Synthesis of [^{14}C]A-62514, a Radiolabelled Derivative of Erythromycin A, via [2-^{14}C]N,N-Dimethylethylenediamine.

Bruce W. Surber, William R. Baker, and Louis Seif

Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, Illinois 60064

SUMMARY

The synthesis of [^{14}C]A-62514, 11-deoxy-11-[carboxy(2-dimethylamino-[1-^{14}C]ethyl)amino]-6-0-methyl-erythromycin A 11,12-(cyclic ester), was performed in five steps. The key intermediate, [2-^{14}C]N,N-dimethylethylenediamine, was obtained in 80% yield by reacting Eschenmoser's salt with K^{14}CN and reducing the resulting [1-^{14}C]N,N-dimethylglycinonitrile with H_2 and Raney Ni in methanol and ammonium hydroxide. The final product was obtained 97% radiochemically pure in an overall radiochemical yield of 14%, with a specific activity of 39 mCi/mmol.

Key Words: [1-^{14}C]N,N-dimethylglycinonitrile, [2-^{14}C]N,N-dimethylethylenediamine, 11-deoxy-11-[carboxy(2-dimethylamino[1-^{14}C]ethyl)amino]-6-0-methyl-erythromycin A 11,12-(cyclic ester)

INTRODUCTION

As part of a program aimed toward the development of erythromycin A analogues, a novel series of compounds was identified which showed improved pharmacokinetics[1]. These erythromycin derivatives possessed a fused oxazolidine ring at the C-11/C-12 position and a C-6 methoxy group. One compound, A-62514, was selected for metabolic evaluation and was thus required in a radiolabelled form. The 2-dimethylaminoethyl side chain was chosen as the site of labelling since the side chain was introduced late in the synthesis and metabolic studies with cold A-62514 showed no degradation to **5a**[2]. The macrolide (**5b**) was labelled in the side chain using [2-^{14}C]N,N-dimethylethylenediamine (**2**) and procedures previously described[1]. However, **2** was unknown and a method for its synthesis was devised.

