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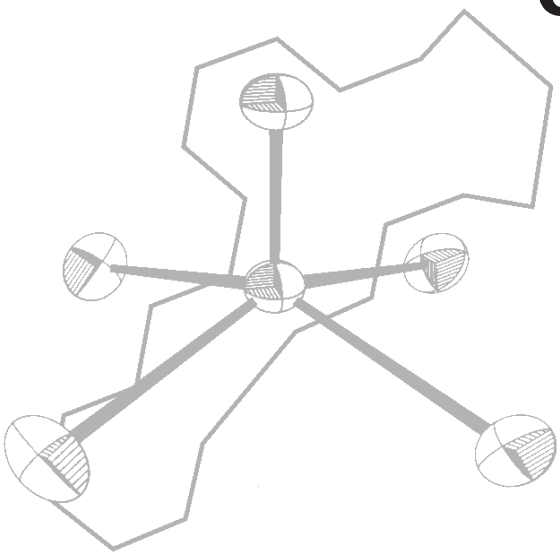

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5-Deoxy, 12-Deoxy, 5,12-Bisdeoxy, and 4,5,12-Trisdeoxy Anthracyclines: Synthesis of New Analogues of Daunorubicin and Doxorubicin by Controlled Deoxygenation of the C-Ring*

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Hydrogenation of the anthracyclines daunorubicin (1) and doxorubicin (2) gave selective deoxygenation at position 5. Hydride reduction of (1) and (2) gave complementary regiocontrol, leading to 12-deoxygenation or 5,12-bis-deoxygenation. This chemistry allows retention of the 7-glycoside and the side-chain carbonyl groups. It has led to new anthracycline families possessing all of the stereochemical and most of the spatial characteristics of the parent compounds (1) and (2). These are typified by 5-deoxy (12), (15); 12-deoxy (22), (23); 5,12-bisdeoxy (34), (35); and 4,5,12-trisdeoxy systems (36). All possess high anticancer activity.

Keywords. Daunorubicin; doxorubicin; anthracycline; anticancer.

Introduction

Deoxy analogues of the important commercial antineoplastic agents daunorubicin (daunomycin) (1) and doxorubicin (adriamycin) (2)† are of interest as second-generation antitumour agents.[1] They modify the redox potential of the parent compound without altering the stereochemistry, leading to an altered and, in some cases, useful spectrum of bioactivity. Known examples include 11-deoxy, 6-deoxy and 4-demethoxy systems, each of which has demonstrated antitumour activity. Gaining access to these systems, the first of which also occurs naturally, has required multi-step total synthesis.[1]

In principle, access to analogous deoxy systems might be considered by simple reduction of parent anthracyclines. However, significant chromophoric modification of (1) and (2) without loss of the glycoside has been reported in only a few cases. Thus 5-imino anthracyclines were formed by reaction of (1) and (2) with ammonia.[2] The products had significantly different reduction potentials from the parent systems and lower cardiotoxicity in test mammals.[3] Another example, *leuco* daunorubicin, resulted as a mixture of diastereoisomers from mild reduction of (1) with sodium dithionite.[4] The glycoside was also retained following acylation of (1) and subsequent catalytic hydrogenation.[5]

If cleavage of the glycoside could be suppressed, direct synthesis of deoxy anthracyclines by reductive deoxygenation of anthracycline parents is chromophorically encouraged by the reported formation of 5,7- and 7,12-bisdeoxy systems (3) and (4). (For convenience of describing such products, the quinonoid grouping in (3) and (4) is considered as occupying a position tautomeric with its placement in (1) and (2).) System (3) was obtained by either electrochemical reduction of 5-iminodaunorubicin[6] or treatment of 7-deoxydaunorubicinone (5) with sodium dithionite.[7] In other work, hydrogenolysis of daunorubicinone (6) (palladium on barium sulfate) gave a product whose reported spectra also appear to correlate with the 5,7-bisdeoxy system (3); this is in preference to the isomeric 7,11-bisdeoxy structure originally suggested.[8] While the present investigation was in progress, the same 5,7-deoxy chromophore was obtained as its 13-ol (7), by reduction of (5) with sodium borohydride, together with its 7,12-bisdeoxy analogue (8).[9]

However, at the outset of this work there was no reported way in which such chromophoric modification of the parent anthracyclines (1) and (2) could be effected without prior reductive cleavage of the 7-glycoside, an *ortho*-hydroxybenzyl system. Thus mild catalytic hydrogenation of daunorubicin (1) mainly led to the 7-deoxy product (5).[10] Retaining the daunosaminyloxy residue is necessary for water solubility and bioactivity.[1]

Following our preliminary reports,[11,12] this paper fully describes efficient reductive synthesis of several new anthracycline families, based on selective 5- and 12-deoxygenation chemistry, without significant loss of the sugar residue.

* Published in preliminary form: *Tetrahedron Lett*., 1988, **29**, 4629; *Tetrahedron Lett*., 1993, **34**, 4685.

† WHO names for these compounds are daunorubicin (1) and doxorubicin (2). For purposes of uniformity, common anthracycline numbering, as on structure (1), is used throughout this paper. This differs from IUPAC numbering, which varies with functionality.

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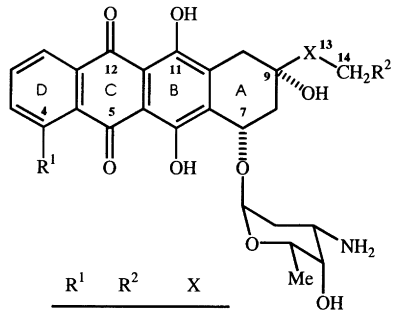
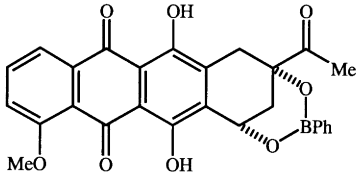


	R^1	R^2	X
(1)	OMe	H	CO
(2)	OMe	OH	CO
(25)	OH	H	CO
(37)	H	H	CO
(39)	OMe	H	CHOH
(44)	OMe	H	$C(OMe)_2$
(46)	OMe	OH	$C(OMe)_2$



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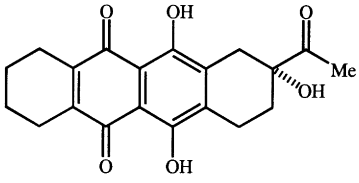

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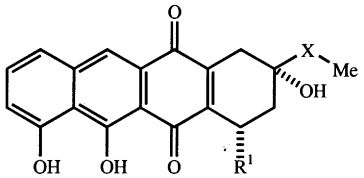
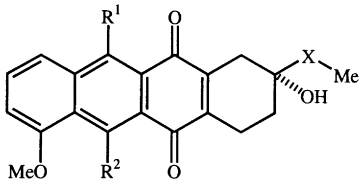
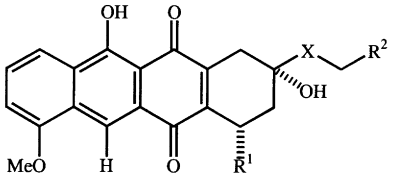


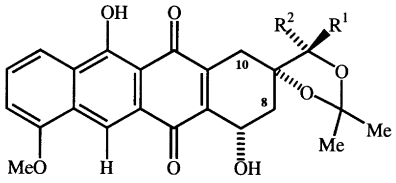
	R^1	X
(27)	H	CO
(28)	H	CHOH
(30)	OH	CO
(31)	OH	CHOH



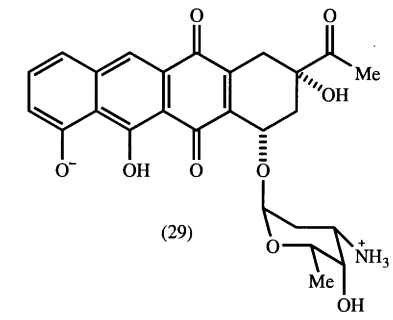
	R^1	R^2	X
(3)	OH	H	CO
(4)	H	OH	CO
(7)	OH	H	CHOH
(8)	H	OH	CHOH



	R^1	R^2	X
(10)	OH	H	CO
(11)	OH	H	CHOH
(17)	H	OH	CO
(18)	OH	OH	CO
(19)	OH	OH	CHOH



	R^1	R^2
(20)	Me	H
(21)	H	Me



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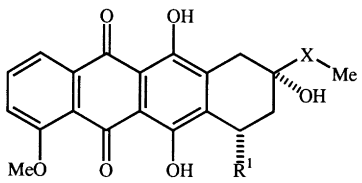
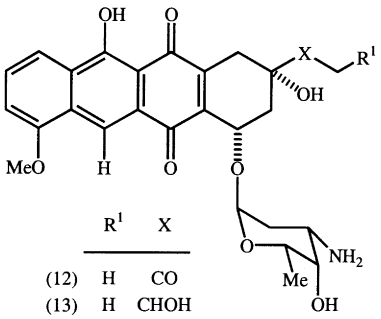
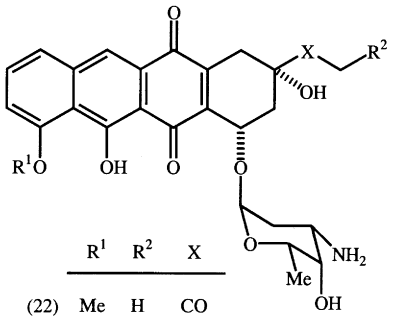


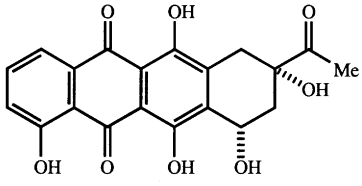
	R^1	X
(5)	H	CO
(6)	OH	CO
(38)	H	CHOH



	R^1	X
(12)	H	CO
(13)	H	CHOH
(15)	OH	CO
(16)	OH	CHOH



	R^1	R^2	X
(22)	Me	H	CO
(23)	Me	OH	CO
(24)	H	H	CO
(26)	H	H	CHOH
(40)	Me	H	CHOH
(45)	Me	OH	CHOH



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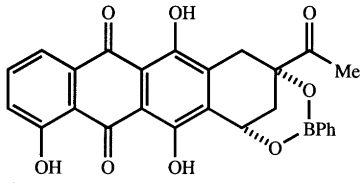


(33)

