*Carbohydrate Research*, 184 (1988) 231–235
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands



## Note

# Preparative procedures for conversion of daunorubicin into doxorubicin (Adriamycin) and 14-*O*-acetyldoxorubicin by way of 14-bromodaunorubicin*

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(Received May 29th, 1987; accepted for publication, July 7th, 1987)

Satisfactory experimental procedures for bromination of daunorubicin (**1**) to afford 14-bromodaunorubicin (**2**), and the subsequent conversion of this product into 14-*O*-acetyldoxorubicin (**3**), and doxorubicin (adriamycin, **4**) are described. The procedures are readily reproducible and give the products in high purity; doxorubicin is obtained in 35% net yield from daunorubicin without recourse to chromatographic steps.

Although the patent literature records[1] the conversion of daunorubicin (daunomycin, **1**) into doxorubicin (adriamycin, **4**) by way of 14-bromodaunorubicin[2–4] (**2**), this procedure was unsuccessful in our hands; the only product obtained was 14-bromodaunomycinone (**6**) resulting from glycosidic hydrolysis. The present procedure describes satisfactory and reproducible conditions for accomplishing this sequential transformation of daunorubicin into doxorubicin. Similar difficulties in converting the bromide **2** into 14-*O*-acetyldoxorubicin (**3**) by the method described[1] were circumvented by a modified procedure.

Attempted bromination of daunorubicin hydrochloride (**1**) in methanol–1,4-dioxane solution with an equimolar amount of bromine for 4 h at room temperature as described[1] led to reaction of only approximately 50% of the starting material. The reaction was still incomplete after 24 h, and chromatography indicated that glycosidic cleavage had taken place to a considerable extent with the release of daunomycinone (**5**) and 14-bromodaunomycinone (**6**). This hydrolysis is attributable to the hydrobromic acid liberated during the bromination. Processing of the product mixture afforded only 14-bromodaunomycinone (**6**); 14-bromodaunorubicin (**2**) was undetectable, even by chromatography. The results indicated glycosidic hydrolysis as a major process accompanying bromination, with further hydrolysis of any bromoglycoside **2** during the isolation procedure.

Use of a larger excess of bromine led to still more extensive hydrolysis, which

*This work was supported, in part, by Grant No. GM-11976 from the National Institute of General Medical Sciences, N.I.H., Bethesda, Maryland.
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0008-6215/88/$ 03.50 

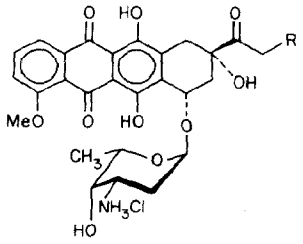
was evident even after only four hours of reaction. Such scavengers for hydrobromic acid as acetamide[5], cyclohexene oxide[6], or cadmium carbonate were unsatisfactory; either hydrolysis was not prevented, or the bromination reaction was arrested. The use of pure methanol as solvent[7] did not give any improvement.

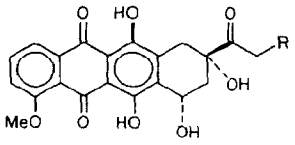
The most satisfactory conditions for bromination involved treatment of **1** with a 3-molar excess of bromine in methanol–1,4-dioxane at 0–5°. After six days of reaction, negligible hydrolysis had taken place, and the 14-bromo derivative **2** was obtained in 54% yield.

The product was isolated by precipitation from solution with ether and filtration. The product was then crystallized from methanol–ether. Evaporation of the bromination solution to dryness[1] was unsatisfactory, as this caused essentially complete hydrolysis of the glycoside.

Conversion of **2** into 14-*O*-acetyldoxorubicin (**3**) by the literature procedure of treating the bromide **2** with potassium acetate in dry acetone[1,8] for 3 h in the boiling solvent under reflux led to incomplete reaction and low yields. The reaction proceeded much more satisfactorily when conducted at room temperature; after 5 h of reaction, 14-*O*-acetyldoxorubicin (**3**) was observed as the sole product.

For conversion of the bromide **2** into doxorubicin (**4**), the literature procedure[1] gave low yields. Instead, 14-bromodaunorubicin (**2**) was dissolved in dimethyl sulfoxide–water and the solution was heated for 2 h at 80° to give pure doxorubicin (adriamycin, **4**) in 64% yield. The net yield of **4** from **1** was reproducibly 35%, and this could be significantly augmented, if desired, by chromatographic processing of mother liquors. N.m.r. parameters for compounds **3** and **4** are recorded in the Experimental Section for reference in synthetic work on novel antitumor anthracyclines[9].




