

TRITIUM ISOTOPE EFFECT IN BIRCH REDUCTIONS

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SUMMARY

D-2-Aminophenylacetic acid (1) (phenylglycine, PG) was subjected to a Birch reduction in the presence of tritium ions. A substantial, but relatively low isotope effect, $k_H/k_T=3.51$, was found for the reduction reaction. The resulting D-2-amino-[3,6-³H](1,4-cyclohexadien-1-yl)acetic acid (2) (dihydrophenylglycine, DHPG) aromatized rapidly by a secondary radio-decomposition to afford mainly P[³H]G, resulting in the predicted loss of half the tritium. When a carbon-14 label was used, the DHP[¹⁴C]G aromatized about seven times faster than DH[³H]PG normalized to the same specific activity, when stored as a solid. This difference is in good agreement with the ratio predicted based on the difference in decay energies for the two isotopes.

Key Words: Birch reduction, D-2-aminophenylacetic acid, Phenylglycine, Isotope effect, Radiodecomposition, Aromatization

INTRODUCTION

In drug metabolic studies, where low specific activities are usually employed, ¹⁴C-labeled compounds are normally preferred over those labeled with tritium. The latter are potential candidates for isotope effects and may release labile tritium, which, if not detected, may result in a distorted picture of the metabolism of the compound.

Labeled DHPG (Fig. 1) was required for the synthesis of two labeled semi-synthetic antibiotics, cephradine and epicillin. DHPG was initially labeled with ¹⁴C, but was found to be very unstable and readily converted to P[¹⁴C]G by aromatization. [¹⁴C]-Epicillin had similar stability characteristics, whereas [¹⁴C]-cephradine decomposed about three times faster than DHP[¹⁴C]G. This aromatization presents a secondary radiodecomposition which, in the case of low

energy β -emitters, is approximately proportional to the specific activity and the energy of decay of the particular isotope, when the compound is stored as a solid and the radiation is absorbed at a relatively high level by the system. $\text{DH}[^3\text{H}]\text{PG}$ at a given specific activity should, therefore, aromatize at about one-eighth of the rate of $\text{DHP}[^{14}\text{C}]\text{G}$ at the same specific activity, since the decay energy for ^3H is 18.6 KeV as compared to 156 KeV for ^{14}C .

$\text{DH}[^3\text{H}]\text{PG}$ was synthesized by a modified Birch reduction as outlined in

Fig. 1:

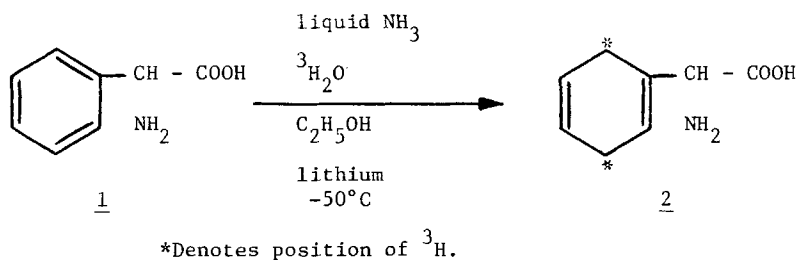


Figure 1

1 D-2-aminophenylacetic acid, (PG)

2 D-2-amino[3,6- ^3H](1,4-cyclohexadien-1-yl)acetic acid, ($\text{DH}[^3\text{H}]\text{PG}$)

A Birch reduction of PG with metallic lithium, if not carefully carried out, will either result in some unreacted PG or will further reduce the DHPG to tetrahydrophenylglycine (THPG). This makes it difficult to prepare pure $\text{DHP}[^{14}\text{C}]\text{G}$ on a small scale. The use of ^3H in the synthesis of labeled DHPG, as opposed to ^{14}C , provides the distinct advantage that any unreacted PG will not be radioactive. For this reason, the reduction was carried out on a small scale and was purposely terminated prior to completion. The resulting $\text{DH}[^3\text{H}]\text{PG}$ was then diluted with unlabeled DHPG, which usually contains 1-1.5% PG as an impurity.

