SYNTHESIS OF DAUNORUBICIN-14-^{14}C AND ADRIAMYCIN-14-^{14}C

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SUMMARY

Reaction of adriamycinone with excess $^{14}CH_3MgI$ and periodate cleavage of the resulting glycol (5) affords daunomycinone-14-^{14}C (6). Bromination and hydrolysis of 6 gives adriamycinone-14-^{14}C (8). Coupling of 6 and the 14-O-p-anisyldiphenylmethyl derivative (9) with 1-chloro-3-N-trifluoroacetyl-4-O-p-nitrobenzoyldaunosamine (10) affords daunorubicin-14-^{14}C (1) and adriamycin-14-^{14}C (2), respectively, after deprotection.

KEY WORDS: Daunorubicin-14-^{14}C and adriamycin-14-^{14}C.

INTRODUCTION AND DISCUSSION

The anthracycline antibiotics adriamycin (2) and daunorubicin (1) are clinically useful antineoplastic agents, adriamycin having an especially broad

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spectrum of activity[1]. The preparation of these drugs with a metabolically stable radiolabel would be most useful for studying their metabolism and mode of action. Heretofore only randomly tritiated daunorubicin and adriamycin have been available. Scheme 1 shows our synthesis of the title compounds.

The dihydroxy acetone side chain at the 9-position of adriamycin (2) seemed to be a promising point of attack for several reasons. Existing metabolic data indicated the suitability of the 14-position as a site for radiolabel[2] because no side chain lability was evident. Reichstein, et al.[3] had reported a transformation similar to our proposed route in the field of steroid chemistry.

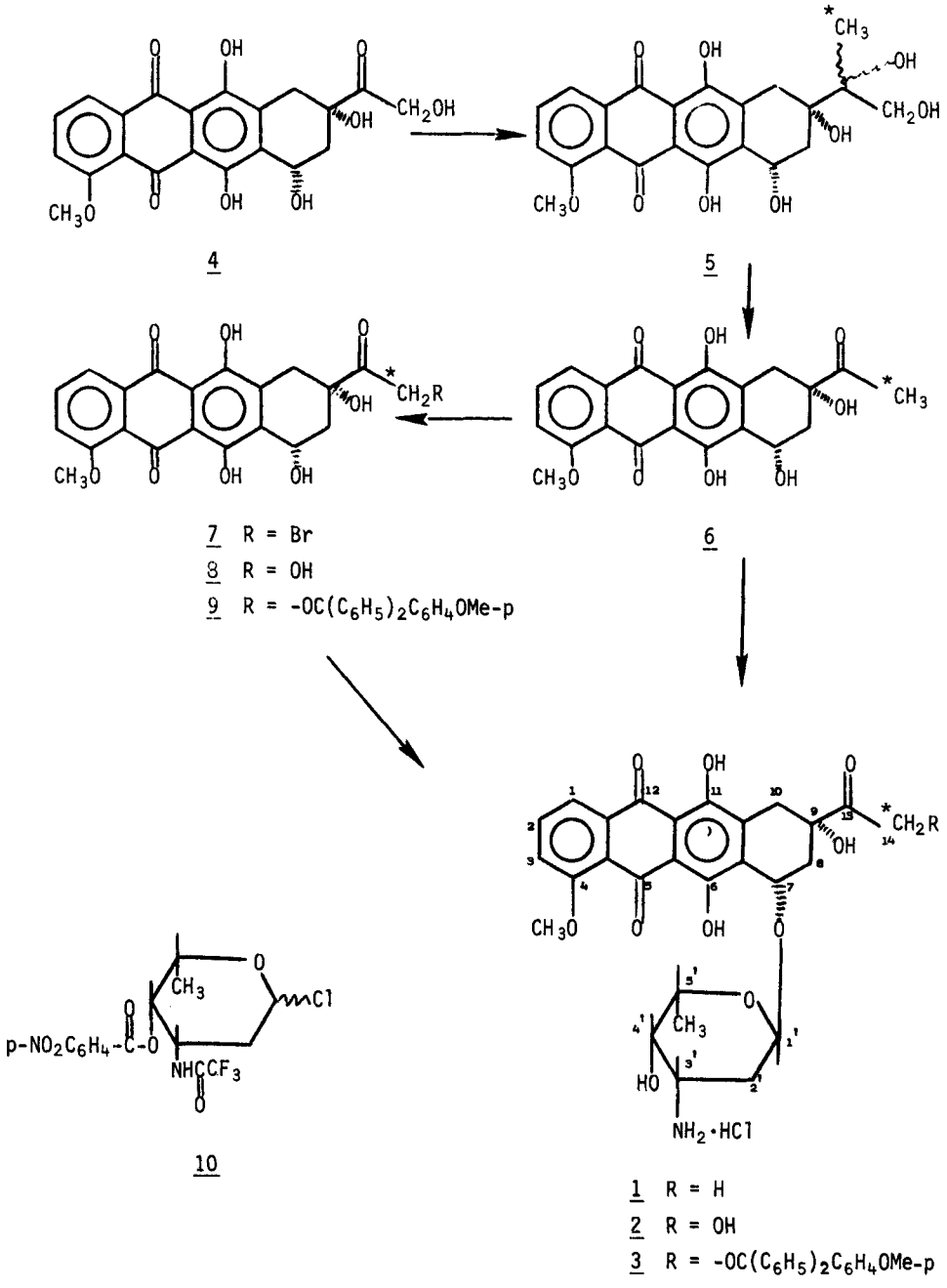
As originally envisaged, our route to daunorubicin-14-^{14}C (1) entailed the 1,2-addition of $^{14}CH_3MgI$ to the 13-carbonyl of adriamycin, followed by periodate cleavage of the resulting glycol. Earlier work had indicated that periodate cleavage of the 3'-4' bond in the daunosamine moiety would not occur[4]. However, reaction of adriamycin or its pertrimethylsilylated derivative with CH_3MgBr under a variety of reaction conditions resulted predominantly in cleavage of the glycosidic bond without addition to the 13-carbonyl. Therefore, we investigated the use of aglycone 4 as starting material although two extra steps, glycoside coupling and deblocking, would be necessary.