**1** R = H
**2** R = Br
**3** R = OAc
**4** R = OH

**5** R = H
**6** R = Br
**7** R = OAc
**8** R = OH

EXPERIMENTAL

*General methods.* — Melting points were determined with a Thomas–Hoover apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. N.m.r. spectra were recorded at 200 MHz for $^1H$ and 50 MHz for $^{13}C$ with a Bruker WP-200 spectrometer. T.l.c. was performed on precoated, plastic sheets (0.2 mm), and glass plates (0.25 mm) coated with Silica Gel 60F-254 (E. Merck, Darmstadt, G.F.R.), with 2:1 toluene–acetone as eluent.

*14-Bromodaunorubicin hydrochloride* (**2**). — To a solution of daunorubicin hydrochloride (**1**, 1.00 g, 1.75 mmol) in a mixture of dry methanol (25 mL) and 1,4-dioxane (80 mL), at 0–5°, was added a solution of bromine (5.10 mmol) in chloroform (8 mL of a solution prepared from 10.0 g of bromine in 100 mL of chloroform). The solution was kept for 6 days at 0–5°, and the reaction was then terminated by first removing the excess of bromine by bubbling air through the solution and then precipitating the product **2** by adding ether (200 mL). The product was filtered off, washed with ether, dried, and crystallized from methanol (10 mL)–ether (20 mL) to afford pure **2**, yield 0.61 g (54%); m.p. 175–177° (lit.[1] 177–178°).

The mother liquors from the crystallization contained an estimated 0.1 g of additional **2**, together with 14-bromodaunomycinone (**6**, $R_F$ 0.60), but no unreacted **1**; the net yield of **2** could be augmented, if desired, by chromatographic manipulation of the mother liquors.

Monitoring of the progress of the reaction was performed by withdrawing aliquot samples at 12-h intervals, removing the bromine by passing a stream of air, and then examining by t.l.c. with reference samples of the aglycons daunomycinone (**5**, $R_F$ 0.50) and 14-bromodaunomycinone (**6**, $R_F$ 0.60) to estimate the extent of glycosidic hydrolysis. Such hydrolysis was negligible up to 4 days of reaction; a limited extent of hydrolysis occurred during the last 2 days. Similar aliquots were independently freed from bromine and treated with ether to afford a red precipitate that was separated by decantation and washed twice with ether. This solid (glycoside plus aglycon) was hydrolyzed by boiling for 5 min in 0.1M HCl, and the resultant red precipitate (the water-insoluble aglycons **5** and **6**) were extracted from the cooled solution with chloroform. T.l.c. indicated the progressive increase of **6** at the expense of **5**, and the proportion of **5** decreased to zero after the 6-day reaction period.

In our hands, the literature procedure[1] for bromination of **1** gave exclusively the brominated aglycon **6**; moreover the use of chloroform for dissolution of the putative **2** was not feasible as **2** is insoluble in this solvent.

*14-O-Acetyldoxorubicin hydrochloride* (**3**). — 14-Bromodaunorubicin hydrochloride (**2**, 100 mg, 0.16 mmol) was suspended in anhydrous acetone (60 mL) and then potassium acetate (0.270 g, 2.75 mmol) was added. The mixture was stirred magnetically at room temperature and monitored every 30 min by evaporating aliquot samples. The resultant syrup (containing potassium acetate and a mixture

