

## Concomitant Oxygen-18 Enrichment in Commercial Carbon-13 Labelled Urea

Katsumi Iida, Takeshi Chiyoda and Masahiro Kajiwara\*

Department of Medicinal Chemistry, Meiji College of Pharmacy,  
1-22-1 Yato-cho, Tanashi-shi, Tokyo 188, Japan

### Summary

By mass spectroscopy, 50-fold oxygen-18 enrichment over natural abundance was observed in commercial  $^{13}\text{C}$ -urea (99 atom %  $^{13}\text{C}$ ) synthesized from  $^{13}\text{C}$ -carbon monoxide that had been  $^{13}\text{C}$ -enriched by cryogenic distillation. In contrast,  $^{13}\text{C}$ -urea synthesized from  $^{13}\text{C}$ -potassium cyanide (a  $^{13}\text{C}$ -labelled compound having no oxygen atom) showed the natural abundance level of oxygen-18.

**Key words:** carbon-13 enrichment, oxygen-18 enrichment, cryogenic distillation, mass spectroscopy,  $^{13}\text{C}$ -urea.

### Introduction

Compounds labelled with stable isotopes are widely used, for example, in biosynthetic studies<sup>1</sup>). Further, a  $^{13}\text{C}$ -urea breath test is used for non-invasive clinical diagnosis of *Helicobacter pylori* infection, which is involved in the pathogenesis of gastritis and gastric ulcers. Carbon-13 labelled compounds are commercially synthesized from  $^{13}\text{C}$ -carbon monoxide enriched in carbon-13 by cryogenic distillation, the only commercial carbon-13 enrichment method currently available. This enrichment method separates  $^{13}\text{C}$ -carbon monoxide and  $^{12}\text{C}$ -carbon monoxide based on the difference of their saturated vapor pressure. Such a procedure should also afford enrichment of oxygen-18, whose natural abundance is 0.20 %, but no

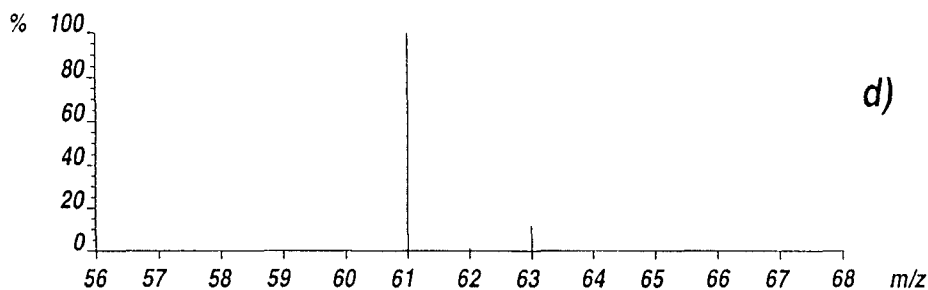
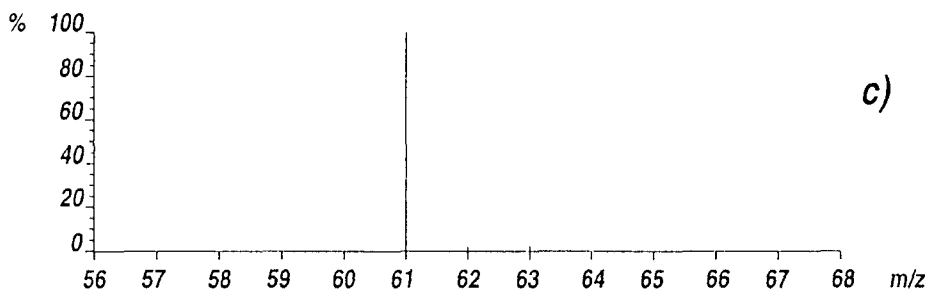
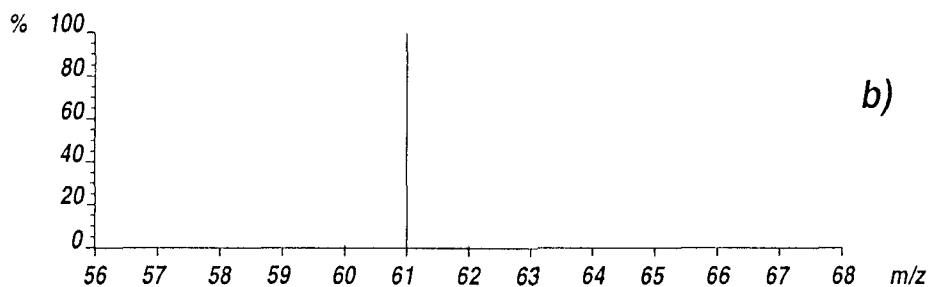
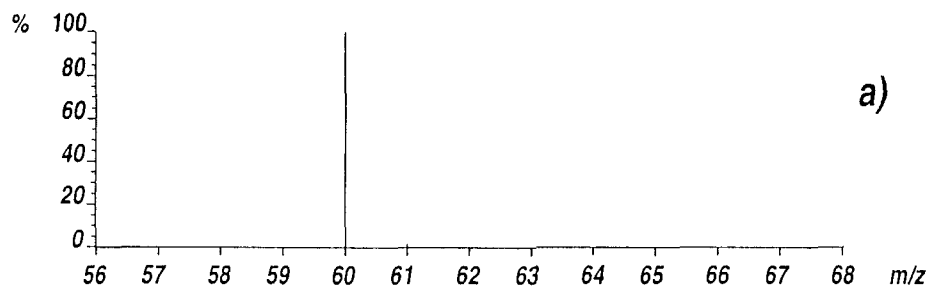
report has yet appeared on this point. Since the oxygen-18 isotope effect could be significant in scientific and clinical applications, we examined the degree of oxygen-18 enrichment in  $^{13}\text{C}$ -urea from several commercial sources.

