Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXIV, No. 11

Synthesis and stability of [1,3-$^{14}C_2$]-glyceryl trinitrate

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Summary

The synthesis of [1,3-$^{14}C_2$]-glyceryl trinitrate ([^{14}C]-GTN) labelled with quasi carrier free carbon-14 atom in the 1,3-positions of the molecule was effected in four reaction steps from $^{14}CO_2$ (3,5 % overall yield). The chemical and radiochemical purity of [^{14}C]-GTN was found better than 99% controlled by HPLC-Radioactivity Monitor and TLC-Digital Autoradiograph methods. Study of the radiochemical stability in dilute solution at 5 °C shows that the product can be stored at least for a month without detectable decomposition.

Key words: anti-anginal agents,carbon-14, [^{14}C]-glycerol, [^{14}C]-nitroglycerin

Introduction

The study of biodegradations[1,2] and metabolism of nitroglycerin which is regarded as the most effective anti-anginal agent, requires the synthesis of [^{14}C]-GTN with high specific activity. Controlling the stability of labelled GTN was also required for biological research.

Results and discussion

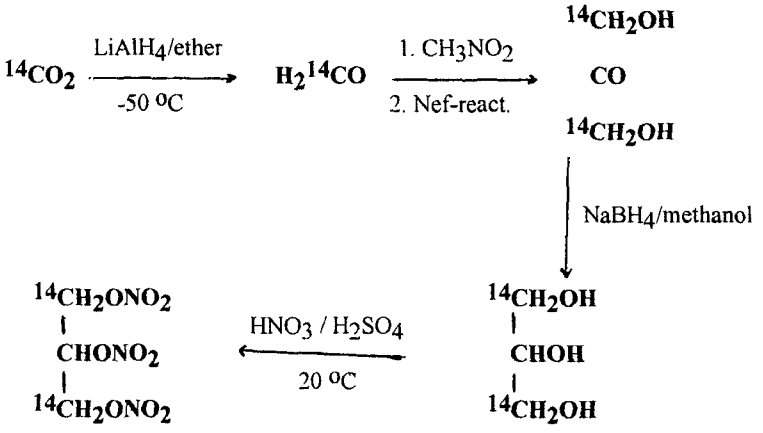
The synthesis of [1,3-$^{14}C_2$]-glycerol is widely discussed in the literature[3]. Some reference[2,4] can be found for the synthesis of [^{14}C]-GTN as well. In our procedure,

CCC 0362–4803/94/111111–05

Received 13 July, 1994
Revised 17 July, 1994

the synthesis of [^{14}C]-GTN from $^{14}CO_2$ was realized in the reaction steps shown in Scheme 1. Vial technique(5) was employed for the production of [^{14}C]-formaldehyde. [1,3-$^{14}C_2$]-Dihydroxyacetone formed in the reaction of [^{14}C]-formaldehyde with nitromethane was reduced with sodium borohydride in the solution of methanol. The crude [1,3-$^{14}C_2$]-glycerine formed was esterified by the well known procedure(6) in HNO_3/H_2SO_4 (1:2) solution at 20°C. After neutralization and prepurification the crude product was further purified by the HPLC method.

The quantity of [^{14}C]-GTN formed was determined by comparison with the USP standard of nitroglycerin. The specific activities of [^{14}C]-GTN calculated from these data and the radioactivities of the samples gave values between 135-140 mCi/mmol. These results are by about 15 per cent higher than that calculated from the specific activity of $^{14}CO_2$ (59 mCi/mmol).



Scheme 1.

Experimental section

[^{14}C]-Formaldehyde

A vial technique developed(3) for the synthesis of ^{14}C-formaldehyde from barium carbonate-^{14}C was used. Formaldehyde formed in dilute solution is suitable for the synthesis.

Barium carbonate-^{14}C (0,8g, 8,73GBq, 236mCi) was decomposed with 7 ml of 10% hydrochloric acid in a vial. The carbon dioxide liberated was swept from the vial with nitrogen (120 ml/min). The water content of CO_2 was filtered with a tube containing a quartz wool plug and the gas was further dried in a trap filled with calcium chloride. The dried gas stream was bubbled through 20 ml of ether containing 0,38g lithium aluminium hydride at -50 °C. The transfer of $^{14}CO_2$ and the reduction were completed within 5 minutes. The unreacted carbon dioxide (about 1-2%) was absorbed in a vial containing 0,1 N NaOH in methanol.

The reaction mixture was hydrolized with 2 ml of water and 4 ml of 10% sulfuric acid at -5 °C. The ether was removed from the reaction mixture by strong nitrogen stream (500 ml/min) at room temperature. The distillate contained about 2-3% of total radioactivity. The residue was further distilled at 130-140 °C with nitrogen stream bubbling through a receiver containing 5 ml of water cooled in an ice bath. Distillation was continued until a residue of about 1 ml remained in the reaction vessel. Formaldehyde content was determined from an aliquot of the solution by iodometric method. The yield was 1,9 mmol.