5-Deoxy Anthracyclines

Successful 5-deoxygenation of daunorubicin (1) and doxorubicin (2) was found to be effected by catalytic hydrogenation under conditions that evolved through a series of preliminary experiments. Initially, hydrogenation of the hydrochloride of (1) in water over palladium on barium sulfate, was confirmed to result in rapid hydrogenolysis of the glycoside, with precipitation of 7-deoxydaunorubicinone (5) (97%) as reported.[13] However, with the same catalyst in methanol, this first product (5) was identified chromatographically but it remained in solution, where it underwent further, slower reduction to a mixture, from which 5,7-bisdeoxydaunorubicinone (3) (88%) was obtained following aerial reoxidation of the derived hydroquinone. A small proportion (8%) of the isomeric 7,12-bisdeoxy compound (4) was also isolated. These isomers were distinguished by their ^{1}H n.m.r. spectra, isomer (3) showing a single chelated hydroxy signal at δ 13.77, whereas for (4) the signal was considerably more deshielded (δ 15.12). For the former isomer, its *meso* proton resonated as a singlet at δ 8.50. This was deshielded relative to the corresponding proton of (4) (δ 8.02), consistent with its *peri* relationship to two oxygen substituents. Other signals were consistent with the assigned structures and each isomer exhibited a molecular ion at *m/z* 366 in its electron-impact (e.i.) mass spectrum. The data for (4) are consistent with relevant values for model tricyclic compounds prepared in our earlier work.[14–16]

Chromophorically, the overall deoxygenation of (5) to the major product (3) is analogous to regioselective hydrogenation of a 1,4-dihydroxy-5-methoxy-9,10-anthraquinone to the corresponding anthrone followed by tautomerism and reoxidation to a 9-hydroxy-5-methoxy-1,4-anthraquinone.[15] With appropriately different regiochemistry, the natural anthraquinone catenarin behaves analogously.[17]

In order to suppress 7-deoxygenation, experiments next compared hydrogenation of the phenylboronate (9), in which the 7-oxy substituent was part of a cyclic system,[18] with the aglycone (6) from which it was derived. The latter has been reported to undergo hydrogenolysis over palladium to give (5).[10] The phenylboronate (9) was synthesized efficiently[18] but when hydrogenated in methanol over palladium on barium sulfate it was found to undergo only 7-deoxygenation to (5). To exclude any possibility of boronate exchange in the alcoholic solvent, the hydrogenation was repeated in ethyl acetate but this led only to recovery of (9), following aerial reoxidation of the corresponding quinol. However, hydrogenation of (9) in ethyl acetate over Adams catalyst instead of palladium gave a pale yellow solution, containing none of the starting quinone. Aerial reoxidation of the filtrate gave a red product clearly different from the orange chromophore of (9). Acid-catalysed exchange of the phenylboronate residue with pentane-2,4-diol and chromatography gave two red compounds, identified as the new 5-deoxydaunorubicinone (10) (45%) and its more polar 13-dihydro derivative (11) (45%). Retention of the 7-hydroxy group in both products was evident from their ^{1}H n.m.r. spectra, which contained deshielded multiplets due to H 7 at δ 5.19 and 5.22, respectively. Singlets at δ 8.54 and 8.63 for the respective *meso* protons confirmed that 5-deoxygenation had occurred, while reduction of the COMe group in (11) was evident from a pair of shielded 14-methyl doublets at δ 1.30 and 1.35 (*J* 6.5 Hz each), representing the two diastereoisomers of this system. Molecular ions were shown at *m/z* 382 (10) and 384 (11) in the respective e.i. mass spectra. The change of catalyst from palladium to platinum thus afforded preferential deoxygenation at position 5 over 7 for the first time, albeit accompanied by competitive reduction at position 13.

By contrast, parallel platinum-catalysed hydrogenation of the unprotected aglycone (6) gave 5,7-bisdeoxydaunorubicinone (3), together with trace amounts of (4) and (7). While retention of the 7-oxy substituent during reduction of (9) may reflect its cyclic nature, it also seemed possible that the difference may have derived from the steric bulk of the phenylboronate residue. This led to experiments involving platinum-catalysed reduction of the anthracycline glycosides (1) and (2) themselves. It was thereby pleasingly established that hydrogenation of the hydrochloride of daunorubicin (1) in methanol over Adams catalyst, followed by the usual aerial reoxidation, gave smooth conversion into a mixture of the new 5-deoxydaunorubicin (12) and 5-deoxy-13-dihydrodaunorubicin (13), the latter as a pair of C 13 diastereoisomers. Subsequent to our original report,[11] compound (12) has also been derived by reduction of 5-iminodaunorubicin with sodium dithionite.[19]

Glycosides (12) and (13) were separated by column chromatography on silica gel and each was isolated as its hydrochloride. Their ^{1}H n.m.r. spectra were similar to those of the corresponding aglycones (10) and (11) except for additional resonances due to the daunosaminyl residue. Thus the spectra of (12) and (13) each contained an appropriate *meso* proton resonance (δ 8.40, 8.39 respectively), while the 14-methyl singlet (δ 2.30) in the spectrum of the former product was deshielded relative to the corresponding resonance (δ 1.29) for the latter. Molecular ions were not observed in their e.i. mass spectra, but f.a.b. mass spectrometry showed weak M+H ions [*m/z* 512 for (12), 514 for (13)] and corresponding M+Na ions. The relative yields of (12) and (13) could be varied by changing the ratio of catalyst to substrate. Compared to 5-deoxygenation of (1), reduction of the side-chain carbonyl group was slow. However, by increasing the ratio of catalyst/substrate to 1 : 1, complete conversion into (13) was achieved in good yield (71%) and isolation did not require chromatography.

Minimizing side-chain reduction so as to achieve optimum yield of (12) would require protection–deprotection of the 13-keto group, as described later. However, satisfactory formation of this product resulted from direct reaction of unprotected (1) by lowering the catalyst ratio to 1 : 5 (20% w/w) and by addition of chloroacetic acid (4 : 1 acid/substrate). After basifying the product solution with sodium hydrogen carbonate followed by aerial reoxidation this led to (12) (57%) along with (13) (25%). The remainder of the product was largely a mixture of (3), (7) and a trace of a red, non-glycosidic component arising from a different mode of reduction. Its ^{1}H n.m.r. spectrum indicated no aromatic proton resonances, but two broad multiplets at δ 1.78

and 2.66 instead, integrating for four protons apiece. There was an acetyl methyl group (δ 2.37) but no methoxy resonance. The spectrum also showed two chelated hydroxy resonances at δ 13.15 and 13.19 and a broad hydroxy resonance at δ 3.82, all D_2O-exchangeable. Other resonances due to the A-ring were similar to those observed for (5). These data, taken with an e.i. molecular ion of *m/z* 356, suggest the compound may have the tetrahydro structure (14).

These optimum conditions were derived empirically by varying the catalyst, reaction time and added acid. Fresh Adams catalyst gave best results. In the absence of chloroacetic acid, 5-deoxygenation to (12) proceeded too slowly, prolonged hydrogenation increasing the proportion of non-glycosidic by-products. An increased catalyst-to-substrate ratio (1 : 1) gave 5-deoxygenation accompanied by excessive side-chain reduction to (13). Adding trifluoroacetic or mineral acid in place of chloroacetic acid gave rapid 7-deoxygenation, while alternative addition of acetic acid had no significant effect.

Under these conditions in methanol, the daunosaminyl residue of (1) both protects the 7-oxy function from hydrogenolysis and leads to greater regioselectivity of deoxygenation of the C-ring. Thus reduction of the aglycone (6) under those same conditions gave only the 7-deoxygenated products (3), (4) and (7). In contrast, the products from parallel reductions of (1) were largely glycosides (12) and (13), and no 12-deoxygenated product analogous to (4) was observed, within the limits of ^{1}H n.m.r. spectroscopic sensitivity.

The clinically important antitumour antibiotic doxorubicin (2) was also hydrogenated in the same way as (1), giving a mixture of the new 5-deoxydoxorubicin (15) (59%) and 5-deoxy-13-dihydrodoxorubicin (16) (20%), which were separated by chromatography and isolated as hydrochlorides. Because of its considerable polarity, compound (16) was difficult to extract from aqueous solution and to elute from silica gel columns. By increasing the w/w ratio of Adams catalyst (1 : 1), (2) gave glycoside (16) exclusively. It was thereby isolated in 76% yield as its hydrochloride, without chromatography being required, as a mixture of C 13 diastereoisomers. Both (15) and (16) showed M+H ions by f.a.b. mass spectrometry (*m/z* 528, 530 respectively) as well as M+Na ions. Such hydrogenations were also accompanied by minor hydrogenolysis of the sugar residue, leading chiefly to the 5,7-bisdeoxy product (17) (15%). Its ^{1}H n.m.r. spectrum resembled that of (3), the 14-methyl signal of the latter (δ 2.38) being replaced by an AB methylene quartet (δ 4.70, 4.74, *J* 21 Hz).

Aqueous acidic hydrolysis of the glycosides (12), (13), (15) and (16) gave the respective aglycones (10), (11), (18) and (19), the first two of which were identical with the reduction products from daunorubicinone phenylboronate (9). The latter pair of aglycones had ^{1}H n.m.r. spectra that were appropriately similar to the former pair but 5-deoxydoxorubicinone (18) showed an AB quartet [δ 4.76 and 4.81 (*J* 21 Hz)] due to the C 14 methylene group and a molecular ion at *m/z* 398 in its e.i. mass spectrum. The ^{1}H n.m.r. spectrum of its 13-dihydro derivative (19) was more complex, consistent with a mixture of diastereoisomers (2 : 1). This particularly affected signals in the δ 1.7–2.5 and 2.6–3.2 regions due to H 8 and H 10, respectively. Its e.i. mass spectrum showed an appropriate molecular ion at *m/z* 400.

Similarly 5-deoxy-13-dihydrodaunorubicinone (11) also showed signals corresponding to a diastereoisomer ratio of 2 : 1. In particular, the C 14 methyl signal resonated as a pair of doublets, as noted earlier. The absolute stereochemistry of the respective isomers was assigned by ^{1}H n.m.r. spectroscopy according to literature procedures for analogous 13-ols.[20–22] To suppress rotational factors affecting the side chain, the mixture (11) was converted into the corresponding isopropylidene derivatives (20) and (21) by treatment with 2,2-dimethoxypropane and *p*-toluenesulfonic acid in dioxan.[20,21] These derivatives were spectroscopically differentiable and chromatographically separable. For the major ketal, chemical shift differences between the non-equivalent methylene protons H 8*eq* and H 8*ax* (δ 2.36, 1.61 respectively; Δδ 0.75), and H 10*eq* and H 10*ax* (δ 2.99, 2.53 respectively; Δδ 0.46) were consistent with values reported for analogous anthracyclinone ketals of defined 13*R* configuration. Corresponding Δδ values for the minor ketal [H 8*eq*, H 8*ax* (δ 2.18, 1.96 respectively; Δδ 0.22) and H 10*eq*, H 10*ax* (δ 3.02, 2.49 respectively; Δδ 0.53)] likewise paralleled isomeric (13*S*)-anthracyclinone ketals.[20,21]

The major ketal in the present study was thereby assigned as the 13*R*-isomer (20), an outcome that was also found to be consistent with n.O.e. experiments. In previous work involving an analogous pair of (13*R*)- and (13*S*)-anthracyclinone ketals, irradiation of the H 13 resonance led to significant enhancement of the H 10 signal for the former isomer and of H 8 for the latter.[22] For the major isomer, now assigned as (20), irradiation of H 13 (δ 4.20) showed largest enhancements (5%) for H 10*eq* and the adjoining methyl signal. For the minor isomer (21), the largest enhancements (7%) were observed for H 8*eq*, along with the adjoining methyl protons.

