Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXVIII, No. 2

SYNTHESES OF CARBON-13 AND CARBON-14 LABELED (METHYLENECYCLOPROPYL)ACETYL-CoA

John E. Baldwin* and Wayne C. Widdison

Department of Chemistry, Syracuse University
Syracuse, New York 13244-4100

SUMMARY

Methylenecyclopropyllithium reacts with ^{13}C- or ^{14}C-oxirane to give labeled 2-(methylenecyclopropyl)ethanol, which may be converted in three steps to the corresponding coenzyme A thioesters of (methylenecyclopropyl)acetic acid.

Key Words: (Methylenecyclopropyl)acetyl-CoA, enzyme inactivators, hypoglycin, general acyl dehydrogenase

INTRODUCTION

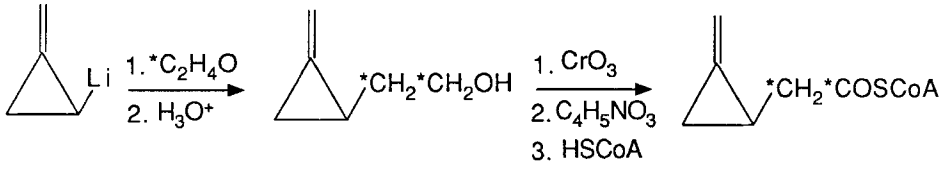
(Methylenecyclopropyl)acetyl-CoA (MCPA-CoA), the metabolite of the naturally occurring amino acid hypoglycin directly responsible for the Jamacian vomiting sickness,[1] is a potent inactivator of the general acyl dehydrogenase from pig kidney (GAD; E.C. 1.3.99.3) and other related acyl dehydrogenases.[1-3] Isotopically labeled versions of MCPA-CoA would facilitate investigations of the structural and mechanistic aspects of this enzyme inactivation process. The desirability of such labeled analogs has long been recognized: with ^{14}C-labeled MCPA-CoA one could, for instance, test for enzyme inactivation through protein modification as well as by reaction between MCPA-CoA and flavin coenzyme.[4,5] ^{13}C-Labeled MCPA-CoA could provide a useful probe for ^{13}C NMR structural studies on the products of inactivation.

0362–4803/90/020175–05$05.00

Received July 13, 1989
Revised August 7, 1989

RESULTS

The synthetic route utilized to prepare carbon-labeled MCPA-CoA thioesters followed well-established precedents. Methylenecyclopropane[6,7] reacts with *tert*-butyllithium and TMEDA in tetrahydrofuran to form methylenecyclopropyllithium (**1**);[8-15] this or related methylenecyclopropyl organometallic reagents may be combined with a variety of electrophilic reagents, such as aldehydes, ketones, nitroalkenes, acid chlorides, α-bromoacetates,[16] and oxiranes,[17] to form through addition or substitution reactions products having a new C-C bond. When ^{13}C- or ^{14}C-labeled oxirane serves as electrophile, the labeled primary alcohol **2** results.



1 **2** **3**

The conversion of **2**-^{14}C in three steps to MCPA-CoA-^{14}C (**3**-^{14}C)was accomplished through a Jones oxidation, preparation of an active ester of (methylenecyclopropyl)acetic acid with *N*-hydroxysuccinimide, and reaction with coenzyme A, as detailed previously.[18] The product was thoroughly purified by HPLC and proved effective as an inactivator of GAD. The same chemistry with oxirane-1,2-$^{13}C_2$ as the source of label afforded **3**-$^{13}C_2$.

DISCUSSION

The synthesis of (methylenecyclopropyl)acetic acid reported in 1987[18] may be adapted through simple variations in labeled reagents and intermediates to provide analogs having a ^{13}C or ^{14}C label in place of any one of the six distinct carbon atoms in the molecule. The present alternative is less flexible but more direct and convenient in applications not requiring labeling at a particular carbon of the methylenecyclopropyl moiety. Under these circumstances it is the preferred synthetic option.