Treatment of adriamycinone (4) in THF with $^{14}CH_3MgI$ (15:1 mole ratio) at room temperature afforded the glycol (5). A larger (30:1) excess of CH_3MgI led to the appearance of products more polar than 5, indicating possible quinone carbonyl involvement; a smaller excess (10:1) gave incomplete reaction.

The hydroxyl groups of 4 consume at least five equivalents of Grignard reagent, presumably before carbonyl addition, and this accounts for part of the required large excess of reagent. Another important factor is probably the heterogeneous nature of the reaction; a solid is present immediately after introduction of the Grignard, but addition to the carbonyl is not complete for several hours. Efforts to conserve $^{14}CH_3MgI$ by prior protection of the hydroxyl groups have been only partially successful and are being investigated further.

Oxidation of 5 with $NaIO_4$ in aqueous methanol resulted in cleavage of the original C_{13}-C_{14} bond to afford daunomycinone-14-^{14}C (6). Koenigs-Knorr

SCHEME 1


4
5
6
7 R = Br
8 R = OH
9 R = -OC(C6H5)2C6H4OMe-p
10
1 R = H
2 R = OH
3 R = -OC(C6H5)2C6H4OMe-p

condensation of 6 with the protected 1-chlorodaunosamine (10), followed by alkaline deacylation of the resulting α-glycoside as previously reported[5], afforded daunorubicin-14-^{14}C (1) which was purified by dilution with "cold" material and recrystallization.