This diastereofacial bias in catalytic reduction of (1) towards 13*R* stereochemistry differs from the outcome of mammalian and microbial reduction in the metabolism of (1). Both have been shown to give only the 13*S* configuration.[20,21]

12-Deoxy, 5,12-Bisdeoxy, and 4,5,12-Trisdeoxy Anthracyclines

Attention was next directed towards alternative regiocontrol of C-ring deoxygenation, so as to gain access to a new, isomeric series of 12-deoxy anthracyclines, represented by 12-deoxydaunorubicin (22), 12-deoxydoxorubicin (23) and 12-deoxycarminomycin (24). Compound (24), hypothetically derivable from the parent carminomycin (25),[23,24] represented a realistic initial target since, by analogy with anthraquinone chemistry,[15,17] it was expected that 12-deoxygenation might be preferred over 5-deoxygenation, for hydrogenation of anthracyclines in which the 4-methoxy substituent was replaced by a 4-hydroxyl. Regioselective 4-*O*-methylation of (24) might then be a reasonable source of (22).

Carminomycin (25) was therefore hydrogenated in methanol containing chloroacetic acid over Adams catalyst,

analogously to (1). Oxidative workup showed poor conversion into a new glycoside initially identified as 12-deoxycarminomycin (24), accompanied by a still more polar glycoside 12-deoxy-13-dihydrocarminomycin (26). However, the major products (46% overall) were water-insoluble, reflecting hydrogenolysis of the glycoside and consisting chiefly of the 7,12-bisdeoxy compounds (27) and (28). Spectroscopic data for the latter pair were analogous to those for their respective *O*-methyl counterparts (4) and (8), except that in the ^{1}H n.m.r. spectrum of (27), for example, the two phenolic hydroxy groups resonated as two singlets at δ 16.14 and 9.92. The considerable deshielding of the former resonance is characteristic of the 5-hydroxy group flanked by two *peri* oxygen substituents.[14] The e.i. mass spectrum of (27) showed an appropriate molecular ion (*m/z* 352).

The more polar (28) was isolated as a (1 : 1) pair of diastereoisomers with doubling of signals due to H 13 (δ 3.84, 3.66, q, q, *J* 6.5, 6.5 Hz) and the C 14 methyl protons (δ 1.33, 1.30, d, d, *J* 6.5, 6.5 Hz). As expected, the *meso* proton H 12 was less deshielded than was H 5 in the 5-deoxy series. For (27), H 12 resonated at δ 8.02 while for the diastereoisomers of (28) it was observed at δ 7.93 and 7.94.

Despite being accompanied by excessive loss of the amino sugar, all of these products reflected the desired 12-deoxygenation. Increasing the acidity of the hydrogenation medium, through addition of dichloroacetic acid (4% w/v) rather than chloroacetic acid, was empirically found to give a much better yield of the glycoside (24) (59%). This was accompanied by a minor proportion of the corresponding 13-dihydro product (26), which was difficult to isolate, on account of its considerable polarity. However, conventional extraction of both these products from aqueous solution, sufficiently basic to avoid protonation of the amino group, proved particularly difficult. Moreover, their colour behaviour differed from that of the non-glycosidic (27) and (28). Sodium hydrogen carbonate solutions of both glycosides were blue, and ethyl acetate extracts were purple, spectacularly different colours from conventional anthracyclines. The products were separated on silica gel or sephadex LH20 columns, with polar mixtures of chloroform, methanol and water as eluent, despite some loss of material thereby occurring, due to strong binding. Evaporated fractions gave (24) and (26) as blue solids. The hydrochloride of (24) was isolated as a red solid after addition of an excess of methanolic hydrogen chloride, but on redissolving in neutral methanol it spontaneously gave a blue solution. Analogous behaviour was observed for the more polar compound (26), on which less work has been done. The electrospray mass spectrum of (24) (acetonitrile/water) showed an M+H ion at *m/z* 498 as the base peak.

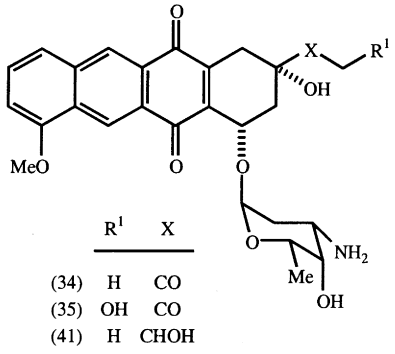
These observations, and the ^{1}H n.m.r. characteristics following, are consistent with (24) existing chiefly as the zwitterion (29) and it is designated as such in subsequent discussion. An analogous zwitterionic structure is inferred also for (26). This implies involvement of the amino sugar with a phenolic hydroxy group of acidity unparalleled in conventional anthracycline chemistry. The visible absorption spectra of buffered solutions of (29) at varying pH revealed that the chromophoric change from red to blue was associated with a pK_a value around 5. Other potentially zwitterionic anthracyclines have been reported but these have always involved carboxy groups as the internal proton source interacting with the amino glycosides.[25,26] A synthetic amino anthracenol has also been reported to form a zwitterionic species in solution.[27]

Solutions of (29) in (D_4)methanol, deuterium oxide or (D_6)dimethyl sulfoxide gave ^{1}H n.m.r. spectra in which every signal except the acetyl methyl was broadened beyond recognition. However, a mixture of (D)chloroform/(D_4)methanol (2 : 1), the maximum proportion of chloroform in which (29) remained in solution, gave a much sharpened spectrum, with recognizable aromatic resonances in particular. The cause of the broadening in protic and dipolar aprotic solvents is unclear. Acidifying a (D_4)methanolic solution of (24) with (D)trifluoroacetic acid, thereby changing the colour from blue to red, sharpened only some signals in the spectrum. This appears to rule out broadening by rapid electron transfer as ascribed to both fredericamycin A[28] and the synthetic amino anthracenol mentioned above.[27] Nevertheless, the dramatic line sharpening on addition of (D)chloroform evidently reflects suppression of radical formation in the other solvents. In many respects compound (29) thus behaves like kinobscurinone, described as 'n.m.r. silent' in (D_6)dimethyl sulfoxide, (D_5)pyridine and (D)trifluoroacetic acid.[29]

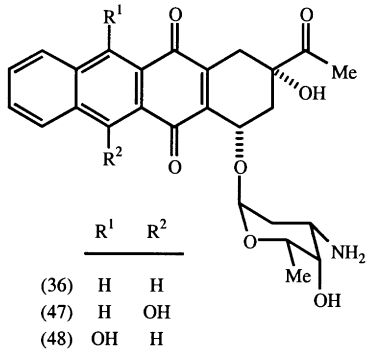
Acidic hydrolysis of (29) and (26) gave the respective red aglycones (30) and (31), which showed none of the spectroscopic anomalies of the amino glycosides. They were also obtained by conversion of carminomycinone (32) into its phenylboronate (33) and hydrogenation of the latter over Adams catalyst, followed by deprotection.

While hydrogenation of carminomycin (25) thus occurred with the desired regiochemistry, the zwitterionic product (29) proved unsatisfactory as a source of 12-deoxydaunorubicin (22). Despite encouragement from simpler model systems,[14,15] selective *O*-methylation of (29) could not be effected. Attention therefore turned to obtaining (22) and its doxorubicin analogue (23) more directly, by hydride reduction of the parent anthracyclines (1) and (2). Extension of methodology so developed has then also led to a further new family of 5,12-bisdeoxy anthracyclines, represented by bisdeoxydaunorubicin (34), bisdeoxydoxorubicin (35) and the 4,5,12-trisdeoxy analogue (36), this last product from analogous bisdeoxygenation of the antitumour antibiotic idarubicin (37). Sodium borohydride was used earlier for converting 1,4-dihydroxy anthraquinones into 9-hydroxy-1,4-anthraquinones.[16,29] It seemed possible that, as nucleophiles, such reductants might lead to different selectivity from hydrogenolysis.

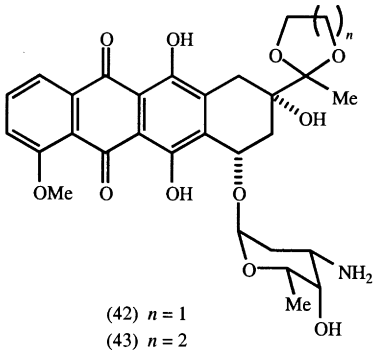
Reported treatment of daunorubicin (1)[30] and carminomycin (25)[31] with sodium borohydride in basic methanol resulted simply in reduction of the side-chain 13-keto group, the quinone nucleus apparently being protected by ionization. More recently,[32] while the present work was in progress, similar reduction of (1) in unbuffered methanol was reported also to give the 7-deoxy 13-ol (38) (58%) along with the side-chain reduced glycosidic system (39) (17%) reported earlier.[30]



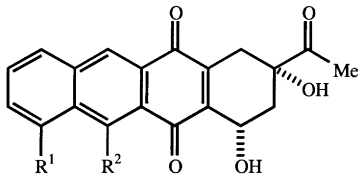
	R^1	X
(34)	H	CO
(35)	OH	CO
(41)	H	CHOH



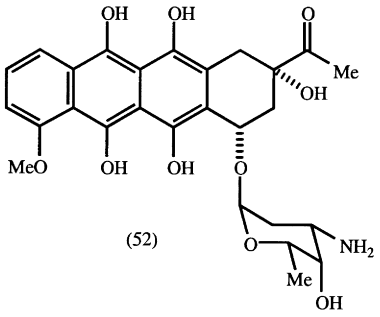
	R^1	R^2
(36)	H	H
(47)	H	OH
(48)	OH	H



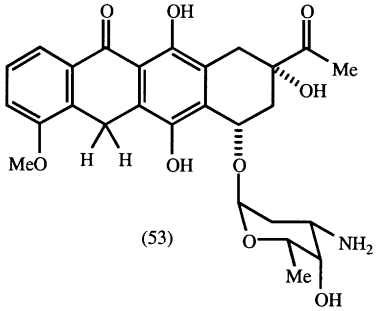
(42) $n = 1$
(43) $n = 2$



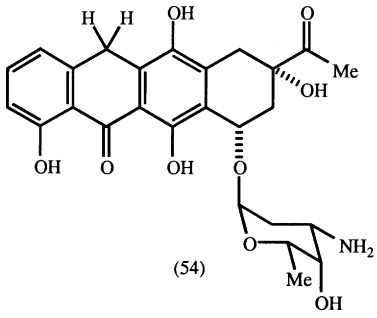
	R^1	R^2
(49)	OMe	OH
(50)	OMe	H
(51)	H	H



