

NOTE

Synthesis of [2'-¹³C]Biotin

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Summary

[2'-¹³C]Biotin was synthesized in 51% yield by reaction of the hydrolysis product of biotin, 3,4-diaminotetrahydro-2-thiophenvalerianic acid, with [¹³C]phosgene.

Key words: Carbon-13; [¹³C]biotin; [¹³C]phosgene; C-C, C-H, C-N spin coupling constants

Introduction

Biotin (1) is the most important prosthetic group for carbon-dioxide transferring enzymes¹. Carbon-13 labelled biotin was required as an NMR-probe to study the mechanism of action at the active site of the transcarboxylase enzyme. The methods of Hofmann et al.² and Melville et al.³ were improved to provide yields over 50% compared with 12% by optimising the reaction conditions.

The shortest route to [2'-¹³C]biotin (3) is synthesis via hydrolysis of biotin to the diamino carboxylic acid (2) which is reacted with [¹³C]phosgene. Hydrolysis of (1) in a glass autoclave at 160° for 4 days and recrystallisation of the resulting product gave 89% yield of (2), but only 65% using the procedure of Hofmann et al.². [¹³C]Phosgene was synthesized by modification of published procedures^{4,5}.

[2'- ^{13}C]Biotin (3) was prepared in 51% yield by slowly warming up a flask with the frozen alkaline solution of diamino acid (2) and the condensed [^{13}C]phosgene followed by stirring overnight. The isotopic purity was determined by mass spectra and integration of inverse gated decoupled ^{13}C -NMR spectra⁶ to be 91.9%. In addition 1.6 g of unreacted diamino acid (2) and 0.8 g of another diastereomer of biotin were isolated.

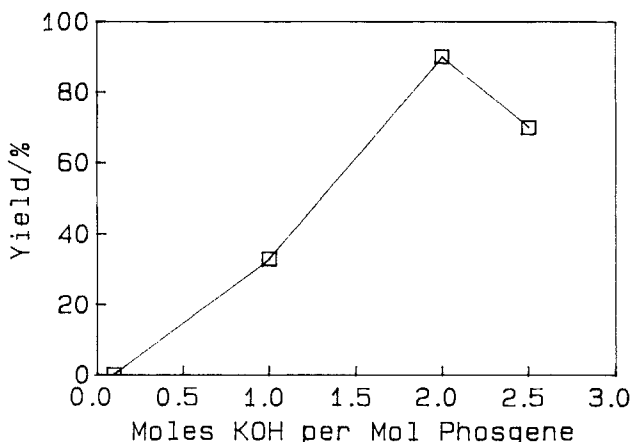


Figure 1. Dependence of the yield on the molar ratio of base to phosgene.

Overall yields were improved because of longer reaction times for the hydrolysis. Only a stoichiometric amount of base was used in the phosgenation reaction compared with nearly 3 equivalents as described by Melville et al.³ relative to the expected amount of HCl liberated by the reaction of phosgene. Model studies on the reaction of the diamino acid (2) with excess phosgene using various amounts of base showed the dependence of the yield on the molar ratio of the base to the amount of phosgene used (see figure 1).

In the proton-NMR-spectrum the labelling was observed by the change of the AB-systems of the 3-H and the 4-H multiplets to the more complicated ABX-systems (see figure 2).

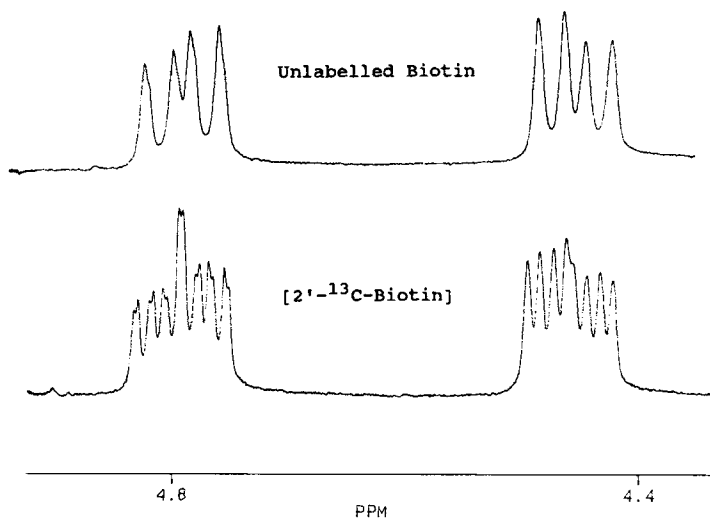


Figure 2. Proton-NMR-spectra of H-3 and H-4 of unlabelled (1) and [2'-¹³C]biotin (3).

³J_{C-H}-couplings were determined by single frequency decoupled ¹³C-spectra: ³J_{C-2',-H-3}=2.3 Hz, ³J_{C-2',-H-4}=1.7 Hz. The ⁴J_{C-H}-couplings could not be resolved. The ³J_{C-C}-couplings were determined by normal ¹H-decoupled ¹³C-spectra: ³J_{C-2',-C-3}=3.9 Hz, ³J_{C-2',-C-4}=3.9 Hz. Two ⁴J_{C-C}-couplings were observed: ⁴J_{C-2',-C-2}=0.9 Hz, ⁴J_{C-2',-C-5}=0.7 Hz. The ¹J_{C-N}-coupling constants were determined by an inverse ¹⁵N-¹H-correlation spectrum⁷: ¹J_{C-2',-N-1},¹J_{C-2',-N-3}=18.5 Hz. ¹J_{N-H}-couplings were determined from this spectrum to 94 Hz. The ¹H and ¹³C-signals for the diamino acid sulfate and the diamino acid were assigned by COSY-spectra and inverse C,H-correlation spectra.

