NOTES

9-α-D-ARABINOFURANOSYL-8-AZAADENINE-2-^{14}C

Received December 15, 1977

SUMMARY

9-α-D-Arabinofuranosyl-8-azaadenine-2-^{14}C (6) was prepared by a procedure previously described for the unlabeled nucleoside. The ^{14}C was incorporated into the 8-azapurine by closure of 5-amino-v-triazole-4-carboxamide with ^{14}C-formic-acetic anhydride. The labeled nucleoside (6) was purified by chromatography.

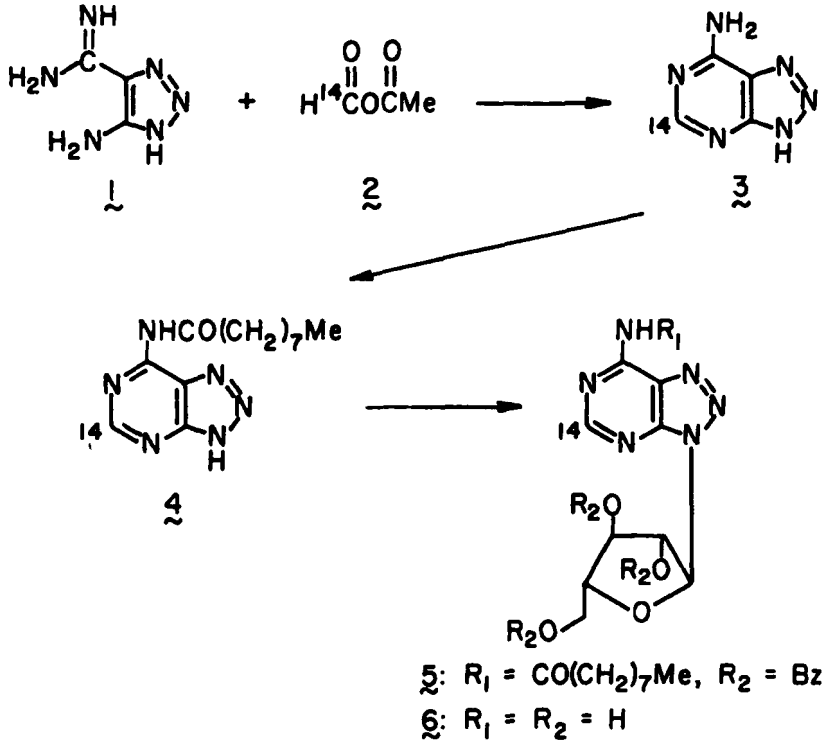
Key Words: Nucleoside, 9-α-D-Arabinofuranosyl-8-azaadenine-2-^{14}C, 8-Azaadenine-2-^{14}C, Metabolism.

INTRODUCTION

Nucleosides and nucleotides isolated from nucleic acid, DNA and RNA, have the β-configuration at the anomeric carbon. Despite this fact, α-arabinonucleosides are more cytotoxic than the corresponding β-anomers and have antiviral activity (1). In order to explain these unexpected results, studies on the metabolism of these compounds were initiated. To facilitate these studies it was necessary to synthesize a radiolabeled substrate. 9-α-D-Arabinofuranosyl-8-azaadenine was chosen because of its relatively high level of biologic activity, and ^{14}C was chosen as the label after considerations of stability and methods of introduction of the label. The most convenient position for labeling is C-2 of the 8-azapurine. Since efforts to prepare 5-amino-1-α-D-arabinofuranosyl-v-triazole-4-carboxamidine—which would allow introduction of the label in one step—by a known procedure (2) were unsuccessful, an alternative, longer route was followed. Reaction of ^{14}C-formic-acetic anhydride (2), prepared from sodium ^{14}C-formate and acetyl chloride (3), with 5-amino-v-triazole-4-carboxamide (1) (4) gave 8-azaadenine-2-^{14}C (3), which was converted

0362-4803/78/0015-0727$01.00

to N-nonanoyl-8-azaadenine (4) for coupling with 2,3,5-tri-O-benzoyl-α-D-arabinofuranosyl bromide (5). After the coupling reaction, the protective groups were removed from 5 by treatment with sodium methoxide in methanol to give the desired 9-α-D-arabinofuranosyl-8-azaadenine-2-^{14}C (6), which was purified by chromatography on thick silica gel plates.



