SYNTHESIS OF 14-^{14}C-Adriamycin

Babu R. Vishnuvajjala, Tadashi Kataoka, Frederick D. Cazer, Donald T. Witiak, and Louis Malspeis
Divisions of Pharmaceutics and Pharmaceutical Chemistry, Medicinal Chemistry, College of Pharmacy and Radiochemistry Laboratory, Cancer Center, The Ohio State University, Columbus, Ohio 43210.
Received September 20, 1976 Revised November 29, 1976

SUMMARY

14-^{14}C-Adriamycin HCl has been prepared from unlabeled adriamycin. The ^{14}C-Diazald served as the source of the label. This synthesis does not require protection of the phenolic hydroxyl groups.

Key Words: 14-^{14}C-Adriamycin, N-Trifluoroacetyladriamycin, ^{14}C-Diazomethane

INTRODUCTION

The metabolism of adriamycin (1), an important anticancer antibiotic,[1] has been investigated in several mammalian species including man.[2-6] Such studies have relied exclusively on the isolation and identification of fluorescent metabolites. We, therefore, considered it timely to report a rather convenient synthesis for 14-^{14}C-adriamycin, the availability of which will enable investigators to study the distribution, pharmacokinetics and detailed metabolism of this drug.

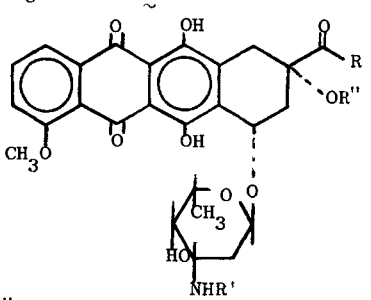
DISCUSSION AND RESULTS

Selective cleavage of the C_{13}-C_{14} bond of N-trifluoroacetyl(TFA)-adriamycin (2)[7] with periodic acid (2 hours; 25°) afforded key intermediate carboxylic acid 3 in approximately 82% yield. A similar reaction was employed by Tong *et al.*[8] for the conversion of adriamycin to the corresponding acid. Reaction of unpurified 3 (0.32 mmol) with 2 equivalents of isobutyl chloroformate and 3 equivalents of triethylamine in 100 ml of chloroform (ice bath; 3 hours) afforded unstable mixed anhydride 4. This solution was added to 60 ml of diazomethane [generated from Diazald (8.0 mmol)] in ether (0-5°). After standing overnight (dark; 25°) the excess diazomethane was removed under a stream of nitrogen and the solvent was concentrated under reduced pressure. Chromatography (tlc; chloroform: methanol, 90:10; silica gel) of the residue afforded diazoketone 5 in 25% yield;

0362-4803/78/0114-0077$01.00/0

ir($CHCl_3$) 2120 cm^{-1} (N=N); nmr * (90 MHz:$CDCl_3$) δ 5.5 (s,1H,-CHN_2). A major byproduct (20%) in this reaction was identified as methyl ester 6 (NMR decoupling experiments on its aglycone confirmed the structure). Diazoketone 5 in dichloromethane was converted utilizing 3 equivalents of hydrogen bromide (0°; 30 min) to bromoketone 7 in excellent yield. Bromoketone 7 was treated with 5% potassium carbonate solution to afford N-TFA-adriamycin 2 (20% yield from the acid 3 **). Protection of the 14-hydroxyl group of N-TFA-adriamycin by tritylation with p-anisyldiphenylmethyl chloride using a modification (75°; 16 hours) of the method of Smith et al.[9] yielded 8: successive removal of the N-TFA group with 0.1N NaOH solution and the p-anisyldiphenylmethyl group with 80% acetic acid yielded adriamycin. Adriamycin was converted to its hydrochloride using methanolic HCl. The physical properties of the resulting product were identical to the properties of an authentic sample of adriamycin HCl.

By this method we have synthesized 9 mg of 14-^{14}C-adriamycin HCl (sp. act. 109 μCi/mmol) from 1.0 mCi of ^{14}C-Diazald (sp. act. 112 μCi/mmol, New England Nuclear) and 200 mg of acid 3.



