SYNTHESES WITH STABLE ISOTOPES: UREA-^{13}C, UREA-^{12}C, AND UREA-^{13}C-$^{15}N_2$

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SUMMARY

Urea has been prepared from the reaction of carbon monoxide and ammonia in the presence of sulfur. The reaction has been applied to the synthesis of urea-^{13}C, urea-^{12}C, and urea-^{13}C-$^{15}N_2$

INTRODUCTION

It has been necessary in this Laboratory to synthesize various isotope isomers of urea containing carbon-13, carbon-12 (depleted in carbon-13), and both carbon-13 and nitrogen-15. There are a variety of methods that have been used to synthesize urea-^{14}C [1] and, in some cases, these methods have been applied to the preparation of urea-^{13}C [2,3]. The radioactive syntheses typically employ starting materials such as sodium cyanide, carbon dioxide, barium carbonate, diphenyl carbonate, or phosgene and ammonia. Since isotopic carbon monoxide and ammonia are available as starting materials, our attention was drawn to a paper by Franz and Applegath [4] in which they reported the preparation of urea (73% crude yield) from stoichiometric quantities of carbon monoxide, ammonia, and sulfur as indicated below.

$$CO + 2NH_3 + S \longrightarrow (H_2N)_2CO + H_2S$$

Since this reaction offered a one-step synthesis from our isotopic starting material and seemed to be applicable to a range of reaction scales, we investigated the reaction as a method of preparing various isotope isomers of urea.

DISCUSSION

For the preparation of urea containing isotopic carbon, nonisotopic reagents were used in excess in an effort to increase the yield of the product. Thus, when the reaction was carried out using a two-fold excess of ammonia and a 30% excess of sulfur, crude yields of 90-95% and isolated yields of 80-90% of recrystallized urea-^{13}C could be obtained. Urea-^{12}C, in which the carbon-13 level was less than 50 ppm, was obtained in comparable yields. The latter urea isotope isomer was subsequently used as a protein denaturing agent in cmr studies on natural abundance proteins.

Preliminary experiments on nonisotopic materials showed that when 50% excess carbon monoxide and 50% excess sulfur were used in the reaction the crude product could be obtained in 83% yield. The yield reported for the preparation of urea-^{13}C-$^{15}N_2$ under these conditions is only 35%, but we feel that mechanical losses were responsible for the low yield since we observed the recovery of ammonia from the ammonium chloride-sodium methoxide reaction described in the experimental section to be 96-100%.

The reaction has been carried out on scales ranging from 0.3 mol to 1.0 mol of carbon monoxide and could be used on larger and smaller scales without difficulty. A moderate-pressure reaction vessel is required, but the method affords a simple one-step synthesis of isotopic urea from carbon monoxide and ammonia.

EXPERIMENTAL

Materials and Methods--A stainless steel gas sampling cylinder (1000 cm^3 or 3000 cm^3, depending on reaction scale) with a pressure rating of 2.8 MPa (400 psig) was used as a reaction vessel. One end of the cylinder was closed with a threaded thermometer (Weston 4303), and the other end of the cylinder was equipped with a removable pipe-tee fitting containing a pressure gauge and inlet valve. Carbon-^{13}C monoxide, carbon-^{12}C monoxide, and ammonium-^{15}N chloride (obtained by hydrolysis of calcium nitride-$^{15}N_2$) were produced at this Laboratory.* Melting points were

*Los Alamos Scientific Laboratory Isotope Separation Facility, Groups CNC-4 and CNC-2.

determined using a Fischer-Johns melting point apparatus and are uncorrected. Ir spectra were recorded on a Perkin-Elmer 710 spectrophotometer using polystyrene calibration lines and are reported to the nearest 5 cm^{-1}. Proton-nmr spectra were recorded on a Perkin-Elmer R-24 60-MHz spectrometer using TMS as an internal standard. Carbon-13 and nitrogen-15 nmr spectra were recorded on a Varian XL-100 spectrometer interfaced with a Supernova computer and using an external D_2O capillary lock.