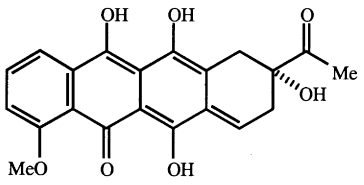
(52)



(53)



(54)



(55)

In the present investigation (1) was first treated with potassium or sodium borohydride in methanol containing aqueous buffer (pH 7). With buffers of lower pH, reduction competed with decomposition of the reagent, while in more basic environments it was impeded by ionization of the substrate. However, at pH 7 there was rapid reduction of the chromophore at 0°, as indicated by a sharp colour change from orange to yellow. Quenching with mineral acid to pH 1–3 caused another colour change to deep red. Workup then gave a dark red solid, isolated as its hydrochloride (93%). ^{1}H n.m.r. spectroscopy identified the major product as the new 12-deoxy-13-dihydrodaunorubicin (40) (71%) accompanied by minor proportions of its 5-deoxy isomer (13)[11] (14%) and a new glycoside (41) (8%). The ^{1}H n.m.r. spectrum of (41) showed two sharp aromatic singlets at δ 8.58 and 9.05, consistent with bisdeoxygenation (discussed later). This mixture was inseparable by chromatography but (40) was obtained by several crystallizations as a 1 : 1 mixture of diastereoisomers at C 13. Despite considerable overlapping, some resonances for the individual isomers were spectroscopically distinct. Thus H 10*eq* for the mixture resonated as a pair of doublets (δ 2.96, 3.02, both *J* 20 Hz). An electrospray mass spectrum showed a strong M+H ion at *m/z* 514. The relative shielding of the *meso* proton singlet (δ 8.04 for both isomers) confirmed that by far the major product of reduction corresponded to the desired 12-deoxygenation.

It was next sought to develop protection–deprotection chemistry, so as to allow the side-chain 13-keto group to be retained during reduction. Treatment of daunorubicin (1) in ethane-1,2-diol or propane-1,3-diol with an excess of trimethyl orthoformate and camphorsulfonic acid catalyst at 20°, gave the respective ketals (42) and (43) without significant hydrolysis of glycoside.[33] However, only the 1,3-dioxan (43) could be deprotected to (1) without undue formation of accompanying aglycone, by treatment with 0.1 M hydrochloric acid at 20°. Indeed, despite (43) being formed efficiently in solution, attempted isolation of (43) as its hydrochloride always gave mixtures with deprotected (1), as differentiated by the chemical shifts of the 14-methyl protons [δ 2.42 for (1) and δ 1.64 for (43)]. Accordingly, the ketalization mixture containing (43) was diluted as such, with methanol and aqueous buffer (pH 7), and treated with potassium borohy-

dride at 0° under nitrogen. After acid-quenching, the intermediate ketal was deprotected as above and the product was isolated as a mixture of hydrochlorides. Its ^{1}H n.m.r. spectrum was consistent with the major component being the desired new 12-deoxydaunorubicin (22) (71%) [δ 2.40 (COMe), 8.03 (H 12)] accompanied by its 5-deoxy isomer (12) (4%) [δ 2.30, 8.40 (H 5)] and the 5,12-bisdeoxy glycoside (34) (8%) (δ 2.40, 8.58, 9.05). These compounds were chromatographically inseparable, but (22) was obtained pure by fractional crystallization. Its electrospray mass spectrum (acetonitrile/water) showed a significant M+H ion at *m/z* 512.

Considerable streamlining of this procedure involved formation of the more labile dimethyl ketal (44), by treating (1) in a mixture of methanol, trimethyl orthoformate and an acid catalyst. After addition of neutral buffer, this was followed by *in situ* reduction and workup with aqueous mineral acid. Under these conditions a separate deprotection step was not required, though the 13-ketone (22) still needed to be separated from the minor products (12) and (34) by fractional crystallization.

While this procedure thus ensured protection of the keto group as desired, attempted isolation of the intermediate ketal (44) as its hydrochloride resembled (43) in always giving mixtures with considerable proportions of deprotected (1), as shown by ^{1}H n.m.r. spectroscopy. Indeed, signals for (44) [δ 3.48, 3.50 (2×OMe) and δ 1.52 (14-Me)] invariably represented the minor component of such mixtures, following isolation. However, the *in situ* protection of (1) as (44) also proved compatible towards 5-deoxygenation by catalytic hydrogenolysis. Hydrogenation of the ketalization mixture over Adams catalyst, oxidative workup and final deprotection with aqueous acid gave 5-deoxydaunorubicin (12) (62%). Despite involving the additional protection and deprotection steps, this overall yield was better than that described earlier for direct hydrogenation of (1); no chromatography of the product was required and there was no significant accompanying formation of the 13-o1 (13).

With these procedures in hand, attention was next directed towards the more difficult problem of synthesizing 12-deoxy analogues of doxorubicin (2). In the absence of side-chain protection, deoxygenation of (2) proceeded analogously to (1), to give the 13,14-diol (45) (95%). However, ketalization of (2) with propane-1,3-diol was significantly slower than for (1). Complete conversion was never achieved and with lengthening reaction time, aglycone by-products became dominant. Moreover, deprotection of the ketal was also slow in 0.2 M hydrochloric acid at 20°, generating more aglycone. Involvement of the dimethyl ketal (46) was considerably better, particularly when 0.1 M methanolic hydrogen bromide instead of camphorsulfonic acid was used as ketalization catalyst, and a mixture of acetone and 0.25 M hydrobromic acid at 20° for deprotection. Although the hydrochloride of (46) was more stable towards isolation than that of analogous (44), it was similarly not able to be obtained sufficiently pure for characterization, because of the presence of (2).

The ^{1}H n.m.r. spectrum of (46) included singlets at δ 3.53 and 3.56 for the new methoxy groups and a further singlet (δ 3.98) for the 14-methylene protons, the latter appropriately shielded with respect to the corresponding group of (2) (δ 4.76). *In situ* reduction of (46) with potassium borohydride as for (1) and deprotection in a separate step proceeded less cleanly than for the latter series. Nevertheless, the major product was the desired new 12-deoxydoxorubicin (23) (52% overall) [δ 4.74, 4.78, ABq, *J* 17 Hz (14-CH_2); 8.05, s (H 12)] accompanied by its 5-deoxy isomer (15) (7%) and the new 5,12-bisdeoxydoxorubicin (35) (5%). The major product (23), separated by fractional crystallization, showed an M+H ion at *m/z* 528 in its electrospray mass spectrum.

We next sought preparative access to the new 5,12-bisdeoxy systems (34) and (35), noted above as by-products accompanying formation of their 12-deoxy counterparts. Initial assessment towards (34) involved extended contact of (1) with an excess of potassium borohydride. Surprisingly this did not lead to a significant increase in the low yield of 5,12-bisdeoxy-13-dihydrodaunorubicin (41). Apparently the major yellow intermediate that led to (40) on quenching with acid is relatively resistant to further reduction during the lifetime of the reagent in the reaction solution. On a spectroscopic scale, the more powerful reductant lithium borohydride in tetrahydrofuran was next added to (1), dissolved in tetrahydrofuran, for solubility reasons as its free base. However, the orange solution instantly became blue, representing ionization of (1) by the strongly basic reagent, and spectroscopic analysis indicated that only the side-chain carbonyl group was then reduced. But, notwithstanding the instability of lithium borohydride towards protic solvents in isolation, addition of a small proportion of methanol (10%) was found to disrupt the lithium complex, allowing reduction of the chromophore to proceed rapidly and giving an almost colourless solution. Quenching by addition of acetone followed by mineral acid then gave a bright yellow solution. This gave a single, yellow, water-soluble product, spectroscopically assigned as the bisdeoxy 13-dihydro glycoside (41), most particularly by its two *meso* proton resonances (δ 8.58, 9.05).

Preparative development of this procedure to give the new 5,12-bisdeoxy anthracycline (34), in which the 13-keto group survived intact, involved initial protection of (1) as the 1,3-dioxan (43) as discussed above, followed by dilution of the ketalizing mixture with methanol, then with a 10-fold excess of tetrahydrofuran. This was followed by reduction with aliquots of lithium borohydride until the solution became nearly colourless. Quenching with acid and associated deprotection then gave 5,12-bisdeoxydaunorubicin (34) as its yellow hydrochloride (69%). The chief contaminant was 5-deoxydaunorubicin (12) (13%), which was removed by fractional crystallization. The same major product (34) was obtained more conveniently by *in situ* reduction of the dimethyl ketal (44) with lithium borohydride. Glycoside (34) showed an M+H ion in its f.a.b. mass spectrum at *m/z* 496 and an M+Na ion at *m/z* 518. The aromatic region of its ^{1}H n.m.r. spectrum showed the two new *meso* protons as sharp singlets at δ 8.58 and 9.05 and other signals consistent with the assigned structure. A singlet acetyl methyl resonance (δ 2.40) confirmed retention of the 13-keto group.

Extension to doxorubicin (2), through its dimethyl ketal (46), gave the new 5,12-bisdeoxydoxorubicin (35) (61%),

accompanied by a small amount of 5-deoxydoxorubicin (15). Quinone (35) had distinguishing ^{1}H n.m.r. signals at δ 4.75, 4.79 [ABq, *J* 17 Hz (14-CH_2)] and at 8.58, 9.06 (H 12,5). It did not show an M+H ion in its f.a.b. mass spectrum but an M+Na ion at *m/z* 534 was observed. However, a strong M+H ion at *m/z* 512 was observed in its electrospray mass spectrum.

Reduction of the clinically important idarubicin (37)[1] was then explored. It was subjected to both of the procedures optimized in preceding paragraphs, for 12-deoxygenation and for 5,12-bisdeoxygenation. Formation of its dimethyl ketal, followed by *in situ* reduction with potassium borohydride and deprotection gave a product spectroscopically assigned as a mixture of the isomeric deoxy glycosides (47) and (48) (87%), together with a small amount of contaminating bisdeoxyidarubicin (36) (8%). The two main products were associated with a pair of ^{1}H n.m.r. singlets at δ 8.11 and 8.14 (2 : 3), assigned to *meso* protons. The lack of significant regioselectivity in deoxygenating (37) is to be expected, given the absence of a 4-oxy substituent and the individual structures (47) and (48) have not been spectroscopically differentiated. All three products co-chromatographed and, because there was no single major isomer, no attempt was made at fractionating the mixture preparatively.

