13}C]Uracil (7)	1701	1736	35	0.40 (KBr)
6-Amino[2-^{13}C]uracil (8)	1697	1731	34	0.40 (KBr)

Experimental

Materials

[^{13}C]Urea (99 atom % ^{13}C) (1) was supplied by Masstrace, Inc..

Instruments

Melting point determinations were carried on a Yanaco micro melting point apparatus, Model MP; values are uncorrected. IR spectra were recorded on a JASCO VALOR-III FT-IR spectrometer. ^1H- and ^{13}C-NMR spectra were recorded on a JEOL GSX-400 (^1H: 400 MHz and ^{13}C: 100 MHz) spectrometer. EI and FAB-MS spectra were obtained on a Fisons Instrument VG Analytical AutoSpec spectrometer at 8 kV with a DEC VAX-4000 Model 60 data system.

[2-^{13}C]Barbital (2)

A solution of sodium (100 mg, 4.35 mmol) in dry ethyl alcohol (3 ml) was added to [^{13}C]urea (1) (230 mg, 3.77 mmol) at 0 °C under argon, and the mixture was stirred for 10 min at room temperature. To this suspension, a solution of diethylmalonic acid diethylester (9) (930 mg, 4.30 mmol) in dry ethyl alcohol (3 ml) was added dropwise at 0 °C, and the whole was stirred for 5 min at this temperature, then heated under reflux for 12 hr. The reaction was quenched with water (5 ml), and the mixture was acidified with concentrated hydrochloric acid (1 ml), and evaporated. The residue was taken up in hot ethyl alcohol (7 ml), and this suspension was stirred for 10 min. The supernatant solution was collected by centrifugation. The process was repeated twice. The combined supernatant solution was evaporated, and recrystallization of the residue from ethyl alcohol gave [2-^{13}C]barbital (2) (400 mg, 57 %), m.p. 189~192°C; ^{1}H-NMR ($CDCl_3$:CD_3OD=5:1) δ: 0.87 (t, 6H, *J*=7.4 Hz, CH_3CH_2), 1.20 (q, 4H, *J*=7.4 Hz, CH_3CH_2), 3.90 (brs, 2H, NH); ^{13}C-NMR ($CDCl_3$:CD_3OD=5:1) δ: 149.9 (^{13}CO); FT-IR (KBr) cm^{-1}: 1737 (^{13}CO); FAB-MS (magic bullet) *m/z*: 186 (MH^+).

Bromovaleryl[^{13}C]urea (3)

A solution of bromoisovaleryl bromide (10) (818 mg, 3.25 mmol) in dry tetrahydrofuran (5 ml) were added dropwise to a solution of [^{13}C]urea (1) (200 mg, 3.25 mmol) in dry tetrahydrofuran (5 ml) for 2 hr under argon with heating under reflux. The mixture was refluxed for 15 hr, then diluted with ethyl acetate. The whole was washed with water, dried over magnesium sulfate and evaporated. Recrystallization of the residue from ethyl alcohol gave bromovaleryl[^{13}C]urea (3) (341 mg, 48 %), ^{1}H-NMR ($CDCl_3$) δ: 1.07 (d, 3H, *J*=6.7 Hz, CH_3), 1.11 (d, 3H, *J*=6.7 Hz, CH_3), 2.31 (d-septuplet, 1H, *J*=6.7, 7.2 Hz, -CHCHBr-), 4.14 (d, 1H, *J*=7.2 Hz, -CHCHBr-); ^{13}C-NMR ($CDCl_3$) δ: 154.7 (^{13}CO); FT-IR (KBr) cm^{-1}: 1706 (^{13}CO); FAB-MS (thioglycerol) *m/z*: 224 (MH^+).

[2-^{13}C]Phenytoin (4)

A solution of [^{13}C]urea (1) (250 mg, 4.09 mmol) and benzil (11) (1.01 g, 4.80 mmol) in 40 % potassium hydroxide (2 ml) was stirred at 100 °C for 3 hr, then diluted with water (20 ml). Concentrated hydrochloric acid was added to this solution until crystallization occurred. The crystals were collected by filtration, and recrystallized from water : ethyl alcohol (1 : 3) to give [2-^{13}C]phenytoin (4) (1.0 g, 65 %), m.p. 299 °C; ^{1}H-NMR ($CDCl_3$) δ: 7.40 (m, 10H, Ar-H); ^{13}C-NMR ($CDCl_3$) δ: 156.2 (^{13}CO); FT-IR (KBr) cm^{-1}: 1691 (^{13}CO); FAB-MS (thioglycerol) *m/z*: 254 (MH^+).

[2-^{13}C]Dimethadione (5)

A solution of sodium (1.14 g, 49.6 mmol) in dry ethyl alcohol (20 ml) was added to 2-hydroxyisobutyric acid ethylester (12) (4 ml, 40.3 mmol) at 0 °C under argon. To this solution was added dropwise a solution of [^{13}C]urea (1) (1.2 g, 19.7 mmol) in dry ethyl alcohol (30 ml) at 0 °C over 3 hr, and the whole was heated under reflux for 30 hr. The reaction was quenched with water (5 ml), and the mixture was acidified with concentrated hydrochloric acid (2 ml), then extracted with chloroform (100 ml x 3). The extracts were dried over magnesium sulfate and evaporated. Recrystallization of the residue from ether gave [2-^{13}C]dimethadione (5) (1.9 g, 74 %), ^{1}H-NMR ($CDCl_3$) δ: 1.60 (S, 6H, CH_3); ^{13}C-NMR ($CDCl_3$) δ: 153.3 (^{13}CO); FT-IR ($CHCl_3$) cm^{-1}: 1720 (^{13}CO); EI-MS *m/z* (rel. int. %): 130 (M^+, 29), 59 (73), 43 (100).