Urea-^{13}C--The 3000-cm^3 stainless steel reaction cylinder was charged with sulfur (32 g, 1.0 mol) and methanol (150 g) previously saturated with hydrogen sulfide by bubbling the gas through the liquid for 20 min. After closing the reaction cylinder with the pipe-tee fitting and cooling the cylinder to Dry Ice temperature, ammonia (45 g, 2.6 mol) was cryogenically transferred to the reaction cylinder from a 300-cm^3 gas cylinder. Carbon-^{13}C monoxide (21 g, 0.71 mol, 89.96 mol % ^{13}C, 6.94 mol % ^{18}O, containing 0.5 g methane) was then added to the reaction cylinder from a similar gas cylinder. After wrapping with flexible heating tape and asbestos tape, the reaction cylinder was fastened to a wrist-action shaker and heated to 100°C with shaking. The maximum pressure in the cylinder, 1.4 MPa (200 psig), was observed as the temperature reached 100°C and declined thereafter. The infrared spectrum of a sample of the reaction gases taken at 3 hr showed 18% unreacted carbon-^{13}C monoxide. A similar spectrum taken at 6 hr showed no residual carbon monoxide, and heating and shaking were ceased. After cooling, the reaction cylinder was vented, drained, and washed with warm methanol. Reduced-pressure evaporation of the solvent from the dark-red reaction solution and combined washings gave a green-grey solid (55 g) which was extracted with warm water (250 cm^3). Filtration of the aqueous extract followed by evaporation of the solvent under reduced pressure afforded crude urea-^{13}C (41 g, 95%). Recrystallization from ethanol (Norit) gave the product (37.5 g, 87%) as colorless needles, mp 134-135°C: nmr (δ, DMSO-d_6) 5.5 broad; ir (KBr) 3440, 3340, 3245, 1670, 1620, 1570, 1435, 1145 cm^{-1}.

Urea-^{12}C--The product was prepared and isolated as described above by substituting carbon-^{12}C monoxide (*ca.* 99.995 mol % ^{12}C) in the reaction: nmr (δ, DMSO-d_6) 5.5 broad; ir (KBr) 3445, 3340, shoulder, 1675, 1610, 1460, 1150 cm^{-1}.

Urea-^{13}C-$^{15}N_2$--A 1000-cm^3 three-neck flask equipped with a magnetic stirrer,

gas inlet tube, and condenser connected to two vacuum traps immersed in liquid nitrogen was charged with ammonium-^{15}N chloride (24.7 g, 0.45 mol, 99 mol % ^{15}N), methanol (200 cm^3), and a solution of sodium (16 g, 0.7 mol) in methanol (160 cm^3). The apparatus was flushed with helium while the ammonium chloride-sodium methoxide slurry was refluxed. After refluxing and flushing for 6 hr, the contents of the vacuum traps (20 g of a methanol-ammonia-^{15}N mixture) were cryogenically vacuum-transferred to a 300-cm^3 gas cylinder.

The 1000-cm^3 stainless steel reaction cylinder was charged with sulfur (10.6 g, 0.33 mol) and hydrogen sulfide saturated methanol (50 cm^3), sealed with the pipe-tee fitting, and cooled to Dry Ice temperature. The methanol-ammonia-^{15}N mixture was transferred to the reaction cylinder, followed by carbon-^{13}C monoxide (10.1 g, 0.34 mol, 91.88 mol % ^{13}C, 6.26 mol % ^{18}O, containing 0.4 g methane). The reaction cylinder was wrapped in heating tape and asbestos tape, fastened to a wrist-action shaker, and heated to 100°C with shaking for 8 hr. Isolation of the product as described above gave urea-^{13}C-$^{15}N_2$ (5.0 g, 35%) as colorless needles, mp 133-135°C: nmr (δ, DMSO-d_6) 5.5, J_{NH} = 89 Hz; ^{13}C-nmr and ^{15}N-nmr gave J_{NH} = 89 Hz, J_{CN} = 19 Hz; ir (KBr) 3435, 3330, 3230, 1660, 1610, 1570, 1425, 1145 cm^{-1}.

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