In situ reduction of the dimethyl ketal of idarubicin (37), with lithium borohydride, gave the new 4,5,12-trisdeoxy anthracycline (36) (62%), accompanied by a small amount of recovered (37) (5%), removed by fractional crystallization. The *meso* protons of (36) resonated at δ 8.63 and 8.66, while the remaining aromatic protons formed two multiplets, each corresponding to two hydrogens, at δ 7.64 and 8.01. There was also an acetyl methyl singlet at δ 2.42. The f.a.b. mass spectrum of (36) showed strong M+H and M+Na ions at *m/z* 466 and 488 respectively.

Hydrolysis of the 12-deoxy (22) and 5,12-bisdeoxy (34) glycosides derived from daunorubicin (1) gave the new aglycones (49) and (50). Subsequent to our original communication,[12] system (50) has also been reported in the patent literature,[34] associated with a multistep synthesis. The ^{1}H n.m.r. spectrum of (49) showed a strongly deshielded, sharp hydroxy singlet at δ 14.90 in (D)chloroform. This was absent from the spectrum of (50). Hydrolysis of bisdeoxyidarubicin (36) gave the yellow trisdeoxy aglycone (51), a system that has been obtained elsewhere by multistep total synthesis.[18]

Regiochemistry of Deoxygenation

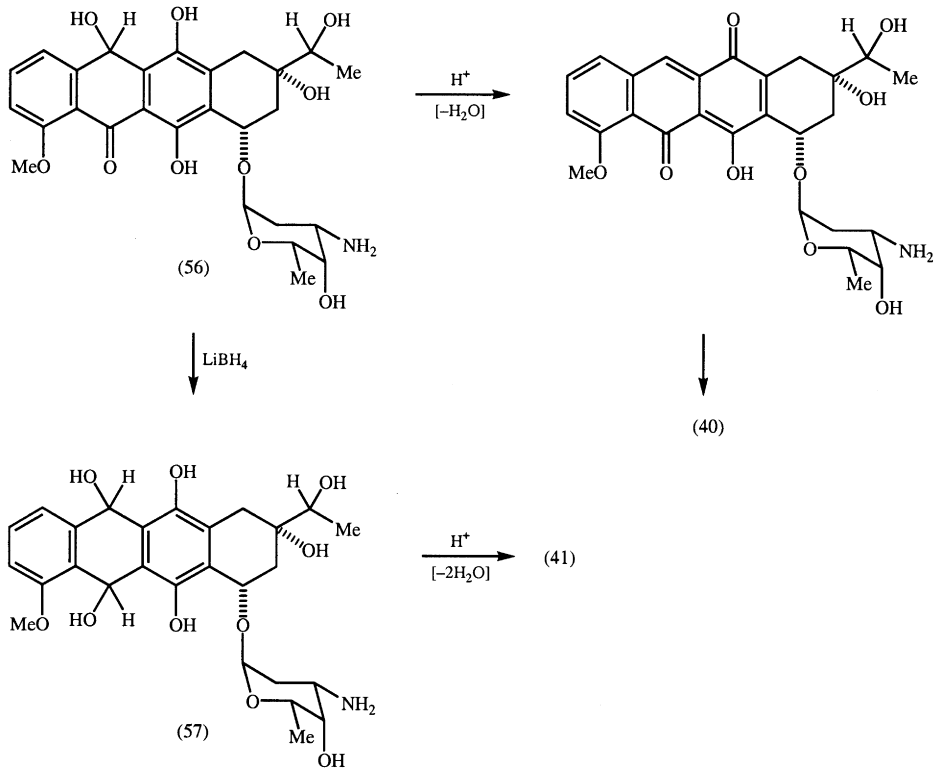
The previous discussion has outlined two new modes of anthracycline reduction. Both of them are mild enough to be effected without loss of the glycosyl residue and both are able alternatively to avoid, or to involve, accompanying reduction of the 13-keto group. Whereas hydrogenation over platinum led chiefly to 5-deoxy products, the action of hydride reagents led to isomeric 12-deoxy glycosides or, with more extensive reduction, to 5,12-bisdeoxy glycosides. The following paragraphs consider these respective processes in more detail.

The course of platinum-catalysed hydrogenation of (1) leading to (12) was followed by analytical thin-layer chromatography on silica gel. After a brief induction period corresponding to formation of platinum metal from the dioxide, the colour of the supernatant phase rapidly changed from orange to yellow. At that point the species present in solution was apparently the hydroquinone (52), since on contact with air it rapidly reverted to (1). Extended hydrogenation led to two new yellow compounds. Unlike (52), they were stable enough to be chromatographed as such, though allowing chromatograms then to stand in air turned them both bright red, corresponding to formation of the 5-deoxy products (12) and (13). The two yellow intermediates were inferred to be the major anthrone (53) and the analogous 13-ol. Chromatography of these compounds was assisted by excluding air as far as possible, and the former was obtained as a yellow-brown hydrochloride. Its ^{1}H n.m.r. spectrum in (D_4)methanol showed minor signals due to (12) (15–20%), representing adventitious oxidation, but these were distinctly resolved from aromatic signals corresponding to the major product. The latter included aromatic doublets at δ 7.30 and 7.89 (*J* 8Hz) and an apparent triplet at δ 7.47 (*J* 8 Hz) consistent with an unaltered D-ring. Importantly, a two-proton singlet at δ 4.04, assigned to a new methylene group, supported the anthrone structure (53).[17] This product could not be purified further because of the ease with which it underwent aerial oxidation, rapidly in solution or more slowly in the solid state, to give (12).

Similar isolation of an analogous intermediate from carminomycin (25) gave a solid, unstable hydrochloride, whose ^{1}H n.m.r. data supported the anthrone structure (54). The different orientation of its nuclear carbonyl group from that of (53) led to less deshielding of the D-ring doublets of its ^{1}H n.m.r. spectrum [δ 6.86 (*J* 8.5 Hz), 7.03 (*J* 7.5 Hz)]. The remaining aromatic signal (H 2) was observed as a doublet of doublets at δ 7.55 (*J* 7.5, 8.5 Hz), while the anthrone methylene signal appeared at δ 4.26.

These observations indicate that in the presence of platinum catalyst, reduction of (1) and (25) proceeds with the same, established selectivity as for analogous, simpler anthraquinones,[15,17] where anthrone intermediates can be isolated more easily.[17] For α-methoxy quinones, deoxygenation with associated change to sp^3 geometry occurs *peri* to the methoxy group. For α-hydroxy quinones it proceeds with the opposite regiochemistry, maximizing intramolecular H-bonding to the anthrone carbonyl group.

Formation of 5-deoxy anthracycline glycosides thereby indicates that, under hydrogenating conditions, the hydroquinone (52) and its tautomers have sufficient lifetime to undergo further reduction to anthrone (53), without significantly competitive 1,4-elimination of daunosamine. The latter process was postulated as occurring rapidly, to convert (52) into the 7-deoxy product (5), through the quinone methide (55);[7] however, subsequent work by Danishefsky's group has separately also concluded that, under mild conditions, (52) was stable towards elimination of the sugar.[5] Preferential 7-deoxygenation of (1) to give (5), such as does occur by catalytic reduction over palladium, is inferred to involve direct hydrogenolysis of the *o*-hydroxybenzyl system.



Scheme 1

These outcomes are strongly contrasted by the 12-deoxygenation of (1), effected by potassium or sodium borohydride. This latter process does not appear to involve an anthrone or any other intermediate stable enough to allow isolation. The yellow mixture, formed by treatment of (1) with an excess of potassium borohydride in methanol and aqueous buffer (pH 7) at 0° under nitrogen, slowly regained the quinizarin chromophore on being stirred in air. Chromatographic analysis then identified 13-dihydrodaunorubicin (39) as the sole product.

While an early report has postulated borohydride reduction of (1) as leading to the corresponding hydroquinone,[35] the yellow mixture above did not show the chromatographic characteristics of either a hydroquinone or an anthrone. No stable intermediate could be isolated from it but, on being applied anaerobically to a t.l.c. layer of silica gel impregnated with oxalic acid, its colour rapidly turned red and it chromatographed as the 12-deoxy anthracycline (40). Under these conditions a solution of the hydroquinone (52) could not be prevented from adventitiously oxidizing completely to (1), while the anthrone (53) was largely stable enough to allow chromatography as such. This chromatographic conversion into (40) parallels the preparative procedure already described. Borohydride reduction of (1) is thus chromophorically reversible up to the point of quenching with acid, the latter step evidently being the one that promotes 12-deoxygenation.

The simplest explanation for these observations is that mild reduction of (1) by potassium borohydride leads directly and selectively to the oxanthrone tautomer (56), or its boron complex,[32,36] as the major product. This reflects reduction by the nucleophilic reagent kinetically favouring attack at the more electron-deficient 12-carbonyl group, the 5-carbonyl being conjugatively affected by electron donation from the 4-methoxy. While intermediate (56) reoxidizes in air, on contact with acid it undergoes 1,4-elimination of water leading to the stable 12-deoxy product (40), without further change of oxidation level (Scheme 1).

Further reduction of (56) is more difficult than of (1) but occurs rapidly with lithium borohydride in methanolic tetrahydrofuran, as described for 5,12-bisdeoxygenation. Formation of the hypothetically derived intermediate (57) has analogy in the reduction of anthraquinone itself to the 9,10-dihydro 9,10-diol, on treatment with sodium borohydride.[37] The nearly colourless reaction solution containing (57) then undergoes twofold elimination on quenching with acid, aromatizing to give the bisdeoxy quinone (41) (Scheme 1).

The borohydride methodology described here represents a useful general basis for deoxygenating quinizarin derivatives. We have applied it earlier to simpler systems[16] but have not previously discussed its regiochemistry.

Conclusion

The new deoxy anthracyclines showed strong anticancer activity, comparable with the parent compounds daunorubicin (1) and doxorubicin (2). For the former series, the 5-deoxy (12), 12-deoxy (22), 5,12-bisdeoxy (34) and 4,5,12-trisdeoxy product (36) gave T/C values of 196% [2.1 mg/kg], 180 [16], 155 [8] and 148 [8] against murine P388 lymphocytic leukaemia *in vivo*; this compared with a value of 173% [1.6 mg/kg] for daunorubicin (1) itself. For the doxorubicin series, corresponding values for the 5-deoxy (13),

12-deoxy (23) and 5,12-bisdeoxy compound (35) were 234% [6.4 mg/kg], 134 [4] and 177 [8], compared with a value of 231% [5 mg/kg] for the parent doxorubicin (2). Deoxy 13-dihydro anthracyclines were also strongly active but at higher concentrations. Thus for the 5-deoxy 13-dihydro compound (16) the corresponding value was 212% [16 mg/kg].