1; R=CH_2OH, R'=R''=H

2; R=CH_2OH, R'=$COCF_3$, R''=H

3; R=OH, R'=$COCF_3$, R''=H

4; R=O_2COi-Bu, R'=$COCF_3$, R''=CO_2i-Bu

5; R=CHN_2, R'=$COCF_3$, R''=CO_2i-Bu

6; R=OCH_3, R'=$COCF_3$, R''=CO_2i-Bu

7; R=CH_2Br, R'=$COCF_3$, R''=CO_2i-Bu

8; R=$CH_2OC(Ph)_2$Ph-p-OMe, R'=$COCF_3$, R''=H

*The nmr spectra for 5 and 7 [δ 0.9(d,6H,J=7.0 Hz,$(CH_3)_2CH$-] indicate that one of the nonphenolic hydroxyl functions in these compounds is esterified with an isobutyl carbonyl group. The aqueous hydrolysis of 4 affords the 2,4-diketo-1,3-dioxolane ring system showing that the 9-hydroxyl group in 4 is present as the isobutyl carbonate. This function is easily removed under the conditions of hydrolysis of the bromoketone and does not interfere with the ultimate preparation of 14-^{14}C-adriamycin.

** Although we were able to introduce a hydroxymethyl function into the tetra-acetate derivative of 3 using methods similar to those described, the synthesis failed for the preparation of 14-^{14}C-adriamycin owing to unsuccessful attempts to remove the protecting groups.

EXPERIMENTAL SECTION

All solvents used were dried and distilled. Radioactivity was measured using the Packard Tricarb Model 3375 liquid scintillation spectrometer. For proof of structure NMR spectra were recorded using a BRUKER HX-90E spectrometer. IR spectra were recorded using the Perkin Elmer Model 257 spectrometer.

7-[(3-N-TFA-Amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-9-carboxy-7,8,9,10-tetrahydro-6,9,11-trihydroxy-4-methoxy-5,12-naphthacenedione (3).

A solution of 25 mg (0.11 mmol) of periodic acid in 1 ml of water was added to a solution of 25 mg (0.039 mmol) of N-TFA-adriamycin (2)[7] in 1 ml of tetrahydrofuran. The mixture was stirred for 2 hours at room temperature. The clear red solution was concentrated under reduced pressure; 5 ml of water was added and the mixture was extracted with ethyl acetate. The organic layer was washed with water, dried over anhydrous sodium sulfate and evaporated to give 20 mg (82%) of 3 m.p. 175-176° (homogeneous on tlc); ir(KBr) 1720, 1620, and 1580 cm^{-1} (C=O's); nmr(90MHz;$CDCl_3$) δ 14.0 and 13.2 (phenolic OH), 4.08 (s,3H,OCH_3), and 1.3(d,3H,J=6.5Hz,CH_3). This material was used in subsequent reactions without further purification.

Formation of Diazoketone 5.

To a stirred suspension of 200 mg (0.32 mmol) of the acid 3 in 100 ml of chloroform, cooled in an ice bath, was added 133 μl (0.96 mmol) of triethylamine followed by 84 μl (0.64 mmol) of isobutyl chloroformate. After stirring at ice bath temperatures for 3 hours, the red solution was added dropwise to a stirred solution of 60 ml of ether containing diazomethane (generated from 8 mmol of Diazald) at 0-5° over a period of 1 hour. The reaction mixture was allowed to stand in the dark for 18 hours; 100 ml of water was added to the mixture. Nitrogen was bubbled through the solution for 1 hour to remove excess diazomethane. The organic layer was washed with water (3 x 50 ml), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The red residue showed 2 major spots on TLC (silica gel; chloroform-methanol 9:1). These compounds were separated by preparative TLC using the same

solvent system. The more polar compound on TLC [60 mg (25%)] was identified as the diazoketone 5. Ir ($CHCl_3$) 2120 cm^{-1} (N≡N), 1720 cm^{-1}, 1620 cm^{-1}, 1580 cm^{-1} (C=O's); nmr (90 MHz;$CDCl_3$) δ 14.0 (s) and 13.3 (s), phenolic hydroxyl groups, 5.5 (s,1H,CHN_2), 4.05 (s,3H,OCH_3), 1.27 (d,3H,J=6.5 Hz,CH_3) and 0.89 [d,6H,J=7.0 Hz,$CH(CH_3)_2$]. The less polar compound [48 mg (20%)] was identified as the methyl ester 6 (characterized by spectral data as well as its degradation to the aglycone).