### Results and Discussion

The theoretical ratios of isotope abundance for urea are 1.96 % at ( $M^{+1}$ ) and 0.21 % at ( $M^{+2}$ ). The  $M^{+1}$  value is influenced by the isotope abundances of all the atoms in urea, one carbon, four hydrogens, one oxygen, and two nitrogen atoms. However, the  $M^{+2}$  value is influenced only by the isotope abundance of the one oxygen atom. That is, the theoretical ratio (0.21 %) of  $M^{+2}$  value of urea is in good agreement with the natural oxygen-18 abundance (0.20 %). Therefore, the measured  $M^{+2}$  value of urea directly reflects its degree of oxygen-18 enrichment. Eight samples of urea of different grades, manufactured by three companies, were examined by mass (MS) spectroscopy. The results (figure 1 and table 1) were in agreement with the theoretical isotope abundance, and the  $M^{+2}$  values were close to

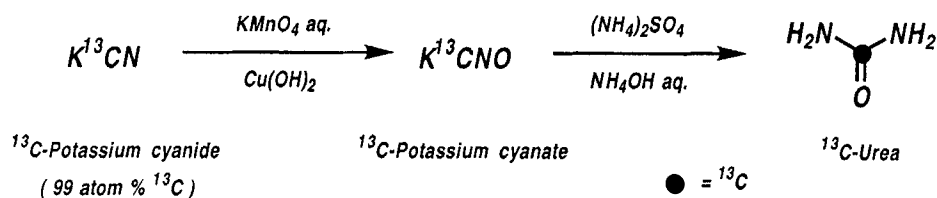
**Table 1; Relative Intensity of Peaks in the Mass Spectra of Unlabelled Commercial Ureas**

Urea \ m/z	60	61	62
<i>ICN</i>	100	1.84	0.20
<i>Junsei</i>	100	1.90	0.20
<i>Wako</i>			
1)	100	1.82	0.20
2)	100	1.94	0.20
3)	100	1.89	0.20
4)	100	1.92	0.22
5)	100	1.94	0.22
6)	100	1.86	0.20



**Figure 1; Mass Spectra of a); Urea Supplied by Wako P. C. I., Ltd., b);  $^{13}\text{C}$ -Urea Synthesized from  $^{13}\text{C}$ -Potassium Cyanide, c);  $^{13}\text{C}$ -Urea Supplied by CIL Inc., d);  $^{13}\text{C}$ -Urea Supplied by Masstrace Inc..**

the natural abundance value. Next,  $^{13}\text{C}$ -urea was synthesized (scheme 1) from  $^{13}\text{C}$ -potassium cyanide (99 atom %  $^{13}\text{C}$ ), which contains no oxygen, in high yield by a modification of the method of Haley and Lambooy<sup>2</sup>). As shown in figure 1 and table 2, the peak at  $m/z$  60 with a relative intensity of 1.11 % reflects the existence ratio of urea ( $^{12}\text{C}$ ) in this synthesized  $^{13}\text{C}$ -urea. The peak with a relative intensity of 0.21 % at  $m/z$  63 ( $M+2$  for  $^{13}\text{C}$ -urea) reflects the oxygen-18 enrichment of  $^{13}\text{C}$ -urea. The abundance of oxygen-18 in this synthesized  $^{13}\text{C}$ -urea was equal to the natural abundance. However, among commercial  $^{13}\text{C}$ -ureas (99 atom %  $^{13}\text{C}$ ), which were synthesized from