Similarly high levels of activity also were exhibited by selected deoxy compounds against murine L1210 lymphoid leukaemia and B16 melanoma *in vivo*. For 5-deoxydoxorubicin (15) the respective T/C values were 171% [3.2 mg/kg] and 235 [6.4]; and for 5-deoxy-13-dihydrodoxorubicin (16) the corresponding values were 162 [16] and 247 [24]. Disadvantageously, however, the new compounds did not give worthwhile activity against a doxorubicin-resistant strain of P388; while long-term administration of 5-deoxydoxorubicin (23) in mice showed it to be more highly cardiotoxic than doxorubicin (2) itself. Consistent with its unusual physical properties, the zwitterion (29) behaved differently from the other anthracyclines. Obtaining its T/C value proved impractical, because its much lower toxicity towards mice prevented a control level from being established.

The chemistry described in this paper has efficiently allowed controlled 5- and/or 12-deoxygenation of anthracyclines, without significant complication from other reductive processes, particularly cleavage of the glycoside, that impeded earlier work. It has led to new anthracycline families possessing most of the skeletal features and all of the stereochemistry of the parent compounds daunorubicin (1), doxorubicin (2) and idarubicin (37), spatially permitting the new systems to function as DNA-intercalators in the same way as the parents. Reduction of the acetyl-containing substrates (1) and (37), protected as 13-ketals, has given 5-deoxy, 12-deoxy, 5,12-bisdeoxy or 4,5,12-trisdeoxy products (12), (22), (34) and (36) respectively by one-pot chemistry and without the need for chromatography. The same applies to reduction of the hydroxyacetyl-containing substrate (2), leading to the 12-deoxy or 5,12-bisdeoxy products (23) and (35), apart from deprotection of the relatively stable 13-ketals in this latter series being best performed as a separate, second stage.

Experimental

General

Melting points were determined on a Kofler hot stage and are uncorrected. Microanalyses were carried out by Chemical and Microanalytical Services, Geelong, or National Analytical Laboratories, Melbourne. Electronic spectra were recorded in methanol containing 1% formic acid (v/v) unless otherwise stated, by using a Varian Superscan 3 spectrophotometer. Infrared spectra were recorded as potassium bromide disks by using a Perkin–Elmer 983 G spectrophotometer. Proton nuclear magnetic resonance (^{1}H n.m.r.) spectra were recorded at 399.65 MHz with a JEOL JNM-GX400 and a Varian Unity plus 400 system. The solvent was (D)chloroform for water-insoluble compounds. Glycosides were run in (D_4)methanol/(D)chloroform mixtures unless otherwise stated. High- and low-resolution mass spectra of aglycones were recorded by using a V.G. Micromass 7070F instrument or a JEOL JMS-AX505H mass spectrometer at 70 ev unless otherwise stated. F.a.b. mass spectra of glycosides were recorded on the latter instrument with thioglycerol as the matrix. Electrospray mass spectra were recorded in acetonitrile/water mixture acidified with formic acid on a V.G. Quattro quadrupole mass spectrometer in positive-ion mode. The mass of each ion is given, followed by its relative intensity. In general, only peaks greater than 20% are quoted, apart from the molecular ion. For all hydrochlorides the molecular ion (M) is taken as of the free base. Analytical and preparative thin-layer chromatography (t.l.c., p.l.c.) were carried out on glass plates coated with a layer of silica gel [Merck Kieselgel 60 GF_{254}, or Merck Kieselgel 60 GF_{254} containing 2% oxalic acid (oxalated silica)]. The separated components were extracted from the silica with ethyl acetate or chloroform. Oxalic acid was removed by washing the extracts with water and then drying over sodium sulfate. Organic extracts generally were dried in the same way before evaporation at reduced pressure. Column chromatography was carried out by using Merck Kieselgel No. 9385 or Pharmacia Sephadex LH20. All solvents were of AR grade or were redistilled prior to use. Petrol refers to the hydrocarbon fraction boiling in the range 40–60°. Adams catalyst refers to freshly opened platinum dioxide obtained from the Aldrich Chemical Co. or type D platinum dioxide from Johnson Matthey and Co.

Associated antitumour testing *in vivo* was performed on $B_6D_2F_1$ mice. Approximately 10^6 tumour cells were injected intraperitoneally in control and test mice. Test mice were dosed at 1, 5 and 9 days over a range of concentrations less than the toxic dose. The antitumour activity is expressed as T/C% calculated from the following: T/C% = median test animal survival time/median control animal survival time×100. The highest T/C value is quoted in each case.

Hydrogenation of Daunorubicin (1) over Palladium

The hydrochloride of daunorubicin (1) (20 mg) in methanol (20 ml) was reduced with hydrogen and palladium on barium sulfate (5%) for 5.5 h at room temperature and pressure. The mixture was filtered through Celite and allowed to stand overnight in air. The red mixture was concentrated under reduced pressure and preparatively chromatographed on oxalated silica layers with toluene/ethyl acetate (9 : 1) as eluent. The red band with R_F 0.35 gave 5,7-bisdeoxydaunorubicinone (3) as a red solid (11 mg, 88%). It formed red needles from acetone, m.p. 231–233° (lit.[10] 229–231°) (Found: C, 69.1; H, 5.3. Calc. for $C_{21}H_{18}O_6$: C, 68.8; H, 5.0%). λ_{max} (log ε) 275 (4.16), 280 (4.15), 307 (3.56), 478 nm (3.96). ν_{max} 3504, 1700, 1654, 1632, 1602 cm^{-1}. δ 13.77, s, OH; 8.50, br s, H 5; 8.00, br d, *J* 8 Hz, H 1; 7.59, t, *J* 8 Hz, H 2; 7.06, br d, *J* 8 Hz, H 3; 4.04, s, OMe; 3.08–2.77, m, 7-CH_2, 10-CH_2; 2.38, s, COMe; 1.98–1.86, m, 8-CH_2. *m/z* 366 (M, 15%), 323 (48), 305 (30), 295 (21).

The band with R_F 0.1 gave 7,12-bisdeoxydaunorubicinone (4) as a red solid (1 mg, 8%). δ 15.12, s, OH; 8.02, s, H 12; 7.62, t, *J* 8 Hz, H 2; 7.51, br d, *J* 8 Hz, H 1; 7.07, br d, *J* 8 Hz, H 3; 4.08, s, OMe; 3.10–2.74, m, 7-CH_2, 10-CH_2; 2.23, s, COMe; 1.96–1.90, m, 8-CH_2. *m/z* 366 (M, 100%), 323 (45), 279 (37).

5-Deoxydaunorubicinone (10) and 5-Deoxy-13-dihydrodaunorubicinone (11) via the Phenylboronate (9)

The hydrochloride of daunorubicin (1) (25 mg) was heated in 0.2 M hydrochloric acid (5 ml) at 90° for 1 h. On cooling, the orange precipitate was filtered off and dried to give daunorubicinone (6) (16.5 mg, 93%), red needles from toluene, m.p. 221–225° (lit.[10] 213–214°). This product (16.5 mg), phenylboronic acid (6 mg) and *p*-toluenesulfonic acid (1 mg) were stirred together in dichloromethane (5 ml) for 43 h at room temperature. The mixture was washed with saturated aqueous sodium hydrogen carbonate and water, then dried. Evaporation under reduced pressure gave *daunorubicinone phenylboronate* (9) as an orange solid (20 mg, 100%), clusters of red needles from ethyl acetate/petrol, m.p. 257–259° (Found: C, 66.5; H, 4.8. $C_{27}H_{21}BO_8$ requires C, 67.0; H, 4.4%). λ_{max} (log ε) 250 (4.39), 288 (3.87), 476 (4.04), 492 (4.05), 530 nm (3.78). ν_{max} 1720, 1618, 1578 cm^{-1}. δ 13.87, 13.20, s, s, 2×OH; 8.02, dd, *J* 1, 7.5 Hz, H 1; 7.82, dd, *J* 1, 7.5 Hz, H 2′, H 6′; 7.76, dd, *J* 7.5, 8.5 Hz, H 2; 7.44–7.30, m, H 3, H 3′, H 4′, H 5′; 5.83, br t, *J* 2.5 Hz, H 7; 4.08, s, OMe; 3.34, dd, *J* 2, 19 Hz, H 10*eq*; 3.24, d, *J* 19 Hz, H 10*ax*; 2.57, s, COMe; 2.35, ddd, *J* 1.5, 2, 14 Hz, H 8*eq*; 2.27, dd, *J* 2.5, 14 Hz, H 8*ax*. *m/z* 485 (M+H, 30%), 484 (M, 100), 483 (22), 407 (68).

Crude phenylboronate (9) (19.5 mg) was hydrogenated over Adams catalyst (10 mg) in ethyl acetate (5 ml) for 40 min. The mixture was then filtered and the filtrate was evaporated to a red solid, which was dissolved in dichloromethane (2 ml) and stirred with pentane-2,4-diol (0.2 ml) and acetic acid (0.1 ml) at room temperature for 64 h. The red mixture was then washed with water, dried and evaporated under reduced pressure. The residue was preparatively chromatographed on oxalated silica layers in toluene/ethyl acetate (7 : 3). The red band with R_F 0.4 gave *5-deoxydaunorubicinone* (10) (7 mg, 45%), red needles from acetone, m.p. 289–291° (Found: C, 65.8; H, 4.9. $C_{21}H_{18}O_7$ requires C, 66.0; H, 4.7%). λ_{max} (log ε) 268 (4.06), 296 (3.72), 306 (3.52), 483 nm (3.91). ν_{max} 3454, 1712, 1654, 1633, 1603 cm^{-1}. δ 13.74, s, OH; 8.54, s, H 5; 8.04, d, *J* 8 Hz, H 1; 7.63, t, *J* 8 Hz, H 2; 7.09, d, *J* 8 Hz, H 3; 5.19, d, *J* 4.5 Hz, H 7; 4.50, br s, OH; 4.05, s, OMe; 3.65, br s, OH; 3.08, dd, *J* 2, 19.5 Hz, H 10*eq*; 2.85, dd, *J* 1, 19.5 Hz, H 10*ax*; 2.43, s, COMe; 2.30, td, *J* 1, 14.5 Hz, H 8*eq*; 2.08, dd, *J* 4.5, 14.5 Hz, H 8*ax*. *m/z* 382 (M, 58%).

