AN IMPROVED SYNTHESIS OF ^{14}C LABELLED GLYCEROL USING SODIUM BOROHYDRIDE

Harish chander, T.V.Ramamurthy and K.V. Viswanathan,*
Labelled Compounds Section,Isotope Group, B.A.R.C.,
Bombay 400 085, India

SUMMARY

[1-^{14}C]Glyceric acid has been reduced to [1(3)-^{14}C]glycerol in high yields via the methyl ester of [1-^{14}C]glyceric acid by sodium borohydride in the presence of t-butyl alcohol and methanol. The importance of the procedure is highlighted in relation to other procedures involving lithium aluminium hydride reduction.

Key words:[1-^{14}C]Glyceric acid, [1(3)-^{14}C]Glycerol, Sodium borohydride.

Carbon-14 labelled glycerol has been widely used to study the metabolism of lipids, carbohydrates and glycerides. The preparation of ^{14}C labelled glycerol has been the theme of many papers. The early references involve the use of labelled precursors such as [1-^{14}C]glyceric acid[1], [1-^{14}C]acetic acid[2], [1-^{14}C]acrylic acid[3], while a recent paper has used a lengthy but novel scheme for the synthesis of [2-^{14}C]glycerol[4] The procedures for [1(3)-^{14}C]glycerol are lengthy and often inefficient. Pichat et al[3] have also tried a direct reduction of glyceric acid as well as its methyl ester using

*Author to whom all correspondence should be addressed.

0362-4803/87/010049–06$05.00

Received March 21, 1986
Revised May 2, 1986

lithium aluminium hydride to synthesise glycerol but the yields were poor. We tried to modify this method by continuously extracting glyceric acid into a refluxing solution of lithium aluminium hydride in ether over a period of 10 hours. However, the yield, though marginally increased, could not be raised to appreciable levels[5]. The poor solubility of glyceric acid in ether and the presence of free hydroxyl groups probably are responsible for the incomplete reduction of glyceric acid despite the use of a large excess of lithium aluminium hydride[6]. We therefore looked for other procedures for reduction of glyceric acid. Recently, sodium borohydride in combination with alcoholic solvents has been successfully applied for conversion of esters into alcohols[7,8,9]. These esters were derived from aromatic and long chain aliphatic acids. Since glyceric acid could be easily converted to its ester, we wanted to extend the sodium borohydride reduction procedures for this ester. Sodium borohydride has also been used in combination with poleythylene glycol but we thought this method if applied to glyceric acid ester would pose problems in the isolation of glycerol from polyethylene glycol at the end of the reaction. We have observed that the sodium borohydride procedure in presence of t-butanol and methanol was highly convenient to prepare glycerol from methyl glycerate in fairly good yields. We have obtained [1(3)-^{14}C]glycerol in 75% radiochemical yield based on [1-^{14}C]glyceric acid. The results of our investigations on the synthesis of [1(3)-^{14}C]glycerol by different methods of reduction of [1-^{14}C] glyceric acid are presented in Table 1.

Table 1.
Synthesis of [1(3)-^{14}C]glycerol

No.	Amount of [1-^{14}C]glyceric acid (mCi)	(mmole)	Method	Radiochemical yield (%)
1.	0.100	1.0	a	6
2	0.100	1.0	b	16
3.	0.150	1.0	c	66
4.	0.150	1.0	c	68
5.	2.000	0.5	c	75

EXPERIMENTAL

All reagents used were of analar grade. Glycolic aldehyde (Fluka) was dissolved in water and kept for 24 hours for depolymerisation prior to use. ^{14}C Potassium cyanide was obtained from Labelled Compounds Section, Isotope Group, B.A.R.C., Bombay, India.

[1-^{14}C]Glyceric acid

An aqueous solution of depolymerised glycolic aldehyde (132 mg, 2.2 mmole) was added to a flask containing ^{14}C potassium cyanide (10 mCi, specific activity 60 mCi/mmole) and carrier potassium cyanide (119.2 mg, 1.83 mmole). The solution was cooled in an ice bath and sulphuric acid (1.12 ml of 1.78 N) was added followed by sodium hydroxide (0.23 ml of 1.72 N). The reaction mixture was kept stirring for 4 hours, at ice temperature. The mixture was then refluxed with concentrated hydrochloric acid (5 ml) for 4 hours. A paper chromatographic analysis of the crude product followed by autoradiography indicated the formation of [1-^{14}C]glyceric acid with a radiochemical purity of about 95%. The solution was repeatedly evaporated to dryness with water to remove excess of hydrochloric acid. [1-^{14}C]Glyceric acid was separated from the remaining hydrochloric acid and other impurities by passing the mixture through a column of Dowex 50 X 4, 200-400 mesh, H^+ form, (120 cm X 1.2 cm). The elution was performed with water at a flow rate of 6 ml/hour. The fraction 60-75 ml contained [1-^{14}C]glyceric acid. It was analysed for radiochemical purity by paper chromatography in two different solvent systems followed by autoradiography. The radiochemical purity was >99% and R_f values for authentic and radioactive glyceric acid coincided with each other. The solution was counted: activity 7.5 mCi, radiochemical yield 75% with respect to ^{14}C potassium cyanide.

[1(3)-^{14}C]Glycerol from [1-^{14}C]Glyceric acid. (Method a.)