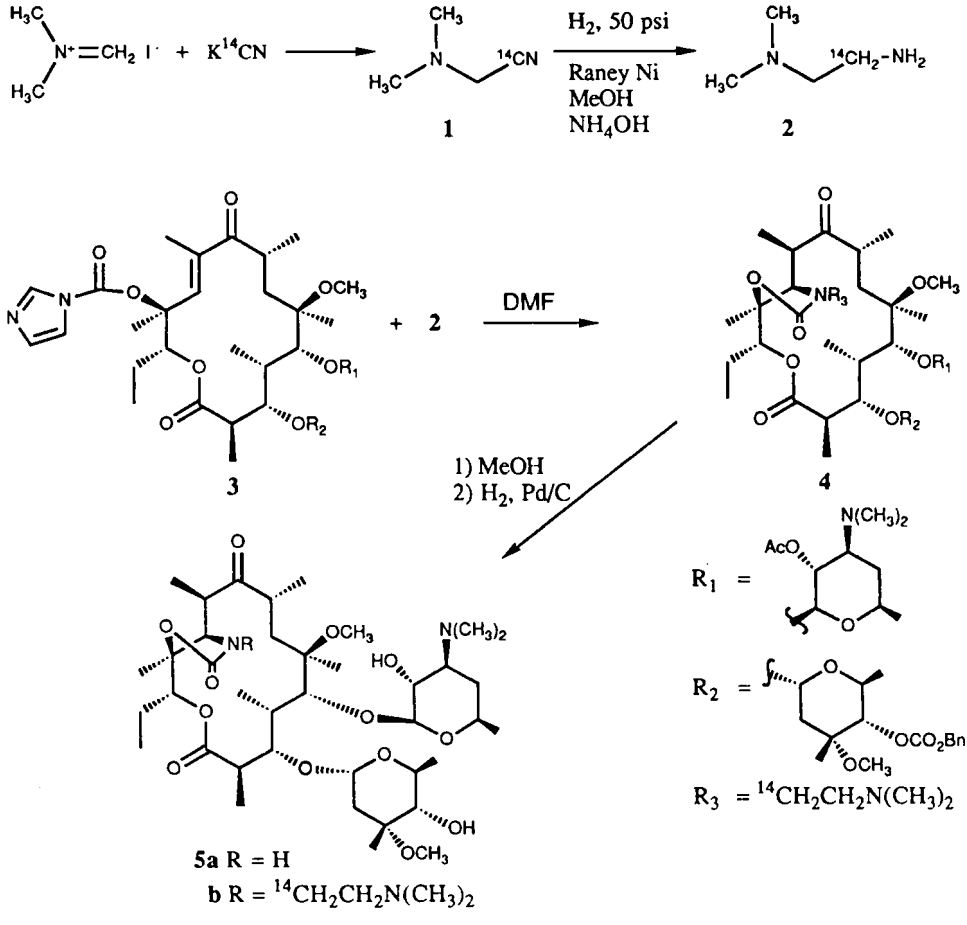
0362–4803/91/010023–05$05.00

Received June 13, 1990
Revised August 15, 1990

RESULTS

Compound **2** was prepared by first reacting Eschenmoser's salt with $K^{14}CN$ to give **1**, similar to a classic approach to alpha-aminonitriles[3,4], followed by reduction to diamine **2**[4,5]. Compound **1** was cleanly and conveniently converted to **2** using hydrogen and Raney nickel in methanol and ammonium hydroxide[5]. Since methanol was the solvent for this reaction, it was used as the solvent in the cyanide coupling with excellent results. The [^{14}C]cyanide was titrated with a solution of the yellow-colored Mannich reagent until the yellow color persisted. Then the catalyst and base were added and **1** was reduced, monitoring the course of the reaction by HPLC. Diamine **2** was isolated as the hydrochloride, liberated with 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU), and distilled as a ~1 M DMF solution. When **3** was added to this

Scheme



solution, the product (**4**) quickly formed and was isolated at 99% radiochemical purity after chromatography. Deprotection by methanolysis

and catalytic hydrogenation completed the synthesis of [^{14}C]A-62514 (**5b**) (see Scheme). Chromatographic purification provided 4.9 mCi at 97% purity (61% yield from **4**).

EXPERIMENTAL

General: Potassium [^{14}C]cyanide was purchased from Chemsyn Science Laboratories. Liquid scintillation counting was performed on a LKB-Wallac 1214 Rack Beta "Excel" counter. TLC plates were coated with Kieselgel 60 (0.25 mm) and were scanned for radioactivity with a Radiomatic RS chromatograph. HPLC was performed using a Waters 60A solvent delivery system and peaks were detected with a Kratos 757 UV/VIS variable wavelength detector connected in series with a Packard Tri-Carb RAM 7500 equipped with a solid scintillator flow cell. Mass spectra were obtained by chemical ionization (NH_3) on a Normag model R30-10 quadrupole mass spectrometer.

[^{14}C]N,N-Dimethylaminoacetonitrile (**1**): In a 100 mL Parr hydrogenation bottle, [^{14}C]potassium cyanide (43 mg, 0.63 mmole, 35 mCi) and unlabeled potassium cyanide (11 mg, 0.16 mmol) were dissolved in methanol (3 mL). A freshly prepared and filtered solution of Eishenmoser's salt in methanol (0.6 M) was added dropwise with stirring until the yellow color persisted (~1 mL). Stirring was continued for 0.5 hours. HPLC analysis (5 micron C-18, 4.6x 50mm eluted with aqueous 100 mM KH_2PO_4, 5 mM $NaSO_3C_8H_{17}$, pH 4.5) indicated that 92% of the radioactivity corresponded to a component with the same retention time as authentic N,N-dimethylaminoacetonitrile (4.6 min).

[2-^{14}C]N,N-Dimethylethylenediamine (**2**): To the solution of **1** was added Raney nickel (~0.5 g) and conc. ammonium hydroxide (0.8 mL). The mixture was shaken under 45 psi of hydrogen for 26 hours. HPLC analysis, using the same conditions as for **1** above, indicated about 5% of **1** remained. The product (**2**), with a retention time of 41.5 minutes, was not UV active so a comparison to authentic material was not made. With the aid of Celite, the catalyst was filtered and washed with methanol. To remove most of the ammonia, the filtrate was cooled to -40°C and, under high vacuum, about half the volatiles were evaporated. Only 3 mCi was in the distillate. Acidifying the remainder with 6 M hydrochloric acid to pH 4 and evaporation under reduced pressure gave a residue which was dissolved in methanol (12 mL) and filtered to remove some potassium chloride. A second evaporation and dissolution in a minimum of dimethyl formamide (DMF) allowed the filtration of the last of the potassium chloride. The filtrate was dried under high vacuum overnight to give a reddish solid. The solid was mixed with DMF (0.7 mL) and DBU (0.29 mL, 1.9 mmole). Distillation under high vacuum to a

graduated "N-trap" cooled in liquid nitrogen provided a solution of **2** in DMF (0.80 mL @ 35 mCi/mL = 28 mCi, 0.64 mmole, 80% yield from [^{14}C]cyanide).