Experimental

Materials

[¹³C]Carbon monoxide was purchased from MSD-Isotopes, Montreal, Canada. Biotin was a gift from BASF, Ludwigshafen, Germany. NMR-spectra were recorded on a Bruker AM-400 (400 MHz) or a Bruker AC-300 (300 MHz) spectrometer. Electron-ionization (EI)-mass spectra were obtained on a Varian CH7 mass spectrometer.

3,4-Diaminotetrahydro-2-thiophenvalerianic acid (2)

A mixture of 8 g (1) with 100 g barium hydroxide in 500 ml water was placed in a glass autoclave and heated to 160°C for 4 days. The resulting mixture was bubbled with carbon dioxide until neutralization. After filtration the solution was concentrated in vacuo. A yellow-brown oil was obtained which was dissolved in 50 ml of water. Acidification with 2N H₂SO₄ to pH 3.5 yielded a small amount of precipitate which was filtered off. Concentration of the solution to 5 ml and addition of 3-4 ml methanol gave on crystallisation 9.2 g of (2)sulfate (colorless crystals, mp. 258°-260° C).

¹H-NMR(D₂O, internal dioxan (=3.53 ppm), pH 4.0): = 4.06-3.96(m, 2H, H-3, H-4), 3.69-3.57(m, 1H, H-2), 3.20-3.13 (dd, 1H, H-5_B), 2.82(t, 1H, H-5_A), 2.18(t, 2H, H-9), 1.74-1.14(m, 6H, H-6,H-7,H-8).

¹³C-NMR(D₂O, internal dioxan (=66.6 ppm), pH 4.0): =178.7(C-10), 56.1(C-3), 53.8(C-4), 46.5(C-2), 33.4(C-9), 29.2(C-5), 27.7, 27.0, 23.7(C-6,C-7,C-8).

Neutral diamino carboxylic acid (2) was prepared by dissolving the diamino acid sulfate in 20 ml of water and titrating this solution with 2N Ba(OH)₂ to pH 7.0. The resulting precipitate was filtered off and the solution was concentrated in vacuo to afford 6.3 g of (2) (slightly yellow crystals, mp. 190°C).

¹H-NMR(D₂O, internal dioxan(=3.53 ppm), pH 7.0): =3.62-3.42 (m, 2H, H-2, H-4), 3.38(t, 1H, J=3.9 Hz, H-3), 2.92(dd., 1H, H-5, 2.55(t, 1H, J=10.7 Hz, H-5), 1.96(t, 2H, J=7.3 Hz, H-9), 1.62-1.07(m, 6H, H-6,H-7,H-8).

¹³C-NMR(D₂O, internal dioxan(=66.6 ppm), pH 7.0): =184.4(C-10), 57.5(C-3), 56.3(C-4), 50.1(C-2), 38.1(C-9), 30.5(C-5), 30.4, 28.4, 26.4 (C-6, C-7, C-8).

$[^{13}\text{C}]$ Phosgene

The apparatus used for the synthesis of $[^{13}\text{C}]$ phosgene and $[^{13}\text{C}]$ biotin is shown in figure 3.

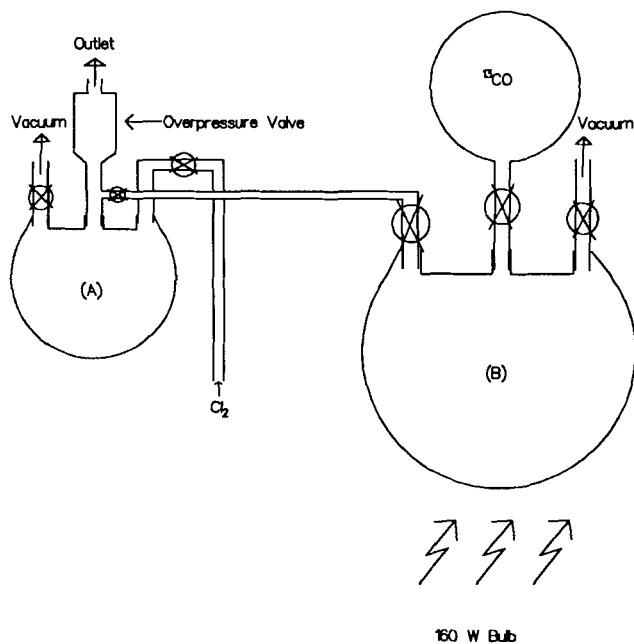


Figure 3. Apparatus for the preparation of ^{13}C -labelled phosgene and reaction with the diamino carboxylic acid (2).

Chlorine was measured by filling the evacuated three necked flask A until bubbling of the overpressure valve, and was condensed into the evacuated, liquid nitrogen cooled three necked flask (B). $[^{13}\text{C}]$ Carbon Monoxide from flask (C) was condensed into flask (B). Warming of flask (B) with parallel irradiation of the flask with the light of a 160 W bulb and continuing the irradiation 3 hours yielded quantitatively $[^{13}\text{C}]$ phosgene.

[2'-¹³C]Biotin (3)

A solution of 4.7 g diamino acid (2) (0.021 mol) and 6.0 g KOH (0.107 mol) in 50 ml of water was placed in flask (A). This solution was cooled with liquid nitrogen and the flask was evacuated. The labelled phosgene was condensed from flask (B) to flask (A). After evacuation of flask (A) the solution was slowly warmed to room temperature and stirred overnight. Precipitation of labelled biotin commenced after 0.5 h.. Filtration of the solution followed by concentration to 10 ml gave 2.7 g (51.1 % yield) of [2'-¹³C]biotin, mp. 232°C.

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