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CHEMISTRY

Synthesis of Deuterated or Tritiated Glycine and Its Methyl Ester

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Abstract—Heating glycine (Gly) and methyl glycinate ($GlyOCH_3$) supported on 5% Pd/C or 5% Pt/C in a deuterium or tritium gas atmosphere gave the isotope-labeled products. The experiments were carried out at 180°C for 10 min. The deuterium atom inclusion under these conditions averaged up to 1.8 atoms per molecule for Gly and up to 1.0 atom per molecule for $GlyOCH_3$. The reaction with tritium gas gave labeled products with a specific radioactivity of 27–31 Ci/mmol for Gly and 18 Ci/mmol for $GlyOCH_3$.

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Usually, glyprolines are deuterated and tritiated using precursors that contain commercial dehydroproline [1–3]. The hydrogen isotope labeling of glycine (Gly) is possible only via isotope exchange. The Gly molecule contains only two non-labile protons; however, for the preparation of a highly labeled product, thorough adjustment of reaction conditions is required. In the synthetic routes to glyprolines using labeled Gly ([^{2}H]Gly), it may be convenient to label the Gly methyl ester ([^{2}H]$GlyOCH_3$). The product thus obtained can be directly condensed with other amino acids.

The purpose of this study is to prepare hydrogen isotope-labeled Gly and Gly methyl ether.

The 5% Pd/C and 5% Pt/C catalysts were used. Glycine, glycine methyl ester, and *N*-methylglycine (sarcosine) were commercial chemicals. The initial compounds and the reaction products were characterized using high-performance liquid chromatography (HPLC) and mass spectrometry. Electrospray ionization mass spectra were recorded on a Thermo Electron LCQ Advantage MAX instrument with direct injection of the sample with 10 μg/mL concentration in 0.1% acetic acid and subsequent molecular ion fragmentation in the analyzer via ion collisions at 35 eV.

Data on the effect of temperature, reaction time, and nature of the catalyst on the isotope exchange efficiency are summarized in Table 1.

The yields of deuterium-labeled Gly and $GlyOCH_3$ in the reaction carried out for 5 min varied in the 30–40% range. As the time of the reaction at 180°C increased, the yield of Gly decreased from 30% (5 min) to 15% (10 min), 10% (15 min), and 0% (30 min). The stability of $GlyOCH_3$ proved to be higher: the yield decreased only to 15% for the reaction time of 30 min. The degrees of deuteration observed for palladium and platinum catalysts differed insignificantly. On temperature rise, the degree of deuteration increased. Both the degree of deuteration and the yield peaked and then declined; the peak levels depended on both time and temperature and were the highest for 180°C and 10–15 min for both Gly and $GlyOCH_3$.

Table 1. Dependence of the deuterium atom incorporation into Gly and $GlyOCH_3$ on time and catalyst nature (20 mg of glycine in 150 μL of water was deposited on 200 mg of 5% palladium or platinum on carbon, then ultrasonicated and freeze-dried)

T, °C	Catalyst	Time, min	Average content of ^{2}H		
			Gly	$GlyOCH_3$	CH_3–NH–CH_2COOH
140	5% Pd/C	5	1.1	0.5	2.0
	5% Pt/C	5	1.2	0.6	2.3
160	5% Pd/C	5	1.2	0.6	2.2
	5% Pt/C	5	1.3	0.7	2.4
180	5% Pd/C	5	1.5	0.8	3.3
		10	1.8	1.0	4.0
		15	1.7	1.1	4.2
		30	–	1.0	3.9
	5% Pt/C	5	1.4	0.8	3.2
200	5% Pd/C	5	1.5	0.9	3.5
	5% Pt/C	5	1.5	0.9	3.4

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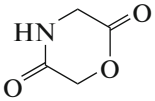
Table 2. Compounds present in the reaction mixture after heating of Gly and $GlyOCH_3$ deposited on 5% Pd/C in a H_2 gas atmosphere (400 hPa, 180°C, 10 min) according to mass spectrometry data

Reaction product	Molecular peaks	
	[M + H]	[M − H]
Products formed from glycine		
Gly	76	74
$CH_3–NH–CH_2COOH$	90	88
$NH_2–CH_2CONHCH_2COOH$	133	131
$HOOC–CH_2–NH–CH_2COOH$	134	132
$CH_3–NH–CH_2COOCH_3$	104	–
Products formed from methyl glycinate		
$GlyOCH_3$	90	–
Gly	76	74
$CH_3–NH–CH_2COOH$	90	88
$CH_3–NH–CH_2COOCH_3$	104	–
$CH_3OOC–CH_2–NH–CH_2COOCH_3$	162	–
	116	–