Scheme 1; Synthesis of  $^{13}\text{C}$ -Urea from  $^{13}\text{C}$ -Potassium Cyanide

Table 2; Relative Intensity of Peaks in the Mass Spectra of Synthetic and Commercial  $^{13}\text{C}$ -Ureas

$^{13}\text{C}$ -Urea \ m/z	60	61	62	63
Synthesis from $\text{K}^{13}\text{CN}$	1.11	100	1.65	0.21
CIL	0.22	100	1.94	2.45
Masstrace	1) 0.75	100	1.68	11.66
	2) 0.51	100	1.40	11.95
Shoko	1) 0.58	100	1.40	11.87
	2) 0.89	100	1.59	10.95

$^{13}\text{C}$ -carbon monoxide enriched in carbon-13 by cryogenic distillation, CIL's  $^{13}\text{C}$ -urea showed a relative intensity of 2.45 % at  $M+2$ , more than ten times the natural abundance. Furthermore,  $^{13}\text{C}$ -ureas from Masstrace and Shoko showed relative intensities of 10.95~11.95 %, implying up to 50-fold enrichment of oxygen-18 over the natural abundance value. Clearly, commercial carbon-13 enrichment by cryogenic distillation involves the concomitant enrichment of oxygen-18.

## Experimental Materials

Ureas were supplied by ICN Pharm. Inc. (purity;  $\geq 99$  %), Junsei Chem. Co., Ltd (purity; 99 %) and Wako P. C. I., Ltd. (purity; 99 %).  $^{13}\text{C}$ -Ureas (99 atom %  $^{13}\text{C}$ ) were supplied by CIL Inc., Masstrace, Inc. and Shoko Co., Ltd..  $^{13}\text{C}$ -Potassium cyanide (99 atom %  $^{13}\text{C}$ ) was supplied by Isotec, Inc..

## Instruments

Melting point determinations were carried out on a Yanaco micro melting point apparatus (Model MP); values are uncorrected. IR spectra were recorded on a JASCO VALOR-III FT-IR spectrometer.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra were recorded on a JEOL GSX-400 ( $^1\text{H}$ : 400 MHz and  $^{13}\text{C}$ : 100 MHz) spectrometer. EI-MS spectra were obtained on a Fisons Instrument VG Analytical AutoSpec spectrometer with a DEC VAX-4000 Model 60 data system. The ionizing voltage was set at 70 eV and the acceleration voltage was set at 8 kV.

### *Synthesis of $^{13}\text{C}$ -Urea from $^{13}\text{C}$ -Potassium Cyanide*

A solution of potassium permanganate (2.1 g, 13.29 mmol) in water (50 ml) was added dropwise to  $^{13}\text{C}$ -potassium cyanide (1.0 g, 15.12 mmol) and copper hydroxide (0.6 g, 6.15 mmol) at 0 °C for 10 min, and the whole was stirred for 5 min at 50 °C. The reaction was quenched with 10 % hydrogen peroxide (15 ml) at 0 °C, and the mixture was filtered. The filtrate was neutralized with 0.5 N sulfuric acid, then freeze-dried. Ammonium sulfate

(2.2 g, 16.65 mmol) was added to a solution of the residue in water (10 ml) and ammonium hydroxide (20 ml), and the reaction mixture was stirred for 4 hr at 70 °C, then freeze-dried. The crude  $^{13}\text{C}$ -urea was extracted with hot ethyl alcohol, and the solution was evaporated. Recrystallization from ethyl alcohol gave  $^{13}\text{C}$ -urea (804 mg, 87 %), m.p. 114~120°C;  $^1\text{H}$ -NMR ( $\text{D}_2\text{O}$ )  $\delta$ : 5.72 ( $\text{NH}_2$ );  $^{13}\text{C}$ -NMR ( $\text{D}_2\text{O}$ )  $\delta$ : 165.2 ( $^{13}\text{CO}$ ); FT-IR (KBr)  $\text{cm}^{-1}$ : 1667 ( $^{13}\text{CO}$ ); EI-MS  $m/z$  (rel. int. %): 61 ( $\text{M}^+$ , 100), 41 (53).

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