of **2** and **3**) was redissolved in 0.1M HCl and boiled under reflux for 5 min, to afford a red precipitate (containing the algycons), which, after cooling, was extracted with chloroform. T.l.c. analysis was performed with aglycons **6** and **7** as standards. After 5 h, the reaction was judged to be complete when **6** was absent from the aliquot samples. Undissolved potassium acetate was filtered off and the filtrate evaporated. The resultant syrup was redissolved in 0.1M HCl (5 mL) and the solution washed with chloroform (3 × 5 mL) to extract a minor amount of aglycon, and subsequently with butanol until no more red product was extracted into the butanol. Evaporation of the butanol gave a red precipitate which crystallized from methanol (5 mL)–ether (10 mL), to give 0.055 g (57%) of pure 14-*O*-acetyldoxorubicin (**3**); m.p. 190–192° (lit.[1] 188–190°), $[\alpha]_D^{20}$ +256° (*c* 0.1, methanol) (lit.[1] +255°, lit.[8] +250°); $^1$H-n.m.r. ($CD_3OD$): $\delta$ 7.71 (2 H, m, H-1,2), 7.50 (1 H, d, $J_{2,3}$ 6.0 Hz, H-3), 5.52–5.03 (4 H, m, H-1′,7,14), 4.35 (1 H, q, $J_{5',6'}$ 6.1 Hz, H-5′), 4.05 (3 H, s, OMe), 3.69 (1 H, bs, H-4′), 3.61 (1 H, m, H-3′), 2.99 (1 H, d, $J_{10ax,10e}$ 18.0 Hz, H-10*e*), 2.74 (1 H, d, H-10*ax*), 2.50–1.82 (4 H, m, H-2′*e*,2′*a*,8*e*,8*ax*), 2.24 (3 H, s, Ac), and 1.28 (3 H, d, H-6′); $^{13}$C-n.m.r. ($D_2O$): $\delta$ 187.0, 186.1 (C-5,12), 174.5 (C=O of Ac), 161.6 (C-4), 157.0, 154.4 (C-6,11), 137.6, 134.5, 134.0 (C-2,6a,10a,12a), 120.5, 120.4, 119.5 (C-1,3,4a), 111.6, 111.4 (C-5a,11a), 100.0 (C-1′), 77.1 (C-9), 69.5 (C-7), 67.9, 67.0 (C-4′,5′,14), 57.2 (OMe), 48.3 (C-3′), 37.1 (C-8), 32.8 (C-10), 28.5 (C-2′), 21.6 ($CH_3$ of Ac), and 16.5 (C-6′).

*Doxorubicin hydrochloride* (**4**). — Treatment of a methanolic solution of **2** with aqueous NaOH, and following the steps exactly as given in the literature[1], afforded **4** in 49% yield. A simplified process and significantly improved yield was achieved by the following modified procedure.

A solution of **2** (0.270 g, 0.42 mmol) in dimethyl sulfoxide (7 mL) and water (1.5 mL) was kept at 80°. The reaction was monitored by taking aliquot samples, hydrolyzing them for 5 min in boiling 0.1M HCl, dissolving the resultant precipitate in chloroform, and examining the product by t.l.c. in comparison with the reference aglycons **6** ($R_F$ 0.60) and **8** ($R_F$ 0.30) as standards. After 2 h the reaction was judged to be complete. The solution was evaporated at 45° to a solid that was redissolved in 0.1M methanolic HCl (5 mL). Addition of ether (10 mL) caused precipitation of pure adriamycin (**4**), yield 0.156 g (64%), identical with an authentic sample; $^1$H-n.m.r. ($CD_3OD$): $\delta$ 7.83 (2 H, m, H-1,2), 7.49 (1 H, d, $J_{2,3}$ 6.1 Hz, H-3), 5.40 (1 H, bs, H-1′), 5.03–4.62 (3 H, m, H-7,14), 4.25 (1 H, q, $J_{5',6'}$ 6.0 Hz, H-5′), 3.95 (3 H, s, OMe), 3.67–3.31 (2 H, m, H-3′,4′), 3.00 (1 H, d, $J_{10e,10ax}$ 18.8 Hz, H-10*e*), 2.82 (1 H, d, H-10*ax*), 2.28–1.81 (4 H, m, H-2′*e*,2′*a*,8*e*,8*ax*), and 1.28 (3 H, d, H-6′); $^{13}$C-n.m.r. ($CD_3SO$): $\delta$ 213.7 (C-13), 186.6 (C-5,12), 160.8 (C-4), 155.9, 154.4 (C-6,11), 136.3, 135.2, 134.7, 134.1 (C-2,6a,10a,12a), 120.0, 119.8, 199.0 (C-1,4a,3), 110.7 (C-5a,11a), 99.1 (C-1′), 74.7 (C-9), 69.7 (C-7), 66.1 (C-4′,C-5′), 63.6 (C-14), 56.6 (OMe), 46.6 (C-3′), 36.4 (C-8), 32.1 (C-10), 28.1 (C-2′), and 16.7 (C-6′).

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