It is worth noting that deuteration of Gly gave a compound differing from $GlyOCH_3$, but with the same molecular weight (Table 2). The methyl group was attached to the amino rather than carboxy group. This was indirectly evidenced by the fact that this methyl group was not removed upon the conventional alkaline hydrolysis. Furthermore, this compound differed from O-methylated Gly in the chromatographic mobility and was identified as *N*-methylglycine (sarcosine). Analysis was carried out on a Reprosil pur DIOL column (Dr. Maisch GmbH, Germany, 4 × 150 mm size, 3 μm particle size) in the acetonitrile—10 mM $NH_4H_2PO_4$ solvent system (75 : 25) at a 1 mL/min flow rate.

This conclusion is not at variance with our earlier data [4, 5]. The tritiation of polyamides (5% Pd/C, 190°C, 15 min) was accompanied by active deamination. For example, ethylenediamine was converted to secondary amines under these conditions. Hence, Gly may be converted to $HOOC–CH_2–NH–CH_2COOH$ and further (upon decarboxylation) to $CH_3–NH–CH_2COOH$ with the molecular weight equal to that of $GlyOCH_3$ (Table 2).

This may account for the appearance of isotopomers of methylated Gly containing 4 (31%) and 5 (28%) deuterium atoms in the mass spectra of the deuterated reaction mixtures obtained from Gly. Meanwhile, much less deuterium atoms are incorporated in the molecule upon the deuteration of $GlyOCH_3$.

The tritiation was conducted by placing 17 mg of 5% Pd/C with Gly (10 : 1) into a tube and evacuating the tube down to 0.1 hPa. Tritium gas was injected up to a pressure of 400 hPa. The reaction was carried out at 180°C for 10 min. After removal of tritium gas, the products were washed on the filter with aqueous methanol (three times, 1 mL each) to remove the catalyst. Labile tritium was removed by evaporation of solvents. Labeled glycine was purified by HPLC on a Reprosil pur Diol column (10 × 150 mm size, 5 μm particle size) in the acetonitrile–10 mM $NH_4H_2PO_4$ solvent system (75 : 25) at a 2 mL/min flow rate. The retention times were as follows (min): Gly, 9.51; $GlyOCH_3$, 7.95; sarcosine, 8.55. The yield of labeled glycine was 50% and the specific radioactivity was 27–31 Ci/mmol. Labeled $GlyOCH_3$ obtained under similar conditions had a specific radioactivity of 18–20 Ci/mmol.

Thus, the simultaneous formation of labeled $CH_3–NH–CH_2COOH$ (sarcosine) with a specific radioactivity of 45–50 Ci/mmol can be considered as an additional result of the study. Sarcosine is known to be investigated for treatment of the prostate cancer and to be used as a nootropic agent against mental depression and schizophrenia symptoms. The presence of the labeled sarcosine analogue will be beneficial for further investigation.

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REFERENCES

1. Shevchenko, V.P., Nagaev, I.Yu., Alfeeva, L.Yu., Andreeva, L.A., Shevchenko, K.V., and Myasoedov, N.F., *Radiokhimiya*, 2004, vol. 46, no. 1, pp. 63–71.
2. V'yunova, T.V., Andreeva, L.A., Shevchenko, K.V., Shevchenko, V.P., Bobrov, M.Yu., Bezuglov, V.V., and Myasoedov, N.F., *Dokl. Akad. Nauk*, 2008, vol. 419, no. 1, pp. 136–137.
3. Shevchenko, V.P., Nagaev, I.Yu., Andreeva, L.A., and Myasoedov, N.F., *Radiokhimiya*, 2010, vol. 52, no. 3, pp. 277–280.
4. Shevchenko, V.P., Nagaev, I.Yu., and Myasoedov, N.F., *Mechennye tritiem lipofil'nye soedineniya* (Tritium-Labeled Lipophilic Comppounds), Moscow: Nauka, 2003.
5. Shevchenko, V.P., Nagaev, I.Yu., Potapova, A.V., and Myasoedov, N.F., *Radiokhimiya*, 1994, vol. 36, no. 5, pp. 440–444.

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