An aqueous solution of the [1-^{14}C]glyceric acid (100 μCi, 0.03 mmole) was transferred to a 25 ml round bottom flask and glyceric acid carrier (102 mg, 0.97 mmole) was added to it. The solution was

evaporated to dryness under a jet of nitrogen gas for 30 minutes at 40°C. An etheral solution of lithium aluminium hydride (95 mg, 2.5 mmole) in 10 ml of dry ether was then added to the glyceric acid and the mixture refluxed for 10 hours. The reaction mixture was then cooled and the complex decomposed by slow addition of water. The solution was centrifuged, and the supernatant containing the [1(3)-^{14}C]glycerol was withdrawn carefully. The residual aluminium hydroxide precipitate was boiled with water and the supernatant was taken out by centrifuging. The process was repeated three times to ensure the complete removal of adsorbed glycerol. The combined supernatant solution was passed through a column of Dowex 50 X 8 resin in H^+ form. The effluent from the column was counted: activity 6 μCi, radiochemical yield 6%.

[1(3)-^{14}C]Glycerol from [1-^{14}C]Glyceric acid. (Method b.)

An aqueous solution of the [1-^{14}C]glyceric acid (100 μCi, 0.03 mmole) was transferred to a liquid-liquid extractor and glyceric acid carrier (102 mg, 0.97 mmole) was added to it. Water was removed under a jet of nitrogen gas for 30 minutes at 40°C. Glyceric acid was continuously extracted into a 50 ml flask containing ether (25 ml) and lithium aluminium hydride (95 mg, 2.5 mmole) for a period of 10 hours. The flask was then disconnected and the complex was decomposed by slow addition of water. Glycerol was isolated as in method (a) and counted: radioactivity 16 μCi, radiochemical yield 16%.

[1(3)-^{14}C]Glycerol from [1-^{14}C]Glyceric acid via methyl ester. (Method c.)

[1-^{14}C]Methyl glycerate.

An aqueous solution of the [1-^{14}C]glyceric acid (2 mCi, 0.50 mmole) was evaporated to dryness in a small round bottom flask under a jet of nitrogen gas at 40°C. To the dry glyceric acid, methanol (2 ml) and dry cation exchange resin, Dowex 50 X 8, H^+ form (20-50 mesh, 300 mg) were added and the mixture refluxed for 5 hours at 90°C. TLC analysis of an aliquot was carried out in the benzene:methanol (80:20, v/v) solvent system. Autoradiography of the TLC indicated that more

than 90% glyceric acid had been converted to methyl glycerate.

Reduction of [1-^{14}C]methyl glycerate to [1(3)-^{14}C]glycerol

The [1-^{14}C]methyl glycerate obtained was carefully transferred into another round bottom flask and methanol washings were also added. The methanol was removed under a current of nitrogen gas, tertiary butanol (2.5 ml) and sodium borohydride (114 mg, 3 mmole) were added to the flask and the mixture was refluxed, adding methanol (0.5 ml) over a period of 1 hour. The refluxing was continued for another two hours. Water (10 ml) was added to decompose the borohydride complex and the reaction mixture was passed through a small column of cation exchange resin, Dowex 50 X 8, H^+ form (20-50 mesh). The effluent was collected until no radioactivity was detected. The solution was rotary evaporated to dryness. Methanol (10 ml) was added to the residue to convert the boric acid into its volatile methyl borate. The mixture was once again rotary evaporated to remove all the volatile methyl borate and the process was repeated twice.

The residue in the flask was taken up in water and the solution was passed through a column of anion exchange resin, Dowex 1 X 8, OH^- form (50-100 mesh) to isolate glycerol from any glyceric acid present. The column was washed with water until the effluent was no more radioactive. The effluent was pooled together and radioactivity was counted. It was found to be 1.5 mCi corresponding to a radiochemical yield of 75%. Aliquots of the [1(3)-^{14}C]glycerol were analysed by paper chromatography in the following two solvent systems followed by autoradiography.

1) n-Butanol : Ethanol : Water :: 104 : 66 : 30 (V/V)

2) n-Propanol : Ammonia (25%) :: 70 : 30 (V/V)

The radiochemical purity was found to be greater than 99%.

REFERENCES

1. Doerschuk A.P. , J. Amer. Chem. Soc., 73, 821 (1951)

2. Gidez L.I. and Karnovsky M.L. , J. Amer. Chem. Soc., 74, 2413 (1952)

3. Seymour Rothchild (Ed.),Advances in tracer methodolgy vol.,3, P. 23, Plenum press, New York (1966)

4. Wirz B. and Kung W., J. Label. Compd. and Radiopharm., 20, 635 (1983)

5. Viswanathan K.V.,Ramamurthy T.V.,and Harish Chander, unpublished work

6. Roger Adams (Ed.), Organic Reactions Vol. VI, P 469, John Wiley, New York (1951)

7. Soai K., Oyamada H., and Ookawa A., Synth. Commun., 12 463 (1982)

8. Santaniello E., Ferraboschi P. and Sozzani P., J. Org. Chem., 46, 4584 (1981)

9. Viswanathan K.V., Garana A.U., Lakshmi R., Padmanabhan D., Int. Symp. Artificial Radioactivity, Pune, India, PAR 23 (1985)