2'-0-Acetyl-4"-0-benzyloxycarbonyl-[^{14}C]A-62514 (**4**): Acylimidazole **3**[6] (570 mg, 0.58 mmole) was added to the DMF solution of **2** (28 mCi, 0.64 mmole) with stirring. As soon as **3** dissolved, the mixture set up to a solid mass. After 0.5 h, the mixture was dissolved in ethyl acetate, washed with water, dried over sodium sulfate, filtered, concentrated *in vacuo*, and chromatographed on silica gel (70-200 mesh, 13 x 300 mm column) eluting with a gradient of methanol in ethyl acetate (100 mL portions: 0, 2, 4, 6, 8, 10 and 12% methanol). Fractions containing **4** were combined to give 8.2 mCi (29% yield) which was >99% pure by HPLC (ret. time 8.8 min on a 5 μm spherical C-8, 4.5mm x 15 cm column eluted with acetonitrile, methanol, 0.01 M aqueous tetramethylammonium perchlorate containing 0.1% (v/v) trifluoroacetic acid (40:5:55) @ 1 mL/min) and another 5.0 mCi of less pure **4**.

[^{14}C]A-62514 (**5b**): Compound **4** (8.2 mCi, >99% pure) was dissolved in methanol (75 mL) and kept at room temperature for 4 days. Palladium on carbon (10%, 200 mg) was added and the mixture was shaken under 45 psi hydrogen for 40 hours. Filtering the catalyst and concentrating under reduced pressure provided an oil which was applied to a column of silica gel, and eluted with a gradient of methanol in chloroform (0, 5, 10, 15, 20%, 200 mL each). Fractions were combined and concentrated *in vacuo* to give 4.9 mCi of **5b** as a white solid (60% yield). It was >97% pure and co-chromatographed with authentic A-62514 by TLC and HPLC (TLC-A: chloroform : methanol (4:5), TLC-B: butanol : acetic acid : water (25:4:10); HPLC: same system as for **4**, above, except the mobile phase was 30:5:65). Specific activity was determined to be 46.4 μCi/mg (39.1 mCi/mmol) both gravimetrically (46.6 μCi/mg) and by HPLC (46.2 μCi/mg, comparing the UV response of a known amount of radioactivity to a standard curve). The MS-FAB spectrum showed the same molecular ion of m/z 844 $(M+1)^+$ and fragmentation pattern as authentic A-62514.

ACKNOWLEDGMENTS

We would like to thank Mr. Steven Cepa for obtaining the mass spectral data and Dr. John Uchic for helpful suggestions in preparing the manuscript.

REFERENCES

1. Baker, W. R.; Clark, J. D.; Stephens, R. L.; Kim, K. H., *J. Org. Chem.* **1988**, 53, 2340-2345.
2. Marsh, K., Abbott Laboratories, private communication.
3. a) Reeve, W.; Fareckson, W. M. III, *J. Am. Chem. Soc.* **1950**, 72, 5195. b) Szantay, C.; Novak, L., *Chem. Ber.* **1963**, 96, 1779. c) Gold, H.; Bayer, O., *ibid*, **1961**, 94, 2594. d) Toye, J.; Ghosez, L., *J. Am. Chem. Soc.* **1975**, 97, 2276. e) Okecha, S.F.; Stansfield, F., *J. Chem. Soc. Perkin Trans. 1* **1977**, 1811. f) Onda, M.; Harigaya, Y.; Horie, J., *Chem. Pharm. Bull.* **1978**, 26, 3330. g) Vedejs, E.; Grissom, J. W., *J. Org. Chem.* **1988**, 53, 1876. h) Billings, R.; Sullivan, H. R., *J. Labelled Compounds Radiopharm.*, **1966**, 3, 17.
4. Walker, J. N.; Engle, A. R.; Kempton, R. J., *J. Org. Chem.* **1972**, 37, 3755.
5. Freifelder, M., "Catalytic Hydrogenation in Organic Synthesis, Procedures and Commentary" John Wiley and Sons, New York, 1978, Ch. 6.
6. Compound **3** was the same material as compound **11a** in reference 1.