The red band with R_F 0.1 gave mixed diastereoisomers of *5-deoxy-13-dihydrodaunorubicinone* (11) (7 mg, 45%), red needles from acetone, m.p. 221–224° (Found: $M^{+\bullet}$, 384.1209. $C_{21}H_{20}O_7$ requires $M^{+\bullet}$, 384.1209). δ 13.91, s, OH (minor); 13.90, s, OH (major); 8.63, br s, H 5; 8.07, d, *J* 8 Hz, H 1; 7.65, t, *J* 8 Hz, H 2; 7.12, d, *J* 8 Hz, H 3; 5.22, m, H 7; 3.88, q, *J* 6.5 Hz, H 13 (major); 3.75, q, *J* 6.5 Hz, H 13 (minor); 3.10, dd, *J* 2, 20 Hz, H 10*eq* (minor); 3.09, dd, *J* 2, 20 Hz, H 10*eq* (major); 2.56, dd, *J* 1, 20 Hz, H 10*ax* (major); 2.52, td, *J* 1, 15 Hz, H 8*eq* (minor); 2.46, dd, *J* 1, 20 Hz, H 10*ax* (minor); 2.37, td, *J* 2, 15 Hz, H 8*eq* (major); 1.83, dd, *J* 5, 15 Hz, H 8*ax* (major); 1.76, dd, *J* 5, 15 Hz, H 8*ax* (minor); 1.35, d, *J* 6.5 Hz, 14-Me (minor); 1.30, d, *J* 6.5 Hz, 14-Me (major). *m/z* 384 (M, 16%), 369 (20), 368 (68), 366 (34), 358 (20), 356 (34), 348 (100).

Hydrogenation of Daunorubicinone (6)

Daunorubicinone (6) (5 mg) in methanol (2 ml) containing chloroacetic acid (20 mg) was hydrogenated over Adams catalyst (2.5 mg) for 55 min. The yellow mixture was filtered, then the filtrate was diluted with methanol (5 ml), and saturated aqueous sodium hydrogen carbonate solution was added dropwise to give a purple suspension. This was stirred in air for 15 min. Water was then added and the mixture was extracted with dichloromethane. The extract was washed with water, dried and concentrated under reduced pressure. The residue was fractionated by p.l.c. on oxalated silica in chloroform/methanol (19 : 1) to give major and minor red bands. The major band, R_F 0.23, gave mixed diastereoisomers of *5,7-bisdeoxy-13-dihydrodaunorubicinone* (7) as a red solid (1.4 mg). It formed red needles from acetone, m.p. 205–210° (Found: $M^{+\bullet}$, 368.1260. $C_{21}H_{20}O_6$ requires $M^{+\bullet}$, 368.1260). λ_{max} (log ε) 275 (4.13), 308sh (3.44), 470 nm (3.83). ν_{max} 3426, 1654, 1629, 1604 cm^{-1}. δ 13.88, s, OH; 8.54, d, *J* 1 Hz, H 5; 7.99, br d, *J* 8 Hz, H 1; 7.58, t, *J* 8 Hz, H 2; 7.05, br d, *J* 8 Hz, H 3; 4.03, s, OMe; 3.85, q, *J* 6.5 Hz, H 13 (isomer A); 3.77, q, *J* 6.5 Hz, H 13 (isomer B); 3.00–2.50, m, 7-CH_2, 10-CH_2; 2.10–1.90, m, 8-CH_2; 1.34, d, *J* 6.5 Hz, COMe (isomer A); 1.31, d, *J* 6.5 Hz, COMe (isomer B). *m/z* 368 (M, 100%), 342 (20), 324 (42), 323 (92), 322 (22).

The minor band, R_F 0.54, gave 5,7-bisdeoxydaunorubicinone (3) (0.4 mg), which co-chromatographed with the product described earlier. A trace of the 5,12-bisdeoxy isomer (4) was also observed by analytical t.l.c. but not isolated. Carrying out the hydrogenation in ethyl acetate chromatographically showed formation of the same three products, compound (3) being the major one.

5-Deoxydaunorubicin (12) and 5-Deoxy-13-dihydrodaunorubicin (13)

Method A

A rapidly stirred solution of the hydrochloride of daunorubicin (1) (50 mg) in methanol (20 ml) containing chloroacetic acid (200 mg) was hydrogenated over Adams catalyst (10 mg) for 50 min at room temperature. The yellow mixture was filtered, and the filtrate was diluted with water (50 ml) and washed with dichloromethane to remove non-glycosidic materials. The aqueous fraction was then brought to pH 8.5 by addition of saturated aqueous sodium hydrogen carbonate while being stirred in an ice bath. Extraction with dichloromethane (3×50 ml) and washing of the organic phase with brine, then drying and evaporation under reduced pressure gave a dark red solid. Separation of the products on a column of silica gel (8×3.5 cm), with chloroform/methanol/water as eluent [first with 100 : 20 : 1, then with 80 : 20 : 1], gave two red bands.

The faster moving, major band on evaporation gave *5-deoxydaunorubicin* (12) as a dark red solid. Addition of methanolic hydrogen chloride (1 equiv.) to a chloroform solution, followed by ether gave (12) as its hydrochloride (26 mg, 57%), m.p. 189–191° (Found: C, 53.6; H, 6.1; N, 2.6. $C_{27}H_{30}ClNO_9 \cdot 3H_2O$ requires C, 53.9; H, 6.0; N, 2.3%). λ_{max} (log ε) 273sh (4.16), 295sh (3.58), 325 (3.58), 480 nm (3.93). ν_{max} 3422, 1710, 1654, 1636, 1608 cm^{-1}. δ [(D_4)methanol] 8.40, s, H 5; 7.94, d, *J* 8 Hz, H 1; 7.62, t, *J* 8 Hz, H 2; 7.21, d, *J* 8 Hz, H 3; 5.45, d, *J* 3.5 Hz, H 1′; 4.96, m, H 7; 4.21, q, *J* 7 Hz, H 5′; 4.02, s, OMe; 3.59, br d, *J* 3 Hz, H 4′; 3.53, ddd, *J* 3, 5, 13 Hz, H 3′; 2.92, 2.88, ABq, *J* 18 Hz, 10-CH_2; 2.30, s, COMe; 2.19, dd, *J* 3, 15 Hz, H 8*eq*; 2.08, dd, *J* 5.5, 15 Hz, H 8*ax*; 1.99, dt, *J* 3.5, 13 Hz, H 2′*ax*; 1.76, dd, *J* 5, 13 Hz, H 2′*eq*; 1.23, d, *J* 7 Hz, Me′. *m/z* (f.a.b.) 534 (M+Na, 3%), 512 (M+H, 3), 383 (20), 347 (50), 321 (40), 305 (75).

The slower moving band on evaporation gave mixed diastereoisomers of 5-deoxy-13-dihydrodaunorubicin (13) as a dark red solid. As for (12), the hydrochloride (11.5 mg) (25%) was obtained as a solvate, m.p. 200–205° (dec.), but because of its higher polarity it retained inorganic contaminant that persisted through crystallization and invariably led to residue on combustion analysis. It was characterized as its aglycone (11), described earlier. λ_{max} (log ε) 270sh (4.14), 295sh (3.86), 320 (3.82), 480 nm (3.80). ν_{max} 3424, 1654, 1636, 1608 cm^{-1}. δ [(D_4)methanol] 8.39, s, H 5; 7.96, d, *J* 8 Hz, H 1; 7.65, t, *J* 8 Hz, H 2; 7.23, d, *J* 8 Hz, H 3; 5.51, br s, H 1′; 4.99, br s, H 7; 4.25, q, *J* 5.5 Hz, H 5′ (minor); 4.23, q, *J* 5.5 Hz, H 5′ (major); 4.05, s, OMe; 3.67, m, H 13; 3.64, br d, *J* 3 Hz, H 4′; 3.54, ddd, *J* 3, 5, 13 Hz, H 3′; 2.97, d, *J* 19 Hz, H 10*eq* (major); 2.88, d, *J* 19 Hz, H 10*eq* (minor); 2.62, d, *J* 19 Hz, H 10*ax*; 2.37, br d, *J* 13.5 Hz, H 8*eq* (minor); 2.07, m, H 8*ax* (major), H 8*eq* (major); 2.02, dt, *J* 3.5, 13 Hz, H 2′*ax*; 1.85, dd, *J* 5.5, 13 Hz, H 8*ax* (minor); 1.78, dd, *J* 5, 13 Hz, H 2′*eq*; 1.29, m, 14-Me, Me′. *m/z* (f.a.b) 536 (M+Na, 8%), 514 (M+H, 6), 367 (30), 305 (22).

Chromatographic analysis of the non-glycosidic fraction (5.3 mg, 16%), obtained above by dichloromethane extraction of the hydrogenation mixture, showed it to consist chiefly of (3), (7) and a new component. This new product was isolated by p.l.c. on oxalated silica in toluene/ethyl acetate (4 : 1). The red band, R_F 0.34, gave *2-acetyl-2,6,11-trihydroxy-1,2,3,4,7,8,9,10-octahydronaphthacene-5,12-dione* (14) (0.9 mg), which formed red needles from acetone, m.p. 290–292° (Found: $M^{+\bullet}$, 356.1262. $C_{20}H_{20}O_6$ requires $M^{+\bullet}$, 356.1260). λ_{max} (log ε) 290 (3.84), 472 (3.78), 504 (3.87), 543 nm (3.26). ν_{max} 3428, 1708, 1652 cm^{-1}. δ 13.19, 13.15, s, s, 2×OH (D_2O-exchanged); 3.82, br s, 2-OH (D_2O-exchanged); 3.05, ddd, *J* 3, 5, 20 Hz, H 4*eq*; 2.96, ddd, *J* 2, 3, 20 Hz, H 1*eq*; 2.88–2.81, m, H 4*ax*; 2.83, br d, *J* 20 Hz, H 1*ax*; 2.66, br s, 7-CH_2, 10-CH_2; 2.37, s, COMe; 2.01–1.88, m, 3-CH_2; 1.78, br s, 8-CH_2, 9-CH_2. *m/z* 356 (M, 19%), 313 (100).

Method B

A solution of the hydrochloride of daunorubicin (1) (10 mg) in propane-1,3-diol (200 mg) containing trimethyl orthoformate (0.1 ml) and camphorsulfonic acid (1 mg) was allowed to stand for 16 h at room temperature. It was then diluted with water, basified with solid sodium hydrogen carbonate and extracted with chloroform, and the extract was washed with water, dried and concentrated to give impure ketal (43) as an oil. This was dissolved in methanol (4 ml) containing chloroacetic acid (40 mg) and hydrogenated as in *A*. After filtration, the resulting solution was then stirred in air in an ice bath, with addition of an excess of aqueous sodium hydrogen carbonate. The purple mixture was extracted into chloroform and the extract was then re-extracted into 0.1 M hydrochloric acid. This red acidic solution was left standing at room temperature for 48 h, then washed with dichloromethane, basified with solid sodium hydrogen carbonate and finally extracted into chloroform. The red extract was washed with a little water, dried and concentrated. Filtration, addition of methanolic hydrogen chloride (1

equiv.) to the filtrate and precipitation with an excess of ether gave the hydrochloride of (12) as a red solid (5.6 mg) (62%), identical with the product from *A*.