[2-^{13}C]Trimethadione (6)

Sodium hydroxide solution (1.9 N, 3 ml) was added dropwise to [2-^{13}C]dimethadione (5) (500 mg, 3.85 mmol) and dimethyl sulfate (13) (550 μl, 5.81 mmol) at 0 °C, and mixture was stirred at 40 °C for 1 hr. It was acidified with 2 N hydrochloric acid (10 ml), and extracted with ether (100 ml x 3). The extracts were dried over magnesium sulfate and evaporated at

0 °C. Chromatography on silica gel with chloroform, followed by recrystallization of the product from ether-hexane gave [2-^{13}C]trimethadione (6) (275 mg, 50 %), ^{1}H-NMR ($CDCl_3$) δ: 1.58 (s, 6H, CH_3), 3.09 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$) δ: 154.7 (^{13}CO); FT-IR ($CHCl_3$) cm^{-1}: 1711 (^{13}CO); EI-MS *m/z* (rel. int. %): 144 (M^+, 35), 129 (10), 43 (100).

[2-^{13}C]Uracil (7)

Propiolic acid (14) (800 µl, 13.02 mmol) was added dropwise to a suspension of [^{13}C]urea (1) (500 mg, 8.19 mmol) in polyphosphoric acid (10 g), and this suspension was heated at 85 °C for 6 hr. It was diluted with water (25 ml), neutralized with ammonium hydroxide, and then evaporated. The residue was dissolved in a minimum volume of ammonium hydroxide (100 ml), and an equal volume of methyl alcohol (100 ml) was added. The resultant crystals were removed by filtration, and the filtrate was evaporated. The residue was chromatographed on silica gel with chloroform:hexane (4:1). The eluates were evaporated, and the residue was taken up in hot methyl alcohol (50 ml). The crystals obtained were collected by filtration to give [2-^{13}C]uracil (7) (615 mg, 66 %), m.p. 230 °C (decomp.); ^{1}H-NMR (1 N-NaOD) δ: 5.64 (d, 1H, *J*=6.3 Hz, 5-H), 7.57 (dd, 1H, *J*=6.3, 11.7 Hz, 6-H); ^{13}C-NMR (1 N-NaOD) δ: 168.6 (^{13}CO); FT-IR (KBr) cm^{-1}: 1701 (^{13}CO); EI-MS *m/z* (rel. int. %): 113 (M^+, 100), 69 (66), 42 (65).

6-Amino[2-^{13}C]uracil (8)

A solution of sodium (790 mg, 34.36 mmol) in dry ethyl alcohol (20 ml) was added to [^{13}C]urea (1) (1.0 g, 16.38 mmol) at 0 °C under argon, then 98 % cyanoacetic acid ethylester (15) (1.8 ml, 16.53 mmol) was added dropwise to the suspension at 0 °C under argon. The whole was heated under reflux for 5 hr. The mixture solidified, and the solid was dissolved in hot water (15 ml). This solution was heated at 80 °C for 15 min, neutralized with acetic acid, and then cooled to 0 °C. The resultant crystals were

collected by filtration. Recrystallization from water gave 6-amino[2-^{13}C]uracil (8) (1.54 g, 74 %), ^{13}C-NMR (0.2 N-NaOD) δ: 163.3 (^{13}CO); FT-IR (KBr) cm^{-1}: 1697 (^{13}CO); EI-MS *m/z* (rel. int. %): 128 (M^+, 100), 100 (14), 84 (22), 68 (47), 43 (52).

References

1) a) Kurumaya K., Okazaki T., Seido N., Akasaka Y., Kawajiri Y., Kajiwara M. and Kondo M. -J. Label. Compds. Radiopharm. 27: 217 (1989), b) Kurumaya K., Kajiwara M., Abei T., Hirano S. and Kokubun N. -Chem. Pharm. Bull. 36: 2679 (1988), c) Kajiwara M., Hara K., Mizutani M. and Kondo M. -Chem. Pharm. Bull. 40: 3321 (1992), d) Kajiwara M., Okazaki T., Iida K., Narumi S., Hirose M., Ijichi M., Abei T., Hirano S. and Iinuma M. -Chem. Pharm. Bull. 44: 1258 (1996)

2) Iida K., Chiyoda T. and Kajiwara M. -J. Label. Compds. Radiopharm. (submitted)

3) Dickey J. B. and Grayr A. R. -Organic Syntheses, coll. vol. 2 (ed. by Blatt A. H.) 60 (1943)

4) Nishizaki K. and Nishihara S. -Yakushi (in Japanese) 35: 1143 (1915)

5) a) Biltz H. -Chem. Ber. 41: 1379 (1908), b) Biltz H. and Seydel K. -Chem. Ber. 44: 411 (1911)

6) a) Stoughton R. W. -J. Am. Chem. Soc. 63: 2376 (1941), b) Spielman M. A. -J. Am. Chem. Soc. 66: 1244 (1944)

7) Harada K. and Suzuki S. -Tetrahedron Lett. 27: 2321 (1976)

8) Sherman W. R. and Taylor Jr. E. C. -Organic Syntheses, coll. vol. 4 (ed. by Rabjohn N.) 247 (1963)