[1,3-$^{14}C_2$]-Glycerol

The water solution (4 ml) of [^{14}C]-formaldehyde (1,9 mmol) was neutralized with potassium carbonate and to this solution 0,060g of nitromethane was added. The reaction mixture was stirred at room temperature for 4 hours, then the solvent was evaporated under vacuum at 40 °C. The residue was dissolved in 1 ml of methanol and added to 1 ml of methanol containing 1,7 mmol of sodium methylate. The precipitated sodium salt was collected and solved in 1,5 ml of water. This solution was added to 1 ml of cold 50% sulfuric acid. The mixture was stirred at room temperature for 10 minutes and poured into 2,5 ml of water containing 0,5g of calcium carbonate. The precipitated calcium sulfate was filtered off and washed with water. The water solution of the product was evaporated to 0,2g of silica and purified by column chromatography (10 g of Kieselgel 60, eluent: ethylacetate: methanol= 9,5:0,5). The pure fractions were collected and evaporated to dryness. The residue (0,030g) was dissolved in 2 ml

of methanol, and 8 mg of sodium borohydride was added to the solution. Reduction took place immediately which was controlled by TLC (eluent: ethylacetate: methanol=8:2, detection with 1% potassium permanganate in 1N sodium hydroxyde, R_f for dihydroxy acetone: 0,5 and for glycerine: 0,3). Radioactivity of crude [1,3-$^{14}C_2$]- glycerol solution (2 ml): 524 MB(14,16 mCi), Radiochemical yield was 6% (based on $Ba^{14}CO_3$). A part (0,4ml) from this solution was evaporated in a suitable vial and the residue was used in the next reaction without further purification.

[1,3-$^{14}C_2$]-Glyceryl trinitrate

To the cooled [1,3-$^{14}C_2$]-glycerol dissolved in 0,01 ml of nitric acid, 0,020 ml of sulfuric acid was added. The reaction mixture was stirred with a magnetic stirrer at room temperature for 30 minutes, than cooled with ice water and diluted with 0,5 ml of water. The product was extracted five times with 1 ml of ether. The etheral solution was washed several times with diluted sodium carbonate solution. To the neutralized solution, 1 g of silica was added and evaporated to dryness. The absorbed product was eluted with 5 ml of methanol.

The resulting solution was concentrated to about 1 ml and purified by prepartive HPLC (Nucleosil C-18 column, methanol:water = 6:4) The pure fractions were collected and stored in a dilute solution (0,29mg/ml) of eluent at 5°C in a refrigerator. Yield:61MBq(1.65 mCi). Specific radioactivity: 135-140 mCi/mmol.

Chemical and radiochemichal purity of [^{14}C]-GTN proved to be greater than 99% determined by the following (TLC-Digital Autoradiograph and HPLC--Radioactivity Monitor) methods.

Thin layer chromatography:

TLC-sheet: DC-Alufolien Kieselgel 60 F_{254} (Merck)

Eluent : benzene:ethylacetate:acetic acid =8:2:0,5

Detection: 1% potassium permanganate in 1N NaOH solution and DigitalAutoradiograph [(EG&G Berthold LB 287, WinDAR Software), Run time: 30 min., Gas flow: 5ml/min, Counter voltage: 1100 V].

R_f-value for gliceryl trinitrate : 0,8.

High-performance liquid chromatography:

ISCO HPLC-system was used under the following conditions:

Column: Nucleosil C-18 (5μm , 250x4mm ID)

Mobile phase: methanol:water = 1:1, Flow rate: 1 ml/min.

Detection: at 210 nm by UV detector.

The radioactivity was measured by HPLC-Radioactivity Monitor (Hewlett-Packard 1090M-EG&G Berthold LB506-C-1), Parameters: Scintillator coctail Quickscint Flow 301 (Zinsser Analitic), Dtector cell type: Z-1000 4 (1ml)

Quantity of [^{14}C]-GTN was determined by comparsion with the standard of nitroglicerin (USP XXII Diluted Nitroglicerin RS).

Radiochemical stability of [^{14}C]-GTN in the dilute solution was controlled on the 1st,3rd,7,15 and 30th days by the above methods. These data showed an unexpected stability of this labelled molecule: the decrease in radiochemical purity was not higher than 1% even on the 30th day.

Acknowledgement

This work was partially supported by grants N^{o} 1753 provided by the Hungarian Research Found (OTKA) and EGIS Pharmaceuticals LTD.

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