EXPERIMENTAL

2-(Methylenecyclopropyl)-1,2-$^{14}C_2$-ethanol (**2**-^{14}C). Methylenecyclopropane[6] (20 μL, 0.9 g/mL, 0.37 mmol) was transferred using a dry ice cooled syringe to a 5-mL round-bottomed flask containing 2 mL of THF cooled to -78 °C under a nitrogen atmosphere. *tert*-Butyllithium (100 μL, 1.8 M in pentane) was added, followed by 24 μL of TMEDA. The reaction mixture was stirred at -78 °C for 45 min and then at 0 °C for 30 min. The solution was cooled to -78 °C and the reaction

flask was fitted quickly to a vacuum transfer line. Ethylene oxide-^{14}C (Sigma Chemical, 13.3 μCi/μmol, 250 μCi) was then transferred to the reaction mixture. The mixture was stirred at -78 °C for 40 min then at 0 °C for 30 min; 1 mL of 5 % HCl and 3 mL ether were added with stirring. The organic layer was separated and dried over $MgSO_4$. Analysis by TLC on silica gel using a 1:1 ethyl acetate:hexane mobile phase gave a spot corresponding to alcohol **2** at R_f 0.35.

Methylenecyclopropyl-1,2-$^{14}C_2$-acetic Acid. The crude dilute 2-(methylenecyclopropyl)-1,2-$^{14}C_2$-ethanol was added to a test tube containing a magnetic stir bar and 3 mL of reagent grade acetone. The solution was cooled in an ice bath and 50 μL of Jones reagent was added dropwise.[18] The mixture was stirred for 3 h; conversion to the acid was confirmed by TLC comparisons with a sample of unlabeled acid (R_f 0.2) using a 1:1 ethyl acetate:5% ethanol-in-hexane mobile phase with anisaldehyde staining. The product was purified by preparative TLC using the same mobile phase and isolated from TLC scrapings by elution with 8 mL of ethyl acetate through a disposable pipette column.

N-[2-(Methylenecyclopropyl)-1,2-$^{14}C_2$-acetoxy]succinimide. One-fourth (2 mL) of the methylenecyclopropylacetic acid solution in ethyl acetate was added to a test tube. A solution of 3 mg of *N*-hydroxy-succinimide in 2 mL of ethyl acetate was added, followed by 6 mg of N,N'-dicyclohexylcarbodiimide in 2 mL of ethyl acetate. The solution was gently swirled and then left undisturbed for 16 h. The reaction mixture was filtered through a cotton plug and concentrated under vacuum to approximately 2 mL. The concentrate was purified by preparative TLC using 1:1 ethyl acetate:hexane as the mobile phase. The product (R_f 0.42) was isolated from the TLC scrapings with 4 mL of THF. The THF solution was concentrated to 2 mL.

Methylenecyclopropyl-1,2-$^{14}C_2$-acetyl-CoA (MCPA-CoA-^{14}C; **3**-^{14}C). Commercial HSCoA (Sigma, 3 mg) was added to a test tube containing a solution of 30 mg of $NaHCO_3$ in 1 mL of distilled H_2O. N-[2-(Methylenecyclopropyl)-1,2-$^{14}C_2$-acetoxy]succinimide (100 μL of the THF solution described above) was added to the HSCoA mixture. The resulting solution was gently swirled for 5 min and then acidified to approximately pH 5 with 5 % perchloric acid. The solution was concentrated to 200 μL and purified by HPLC using a Hewlett Packard 1090 series instrument equipped with a 3.9 mm x 30 mm Waters C_{18} μBondapak column and C_{18} μBondapak guard column insert. The mobile phase consisted of (20 mM KH_2PO_4, 0.3 mM EDTA, pH 6.0) buffer and methanol. A linear gradient of 20% methanol to 35% methanol was applied over 30 min at a flow rate of 0.5 mL/min; the mobile phase was then maintained at 35% methanol for an additional 30 min. The MCPA-CoA-^{14}C had a retention time of 29 min at 20 °C and a concentration of 17.7 μM, derived from a plot of unlabeled MCPA-CoA concentration versus integrated HPLC detector response at 250 nm. Scintillation counting

(Beckman Model LS-255; ICN Ecolume scintillation fluid) efficiency was calculated to be 79%, based on a specific activity of the ethylene oxide-^{14}C, 13.3 μCi/μmol.