Bromination of daunomycinone-14-^{14}C (6) gave 7, and subsequent hydrolysis with 0.1N sodium hydroxide in 80% aqueous acetone provided adriamycinone-14-^{14}C (8)[6]. The 14-OH of 8 was protected by reaction with p-anisylchlorodiphenylmethane[6d] to give 14-O(p-anisyldiphenylmethyl)adriamycinone-14-^{14}C (9), which was then condensed with 10 to give the α-glycoside. Deacylation with NaOH followed by detritylation with acetic acid afforded the adriamycin-14-^{14}C (2). This was purified by dilution with "cold" material and recrystallization.

EXPERIMENTAL

(13R,S)-13-Hydroxy-13-Methyl-^{14}C-adriamycinone (5)--A mixture of anhydrous ether (4 ml) and methyl-^{14}C iodide (719.8 mg, 5.0 mmole, 100 mCi) was distilled under vacuum into a flask containing magnesium turning (33.2 mg, 5.5 mmole). Then the Grignard solution was added to a solution of adriamycinone (4) (138 mg, 0.33 mmole) in THF (50 ml). The mixture was stirred at room temperature overnight, cooled, decomposed with 0.1N HCl (50 ml) and extracted with $CHCl_3$. The extract was washed with saturated $NaHCO_3$ and then with water, dried over Na_2SO_4, and evaporated to give the glycol (5) (100 mg, crude, 5.3% from $^{14}CH_3I$).

Daunomycinone-14-^{14}C (6)--To a solution of 5 (100 mg, 0.23 mmole) in MeOH (30 ml) and H_2O (5 ml), cooled to 0°, was added $NaIO_4$ (50 mg, 0.23 mmole) in H_2O (12 ml). The mixture was stirred at 0° for 4 hr, diluted with H_2O (30 ml) and extracted with $CHCl_3$. The extract was washed with saturated $NaHCO_3$ and then with water, dried over Na_2SO_4, and evaporated to give a residue that was purified by preparative TLC (Silica Gel F-254 2 mm, E. Merck; $CHCl_3$:MeOH, 9:1) to afford 65 mg of crude 6.

Daunorubicin-14-^{14}C HCl (1)--A mixture of 6 (125 mg, 0.31 mmole), $Hg(CN)_2$ (722 mg), $HgBr_2$ (310 mg) and powdered molecular sieve 3A (1.62 g), in anhydrous

THF (30 ml) was heated at 50-55° for 2 hr. To the mixture were added four 1-molar equivalents of freshly prepared chloro sugar (10) in CH_2Cl_2 (2 ml) after 0, 5, 22, and 27 hr. The chloro sugar was prepared by bubbling HCl into a chilled solution of N-trifluoroacetyl-1,4-di-O-p-nitrobenzoyldaunosamine (188.2 mg, 0.31 mmole)[5] in CH_2Cl_2 (8 ml) for 5 min, stirring at room temperature for 10 min, filtering, evaporating, and drying under vacuum to give 10 as a white foam. Additional portions of $Hg(CN)_2$ (722 mg), $HgBr_2$ (310 mg) and powdered molecular sieve 3A (1.62 g) were added at 5, 22, and 27 hr. After 47 hr, the reaction mixture was filtered and evaporated. The residue was triturated with $CHCl_3$, filtered, washed with 30% KI and then with water, dried over Na_2SO_4, and evaporated.

The residue in THF (40 ml) was cooled to 0° and treated with 0.1N NaOH (60 ml) and the solution (pH 11) was stirred for 8 hr. The pH of the aqueous phase was adjusted to 8-9 with 0.1N HCl, and the solution was extracted with $CHCl_3$. The extract was washed with saturated $NaHCO_3$ and water, dried over Na_2SO_4, and evaporated.

The residue (1) as the free base was dissolved in $CHCl_3$ (8 ml) and filtered. To the filtrate were added 0.4N ethereal HCl (0.72 ml) and ether (6 ml). The precipitate was collected by centrifugation, washed with ether, and dried to give 1 (50 mg, 26.3%). Further purification was accomplished by dilution with cold daunorubicin HCl (50 mg) in MeOH (2 ml) and precipitation with ether (6 ml) to yield final 1 (72 mg, sp. act., 6.9 mCi/mmole, total act., 805 μCi).