RESULTS AND DISCUSSION

The radiochemical yield for the Birch reduction, assuming a complete exchange with the three hydrogen atoms of ammonia, the two hydrogen atoms of water and the one hydrogen atom of ethanol, was calculated to be 0.59%. The experimental yield, however, was found to be only 0.17%, indicating an isotope effect.

As the $\text{DH}[^3\text{H}]\text{PG}$ was found to contain some semilabile tritium, the total radiochemical yield of the Birch reduction was calculated from the end product, $[^3\text{H}]\text{-cephradine}$, which was purified by Preparative High Pressure Liquid Chromatography (C-18 Reverse Phase Column, 4% acetonitrile in water) to a radiochemical purity of 98.6%, and which had a molar specific activity 6.5% lower than $\text{DH}[^3\text{H}]\text{PG}$.

When DHPG, as a solid, is exposed to air, a small amount aromatizes back to PG. The presence of β -radiation greatly accelerates this process, due perhaps to the formation of NO_2 from atmospheric nitrogen and oxygen. (Exposure of DHPG to NO_2 resulted in the formation of the same decomposition products.) About 60% of the degradation products was accounted for as PG; the other three major degradation products were not identified. Since the rate of decomposition is a function of surface area, the presence of certain trace impurities, and many other parameters, an accurate comparison of the decomposition rates is not possible (1). The decomposition rates, when the solid was stored at 5°C , were 0.3% per mCi/mMole per month for $\text{DH}[^3\text{H}]\text{PG}$ (G(-M) value:340) and 2.0% per mCi/mMole per month for $\text{DHP}(^{14}\text{C})\text{G}$ (G(-M) value:250). The G(-M) value (1) indicates how many molecules are decomposed per 100 eV (electron Volts) absorbed by the system. Values were calculated with the equations cited in the reference, except that the radiation was assumed to be 100% absorbed, since the actual values were not determined. When the percentage of absorbed radiation is known and is introduced into the equations, the G(-M) values of the tritium and carbon-14 labeled DHPG should be identical. For reference, a compound having a G(-M) value larger than 10 is generally considered unstable.

EXPERIMENTAL

Materials and Methods

Tritiated water, 30.5 Curies, was prepared from tritium gas and platinum oxide by Dr. J. Moravek of Moravek Biochemicals, 15302 E. Proctor Ave., City of Industry, California, 91745.

Liquid scintillation counting was carried out on a Model 2425 Packard Tri-Carb® Liquid Scintillation Spectrometer. Samples were counted in 15 ml TX-114

cocktail (2). Efficiencies were determined by the external standardization method.

Thin-Layer Chromatography

Support: Cellulose, Eastman Chromagram® No. 6065
Spotting Solution: 1 mg/ml 1 N HCl
Amount spotted: 4 microliters, 4 micrograms
Developing solvent: 1-butanol/pyridine/acetic acid/water (30:20:6:24)
Visualization: Cadmium-ninhydrin spray (3)

The R_f values of PG, DHPG and THPG increase in this order. The compounds chromatograph close together but are reasonably well separated at R_f ca. 0.7. Ninhydrin gives a red color with PG, and a yellow color with DHPG and THPG, which turns a stable red color after several hours. The amount of unreduced PG was determined by visual comparison of the sample with a series of DHPG standards containing 2, 5, 10, 15 and 20% PG.

Birch Reduction

D-2-Aminophenylacetic acid (PG), 60 mg*, was placed into a 30 ml reaction flask equipped with a movable sidearm, a septum, and a stopcock. Metallic lithium, 12 mg (four pieces), was charged into the sidearm, connected to the reaction flask and evacuated on a vacuum line. Using liquid nitrogen to cool the receiver, about 20 mg $^3\text{H}_2\text{O}$ (30.5 Curies) was distilled into the flask, followed by 0.20 ml ethanol and 1.0 ml liquid ammonia. The temperature was increased to -50°C and the mixture stirred until a solution was obtained. The Birch reduction was started by the addition of the metallic lithium. After stirring for 5 to 15 minutes, the reaction was stopped by the addition of 1 ml methanol through the septum. Volatile substances were distilled into a flask for the collection and radiometric assay of the total labile tritium, which for practical purposes represented the total radioactivity used in the reduction.