9-O-Isobutoxycarbonyl-N-trifluoroacetyl-14-bromodaunomycin (7)

To a solution of 60 mg (0.08 mmol) of diazoketone 5 in 50 ml of dichloromethane, cooled in an ice bath, was added 1.6 ml (0.192 mmol) of a stock solution*** of HBr in dichloromethane. After stirring at ice bath temperatures for 30 minutes the solution was washed with water (3 x 25 ml), dried over anhydrous sodium sulfate, and concentrated under reduced pressure to afford 61 mg (95%) of bromoketone 7. Ir ($CHCl_3$) 1730, 1620 and 1580 cm^{-1} (C=O's); nmr* (90 MHz; $CDCl_3$) δ 4.23 (CH_2Br, AB pattern partially obscured by other peaks).

N-Trifluoroacetyl adriamycin (2)

To a solution of 61.0 mg (0.076 mmol) of bromoketone 7 in 40 ml of THF was added 40 ml of freshly prepared 5% potassium carbonate solution at room temperature. After stirring for 30 minutes, 40 ml of water was added followed by solid tartaric acid until the solution turned red. The solution was extracted with ethyl acetate, washed with water, dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford 39 mg (80%) of N-TFA adriamycin 2 identical in all respects to an authentic sample.

14-O-p-Anisyldiphenylmethyl-N-TFA adriamycin (8)

A solution containing 39 mg (0.061 mmol) of N-TFA adriamycin, 383 mg (1.24 mmol) of p-anisyldiphenylchloromethane and 20 ml of pyridine was heated on an oil bath at 75° for 16 hours. Pyridine was removed under reduced pressure at room temperature. The residue was dissolved in $CHCl_3$, washed with water,

*** The stock solution was prepared by diluting 1 g of a 32% HBr in acetic acid solution with 100 ml of dichloromethane.

dried over anhydrous sodium sulfate and concentrated under reduced pressure. Pure tritylether 8 was isolated in 56% yield by preparative TLC (silica gel) using chloroform-methanol, 95:5 as solvent.

Adriamycin HCl (1)

To a solution of 31.6 mg (0.034 mmol) of tritylether (8) in 3 ml of THF cooled in an ice bath under a nitrogen atmosphere was added 3 ml of 0.1N sodium hydroxide solution. The mixture was stirred at ice bath temperatures for 3 hours and acetic acid was added until the color changed from blue to orange. The pH of the solution was adjusted to 8.3 by addition of solid sodium bicarbonate. The solution was extracted with chloroform, washed with water, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was dissolved in 5 ml of 80% acetic acid and stirred at room temperature for 3 hours. Ten ml of water was added and the pH of the solution was adjusted to 8.3 with sodium bicarbonate. The aqueous solution was extracted with chloroform and the organic layer was washed with water, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to ca. 3 ml. To this solution was added 3 ml of ether followed by 3 drops of 1.5 N methanolic-HCl solution. The precipitated red solid was collected by centrifugation, washed with ether followed by chloroform and dried under reduced pressure yielding 9 mg (45% from 8) of adriamycin HCl identical in all respects to an authentic sample.

ACKNOWLEDGMENT

We are grateful to the National Cancer Institute for support of this work under Grants CA-15147 and CA-16058 and to the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment for supplying 1 gram of adriamycin hydrochloride.

REFERENCES

1. Arcamone F., Cassinelli G., Franceschi G., Penco S., Pol C., Redaelli S., and Selva A. - Int. Symp. Adriamycin (Proc.), Carter S. K., DiMarco A., Ghione M., Krakoff I. H., and Mathe G., Ed., Springer-Verlag, New York, N.Y., 1972, p 9.

2. Takanashi S., and Bachur N. R. - Drug Metab. and Disp., 4: 79 (1976).

3. Bachur N. R., Egorin M. J., and Hildebrand R. C. - Biochem. Med., 8: 352 (1973).

4. Bachur N. R., Egorin M. J., Hildebrand R. C., and Takanashi S. - Proc. Amer. Assoc. Cancer Res., 14: 14 (1973).

5. Bachur N. R., Hildebrand R. C., and Jaenke R. S. - J. Pharmacol. Exp. Ther., 191: 331 (1974).

6. Benjamin R. S., Riggs C. E., Jr., and Bachur N. R. - Clin. Pharmacol. Ther., 14: 592 (1973).

7. Arcamone F., Barbieri W., Franceschi G., and Penco S. - Chim. Ind. (Milan) 51: 834 (1969).

8. Tong G., Lee W. W., Black D. R., and Henry D. W. - J. Med. Chem., 19: 395 (1976).

9. Smith T. H., Fijiwara A. N., Henry D. W., and Lee W. W. - J. Amer. Chem. Soc., 98: 1970 (1976).