Adriamycinone-14-^{14}C (8)--To a $CHCl_3$ solution (15 ml) of 6 (103 mg, 0.26 mmole) was added a solution of bromine in $CHCl_3$ (0.37N, 2.5 ml, 0.91 mmole). The reaction mixture was stirred at room temperature for 5.25 hr. The solution was evaporated, and the residue was coevaporated with benzene. The residue was dissolved in 80% aqueous acetone (30 ml) and 0.1N NaOH (4 ml) was added, and the solution was heated over a steam bath for 5 min and cooled to room temperature for 10 min. After the solution was concentrated, water was added and the product was extracted with $CHCl_3$:MeOH (1:1). The solution was washed with water, dried over Na_2SO_4, and evaporated to give 8 (71 mg, 66%).

14-O-(p-Anisyldiphenylmethyl)adriamycinone-14-^{14}C (9)--To a chilled solution of 8 (54 mg, 0.13 mmole) in pyridine (10 ml) was added p-anisylchlorodiphenylmethane (400 mg, 1.3 mmole). The solution was kept at 5° for 5 days and then poured into ice water and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with saturated $NaHCO_3$ and with water and evaporated. The residue was coevaporated with toluene, and the resulting red oil was dissolved in $CHCl_3$ (4 ml) and added to petroleum ether (bp, 30-60°, 30 ml). The resulting precipitate was recovered by centrifugation and decantation. Further purification by the above method gave pure 9 (58 mg, 65%).

14-O-(p-Anisyldiphenylmethyl)adriamycin-14-^{14}C (3)--A solution of 9 (58 mg, 0.085 mmole), $Hg(CN)_2$ (250 mg), $HgBr_2$ (130 mg) and powdered molecular sieve 3A (500 mg) in THF (9 ml) was refluxed for 2 hr. Ten 1-molar equivalent portions of freshly prepared 10 (see procedure for 1) were added at 0, 2.5, 5.5, 10.5, 24, 27.5, 30.5, 35.5, 47.0 and 48.5 hr. Additional portions of $Hg(CN)_2$ (250 mg), $HgBr_2$ (130 mg) and molecular sieve 3A (500 mg) were added at 23 hr. The reaction temperature was kept at 60° throughout. Total reaction time was 50 hr, after which time the reaction mixture was filtered, and the filtrate washed with 30% KI, saturated $NaHCO_3$, and water, dried over Na_2SO_4, and evaporated to give an orange residue.

To a chilled solution of the residue in THF (8 ml) was added 0.2N NaOH (17 ml). The deacylation reaction was followed by TLC (Silica Gel F-254; $CHCl_3$:MeOH, 10:1) and was complete after stirring in an ice bath for 5.25 hr. After neutralization with 0.2N HCl to pH 8, the solution was extracted with $CHCl_3$:MeOH (4:1). The organic solution was washed with water, dried over Na_2SO_4, and evaporated to give 80 mg of crude 3.

Adriamycin-14-^{14}C HCl (2)--The 80 mg of crude 3 was dissolved in 80% acetic acid (4 ml) and the solution was stirred at room temperature overnight. The solution was lyophilized while the temperature was kept below 0°. The residue was dissolved in MeOH:$CHCl_3$ (2:1) (5 ml) and filtered. To the filtrate was added 0.1N methanolic HCl (1.5 ml) followed by ether (50 ml). The precipitate was collected by centrifugation and decantation. Further purification--precipitation

from MeOH with Et_2O--gave 2 (20 mg, 40% for three steps). Final purification by dilution with cold adriamycin·HCl (14 mg) gave 17 mg of product (sp. act. 6.5 mCi/mmole; total act. 190 μCi).

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