A more concentrated sample of MCPA-CoA of lower specific activity was prepared through dilution of labeled with unlabeled methylene-cyclopropylacetoxysuccinimide and continuation of the synthesis as described; the MCPA-CoA-^{14}C obtained was 187 μM and 0.87 μCi/μmol (96 % radioactive purity, 95 % pure by HPLC, A_{232}/A_{260} = 0.56 at pH 7). Incubation of 15 μL of this MCPA-CoA-^{14}C with 45 μL of 12 μM general acyl dehydrogenase isolated from pig kidney[19] for 10 min reduced enzymatic activity[20] by 84%.[5,18]

Methylenecyclopropyl-1,2-$^{13}C_2$-acetyl-CoA (MCPA-CoA-$^{13}C_2$; **3**-$^{13}C_2$). Reaction of methylenecyclopropylithium from 24 mmol of methylenecyclopropane with 0.5 g (11.3 mmol) of ethylene oxide-1,2-$^{13}C_2$ (99%, Cambridge Isotope Laboratories), followed by oxidation with Jones reagent, gave 0.76 g of distilled product (60% overall yield, based on ethylene oxide-1,2-$^{13}C_2$); ^{13}C NMR ($CDCl_3$) δ 37.56 (d, J ^{13}C-^{13}C = 55.5 Hz), 178.86 (d, J ^{13}C-^{13}C = 55.5 Hz). The ^{13}C-labeled MCPA-CoA prepared from this acid had identical chromatographic behavior, ultraviolet spectral properties, and inactivator potency toward GAD as shown by the unlabeled and the ^{14}C-labeled thioesters.

Acknowledgment: We thank the National Institues of Health for support of this work through GM 38262.

REFERENCES

1. Tanaka, K. - *in* Handbook of Clinical Neurology Vol 37: Intoxications of the Nervous System, Part 2 (Vinkne, P. J. and Bruyn, G. W., eds.), pp. 511-539, Elsevier North-Holland, Amsterdam, 1979.

2. Tanaka, K. and Rosenberg, L. E. - *in* The Metabolic Basis of Inherited Disease, 5th Ed. (Stanburg, J. B., Wyngaarden, J. B., Fredrickson, D. S., Goldstein, J. L. and Brown, M. S., eds.), pp. 440-473, McGraw-Hill, New York, 1983.

3. Ghisla, S., Wenz, S. and Thorpe, C. - *in* Enzyme Inhibitors (Brodbeck,U., ed.), pp. 43-60, Verlag Chemie, Weinheim, 1980.

4. Wenz, A., Thorpe, C. and Ghisla, S. - J. Biol. Chem. 256: 9809-9812 (1981).

5. Lenn, N. D., Shih, Y., Stankovich, M. T. and Liu, H. W. - J. Am. Chem. Soc. 111: 3065-3067 (1989).

6. Salaun, J. R., Champion, J. and Conia, J. M. - Org. Synth. 57: 36-40 (1977).

7. Köster, R., Arora, S. and Binger, P. - Liebigs Ann. Chem.: 1219- 1235 (1973).

8. Akhachinskaya, T. V., Bakhbukh, M., Grishin, Yu. K., Donskaya, N. A. and Ustynyuk,Yu. A. - J. Org. Chem. USSR 14: 2139-2144 (1978).

9. Akhachinskaya, T. V., Donskaya, N. A., Grishin, Yu. K., Roznyatovskii, V. A. and Shabarov, Yu. S. - J. Org. Chem. USSR 17: 1271-1276 (1981).

10. Graber, F. D. - Ph. D. Thesis, University of Kansas (1982); Dissert. Abst. Internat. 43: 2555-B (1983).

11. Thomas, E. W. - Tetrahedron Lett. 24:1467-1470 (1983).

12. Duff, S. R. - Ph. D. Thesis, University of Kansas (1983); Dissert. Abst. Internat. 44: 3404-B (1984).

13. Akhachinskaya, T. V., Shapiro, I. O., Donskaya, N. A., Shabarov, Yu. S. and Shatenshtein, A. I. - J. Org. Chem. USSR 21: 421-426 (1985).

14. Akhachinskaya, T. V., Donskaya, N. A., Grishin, Yu. K. and Shabarov, Yu. S. - J. Org. Chem. USSR 23: 295-301 (1985).

15. Sternberg, E. and Binger, P. - Tetrahedron Lett. 26: 301-304 (1985).

16. Widdison, W. C. - unpublished.

17. Leandri, G., Monti, H. and Bertrand, M. - Bull. Soc. Chim. France: 3015-3020 (1974).

18. Baldwin, J. E. and Parker, D. W. - J. Org. Chem. 52: 1475-1477 (1987).

19. Gorelick, R. J., Mizzer, J. P. and Thorpe, C. - Biochemistry 21: 6936-6942 (1982).

20. Stadtman, E. R. - Methods Enzymol. 3: 931-941 (1957).