EXPERIMENTAL

8-Azaadenine-2-^{14}C (3)

A 10-ml round-bottom flask containing 853 mg (12.5 mmol) of ^{14}C-sodium formate (3 mCi/mmol) in 5 ml of anhydrous ether was placed in a dry bag of nitrogen atmosphere. The contents were stirred magnetically while 838 mg (10.68 mmol) of acetyl chloride was added. The resulting suspension was stoppered, stirred for 4 hr at ambient temperature, and then filtered directly into a mixture of 138 mg (1.1 mmol) of 1 (4) in 5 ml of anhydrous dimethylsulfoxide. The yellow solution that resulted was heated at 70° for 45 min and

left 20 hr at ambient temperature. It was then removed from the dry bag, evaporated at 70° in vacuo for 5 min, taken to pH 8 with 10–15 ml of 0.5 N potassium bicarbonate solution, heated at 105° for 20 min, and evaporated to dryness in vacuo. An aqueous solution of the residue, upon cooling, produced a precipitate of inorganic material that was removed by filtration. Cooling and seeding the filtrate produced 3 as a gelatinous solid; yield 115 mg (77%).

N-Nonanoyl-8-azaadenine-2-^{14}C (4)

A solution of 115 mg (0.84 mmol) of 3 in 2 ml of anhydrous pyridine containing 151 mg (0.86 mmol) of nonanoyl chloride was refluxed for 1.5 hr, left 20 hr at ambient temperature, and then poured into 20 ml of ice water. The precipitate of 4 that resulted was collected by filtration and dried; yield 142 mg (61%).

9-α-D-Arabinofuranosyl-8-azaadenine-2-^{14}C (6)

A mixture of 140 mg (0.51 mmol) of 4, 236 mg (0.51 mmol) of 2,3,5-tri-O-benzoyl-α-D-arabinofuranosyl bromide, and 1 g of AW-500 molecular sieve (Linde) in 25 ml of dry benzene was refluxed for 1 hr, treated with another 1 g of molecular sieve, refluxed 6 hr more, and filtered. The insoluble material was washed several times with hot benzene. The combined filtrate and wash was evaporated to dryness in vacuo, giving 5 as an orange glass.

A solution of 5 in 21 ml of 0.048 N methanolic sodium methoxide was refluxed for 45 min, neutralized with glacial acetic acid, and streaked across 4–8 in. Brinkmann silica gel plates (20 x 20 cm, 2-mm thickness). The plates were twice developed in 3:1 chloroform-methanol solvent system. The major uv-absorbing band was removed and extracted with 150 ml of boiling methanol. Evaporation of the methanol solution gave 6 as a white solid; yield 72 mg (53%): specific activity determined by liquid scintillation counting 1.83 mCi/mmol. The radiochemical purity determined by paper chromatography [Whatman No. 1 paper in solvents of the following compositions: A, equal volumes of 93.8%

aqueous 1-butanol and 44% aqueous propionic acid; B, 5 N ammonium acetate, saturated sodium tetraborate, ethanol, and 0.25 M EDTA, pH 9.5 (20:80:220:1, v/v)] was 99%. The chemical purity was determined by thin-layer chromatography (Analtech silica gel GF, 3:1 chloroform-methanol) and by reverse-phase high-pressure liquid chromatography; elution was accomplished with a water-acetonitrile solvent (98:2, v/v) and was monitored by measurement of optical density at 254 nm.

REFERENCES

1. Bennett L. L., Jr., Shannon W. M., Allan P. W., and Arnett G. - Ann. N. Y. Acad. Sci. 255: 342 (1975)
2. Montgomery J. A. and Thomas H. J. - J. Med. Chem. 15: 182 (1972)
3. Krimen L. I. - Org. Syn. 50: 1 (1970).
4. Stevens M. A., Smith H. W., and Brown G. B. - J. Am. Chem. Soc. 82: 3169 (1960).
5. Montgomery J. A. and Thomas H. J. - J. Med. Chem. 15: 305 (1972)

John A. Montgomery and H. Jeanette Thomas
Kettering-Meyer Laboratory
Southern Research Institute
Birmingham, Alabama 35205