Labile tritium from $\text{DH}[^3\text{H}]\text{PG}$ was largely but not completely removed by the addition and subsequent removal of 1.0 ml 2N NH_4OH and 1.0 ml 2 N HCl. The

*Larger amounts give solubility problems.

residue, $\text{DH}[^3\text{H}]\text{PG}$, was dissolved in 60.0 ml 2 N HCl and assayed. Thin-layer chromatography indicated 5% PG and 95% $\text{DH}[^3\text{H}]\text{PG}$. The radiometric assay showed a total of 62.0 mCi.

Unlabeled DHPG, 5.00 g, was added to the above solution and the solution was stored at room temperature for 1 to 2 hr[†]. The pH of the solution was then adjusted to 6.5 by the dropwise addition of 28% ammonium hydroxide solution, which precipitated the $\text{DH}[^3\text{H}]\text{PG}$. Crystals were removed by filtration, washed with 80 ml distilled water and 25 ml ethanol, and dried in vacuo (yield: 4.14 g, 85% by mass).

The radiometric assay of $\text{DH}[^3\text{H}]\text{PG}$ gave a specific activity of 10.7 $\mu\text{Ci}/\text{mg}$. When $[^3\text{H}]$ -cephradine was synthesized from this material without the addition of carrier, the molar specific activity of $\text{DH}[^3\text{H}]\text{PG}$ was found to be 1.55 mCi/mMole (specific activity of 10.1 $\mu\text{Ci}/\text{mg}$).

Radioassay of Total Labile Tritium

All labile tritium was collected in one container, the volume adjusted to 50 ml and two aliquots were assayed. A total of 30.5 Curies of ^3H was found.

Isotope Effect

The following equation (4) was used:
$$\frac{k_{\text{H}}}{k_{\text{T}}} = \frac{\log(1-F_1)}{\log[1-(F_1 R_p/R_o)]}$$

F_1 = fraction of starting material (^3H) that had reacted

R_p = specific activity of accumulated product; $\text{DH}[^3\text{H}]\text{PG}$

R_o = specific activity of initial reactant; ^3H

Total milliatoms of hydrogen (mA_{H}) in the reaction: 126.

$\text{DH}[^3\text{H}]\text{PG}$ (MW 153.2)

Yield, mass: 60 mg x 0.95 = 57 mg

Yield, total milliatoms hydrogen: $\frac{57 \times 2}{153.2} = 0.74 \text{ mA}_{\text{H}}$

Yield, total radioactivity: 5057 mg x 10.1 $\mu\text{Ci}/\text{mg}$ = 51.1 mCi

[†]Earlier experiments had shown that some semilabile tritium is liberated in acidic solution.

$$F_1 = \frac{0.74 \text{ mA}_H}{126 \text{ mA}_H} = 0.0059 \quad R_p = \frac{51.1 \text{ mCi}}{0.74 \text{ mA}_H} = 69.1 \text{ mCi/mA}_H$$

$$R_o = \frac{30500 \text{ mCi}}{126 \text{ mA}_H} = 242 \text{ mCi/mA}_H$$

$$\frac{k_H}{k_T} = \frac{\log(1-0.0059)}{\log[1-(0.0059 \times 69.1/242)]} = 3.51$$

Aromatization

DHP[¹⁴C]G as a solid (97 μCi/mg; 14.9 mCi/mMole) decomposed by 28% during one month at 5°C; ca. 60% of the decomposition product was identified as P[¹⁴C]G. DH[³H]PG (10 μCi/mg; 1.5 mCi/mMole) decomposed by 0.5% when stored for one month under identical conditions. [³H]PG, isolated from a decomposed sample, was found to have half the specific activity of the DH[³H]PG from which it originated.

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