5-Deoxy-13-dihydrodaunorubicin (13)

A solution of daunorubicin hydrochloride (1) (10.4 mg) in methanol (6 ml) was hydrogenated over Adams catalyst (8.7 mg) for 70 min at room temperature. The mixture was filtered, and the filtrate was diluted with water (10 ml) and washed with dichloromethane. The aqueous phase was brought to pH 8.5 by addition of saturated aqueous sodium hydrogen carbonate, and then extracted with dichloromethane. The extract was washed with brine, dried and evaporated, to give 5-deoxy-13-dihydrodaunorubicin (13) as a dark red solid (6.7 mg, 71%), identical with the product from the previous section.

5-Deoxy-13-dihydrodoxorubicin (16)

Doxorubicin hydrochloride (100 mg) was hydrogenated in methanol (40 ml) over Adams catalyst (100 mg) for 90 min at room temperature. The yellow solution was then filtered through a Celite pad and the filtrate was cooled in an ice-bath and then stirred with saturated aqueous sodium hydrogen carbonate (15 drops) for 10 min to give a dark red mixture. Brine (20 ml) was added and the mixture was extracted four times with chloroform. The combined organic layers were washed with a little brine and then dried over anhydrous sodium sulfate. Evaporation of the filtered extract under reduced pressure below 30° gave a dark red solid which was dissolved in chloroform/methanol, filtered, and concentrated to *c.* 1 ml in a centrifuge tube. The tube was chilled at –10° and then methanolic hydrogen chloride (1 equiv.) was added followed by an excess of diethyl ether. Centrifugation gave a dark red solid which was redissolved in methanol and precipitated again with diethyl ether to give a pair of diastereoisomers of *5-deoxy-13-dihydrodoxorubicin* (16) as the hydrochloride (74 mg, 76%), m.p. 195–200° (dec.) (Found: C, 57.0; H, 5.7. $C_{27}H_{32}ClNO_{10}$ requires C, 57.3; H, 5.7%). λ_{max} (log ε) 270sh (4.07), 308sh (3.54), 480 nm (3.84). ν_{max} 3416, 1654, 1636, 1604 cm^{-1}. δ 8.52, s, H 5 (minor); 8.51, s, H 5 (major); 8.02, d, *J* 8 Hz, H 1 (minor); 8.01, d, *J* 8 Hz, H 1 (major); 7.64, t, *J* 8 Hz, H 2; 7.03, d, *J* 8 Hz, H 3; 5.57, br d, *J* 3 Hz, H 1′; 5.09, br s, H 7; 4.21, q, *J* 6.5 Hz, H 5′ (minor); 4.19, q, *J* 6.5 Hz, H 5′ (major); 4.05, s, OMe; 3.94, dd, *J* 3.5, 12 Hz, H_A 14; 3.78, m, H_B 14; 3.74, br d, *J* 3 Hz, H 4′; 3.60, m, H 13; 3.50, ddd, *J* 3, 5, 13 Hz, H 3′; 3.06, d, *J* 20 Hz, H 10*eq* (major); 3.04, d, *J* 20 Hz, H 10*eq* (minor); 2.72, d, *J* 20 Hz, H 10*ax* (major); 2.70, d, *J* 20 Hz, H 10*ax* (minor); 2.48, br d, *J* 15 Hz, H 8*eq* (minor); 2.21, br d, *J* 15 Hz, H 8*eq* (major); 2.07, dd, *J* 5, 15 Hz, H 8*ax* (major); 2.03, dt, *J* 3.5, 13 Hz, H 2′*ax*; 1.87, br dd, *J* 5, 15 Hz, H 8*ax* (minor); 1.82, br dd, *J* 5, 13 Hz, H 2′*eq*; 1.29, br d, *J* 6.5 Hz, Me′. *m/z* (f.a.b.) 552 (M+Na, 2%), 530 (M+H, 1).

5-Deoxydoxorubicin (15) and 5-Deoxy-13-dihydrodoxorubicin (16)

A solution of the hydrochloride of doxorubicin (2) (50 mg) in methanol (20 ml) and chloroacetic acid (200 mg) was hydrogenated by rapidly stirring over Adams catalyst (5 mg) for 50 min at room temperature. The yellow solution was filtered and the filtrate was then brought to pH 8.5 by addition of saturated aqueous sodium hydrogen carbonate while being stirred in an ice bath. Extraction with chloroform (4×25 ml) and washing of the organic phase with brine, then drying and evaporation under reduced pressure gave a dark red solid. Separation of the products on a column of silica gel, with chloroform/methanol/water (initially 80 : 20 : 0.5 and then 75 : 25 : 0.5) as eluent, gave two red bands.

The faster moving, major band on evaporation gave a dark red solid which was redissolved in chloroform and treated with methanolic hydrogen chloride (1 equiv.) followed by ether to give *5-deoxydoxorubicin* (15) as its hydrochloride (29 mg) (59%), m.p. >200° (dec.) (Found: C, 54.1; H, 6.4; N, 1.9. $C_{27}H_{30}ClNO_{10}$·3.5MeOH requires C, 54.2; H, 6.5; N, 2.1%). λ_{max} (log ε) 272 (4.22), 310 (3.69), 480 nm (3.99). ν_{max} 3416, 1724, 1654, 1636, 1604 cm^{-1}. δ 8.53, s, H 5; 8.02, d, *J* 8.5 Hz, H 1; 7.65, t, *J* 8.5 Hz, H 2; 7.15, d, *J* 8.5 Hz, H 3; 5.56, d, *J* 3.5 Hz, H 1′; 5.10, m, H 7; 4.78, 4.74, ABq, *J* 20 Hz, 14-CH_2; 4.18, q, *J* 7 Hz, H 5′; 4.06, s, OMe; 3.75, br d, *J* 3 Hz, H 4′; 3.53, ddd, *J* 3, 5, 13 Hz, H 3′; 3.11, dd, *J* 1, 20 Hz, H 10*eq*; 2.93, dd, *J* 1, 20 Hz, H 10*ax*; 2.28, dt, *J* 1, 15 Hz, H 8*eq*; 2.13, dd, *J* 5, 15 Hz, H 8*ax*; 2.03, dt, *J* 3.5, 13 Hz, H 2′*ax*; 1.83, dd, *J* 5, 13 Hz, H 2′*eq*; 1.28, d, *J* 7 Hz, Me′. *m/z* (f.a.b.) 550 (M+Na, 5%), 528 (M+H, 2).

The slower moving band on evaporation and treatment with hydrogen chloride, as for (15), gave mixed diastereoisomers of 5-deoxy-13-dihydrodoxorubicin (16) as its hydrochloride (9.6 mg) (20%), identical with the product from the previous section.

Non-Glycosidic Derivatives of 5-Deoxydoxorubicin

Doxorubicin hydrochloride (50 mg) was hydrogenated as in the previous section but the crude chloroform extract was partitioned rather than chromatographed. Extraction with 0.2 M hydrochloric acid gave aqueous glycosidic and organic non-glycosidic fractions.

The latter fraction gave a red solid (13 mg). Analytical t.l.c. showed it to consist of a major component and several minor ones. The major component was isolated by p.l.c. on oxalated silica in toluene/acetone (7 : 3). The band with R_F 0.53 was consistent with 5,7-bisdeoxydoxorubicinone (17) as an incompletely characterized red solid (5 mg, 15%), red microneedles from acetone (Found: $M^{+\bullet}$, 382.1052. Calc. for $C_{21}H_{18}O_7$: $M^{+\bullet}$, 382.1052). ν_{max} 3427, 1717, 1649, 1629 cm^{-1}. δ 13.65, s, OH; 8.46, s, H 5; 7.95, d, *J* 8 Hz, H 1; 7.59, t, *J* 8 Hz, H 2; 7.06, d, *J* 8 Hz, H 3; 4.74, 4.70, ABq, *J* 21 Hz, 14-CH_2; 4.04, s, OMe; 3.00–1.50, m, 7-CH_2, 8-CH_2, 10-CH_2. *m/z* 382 (M, 54%), 323 (100).

The acidic glycoside fraction was heated on a steam bath for 1 h and then cooled. The precipitated solid was filtered off, and then subjected to preparative chromatography on oxalated silica in toluene/acetone (7 : 3). The red band, R_F 0.53, gave *5-deoxydoxorubicinone* (18) (10 mg, 28%), red needles from acetone, m.p. 188–190° (Found: $M^{+\bullet}$, 398.1002. $C_{21}H_{18}O_8$ requires $M^{+\bullet}$, 398.1002). λ_{max} (log ε) 270sh (4.13), 306sh (3.60), 482 nm (3.97). ν_{max} 3420, 1724, 1654, 1636, 1604 cm^{-1}. δ 13.80, s, OH; 8.59, s, H 5; 8.04, d, *J* 8 Hz, H 1; 7.64, t, *J* 8 Hz, H 2; 7.10, d, *J* 8 Hz, H 3; 5.20, br d, *J* 5 Hz, H 7; 4.81, 4.76, ABq, *J* 21 Hz, 14-CH_2; 4.05, s, OMe; 3.16, dd, *J* 2.5, 20 Hz, H 10*eq*; 2.90, dd, *J* 1, 20 Hz, H 10*ax*; 2.35, td, *J* 1.5, 15 Hz, H 8*eq*; 2.09, dd, *J* 5, 15 Hz, H 8*ax*. *m/z* 398 (M, 9%), 362 (65), 381 (100), 303 (71).

The red band with R_F 0.18 gave mixed diastereoisomers of *5-deoxy-13-dihydrodoxorubicinone* (19) (5 mg, 15%), red needles from methanol/acetone, m.p. 214–217° (Found: $M^{+\bullet}$, 400.1158. $C_{21}H_{20}O_8$ requires $M^{+\bullet}$, 400.1158). λ_{max} (log ε) 268sh (3.99), 307sh (3.48), 482 nm (3.86). ν_{max} 3417, 1653, 1635, 1602 cm^{-1}. δ 8.59, s, H 5; 8.03, br d, *J* 8 Hz, H 1; 7.64, t, *J* 8 Hz, H 2; 7.13, br d, *J* 8 Hz, H 3; 5.14, br d, *J* 5 Hz, H 7; 4.06, s, OMe; 3.94, dd, *J* 4, 11.5 Hz, H_A 14 (minor); 3.92, dd, *J* 4, 11.5 Hz, H_A 14 (major); 3.81, dd, *J* 7, 11.5 Hz, H_B 14 (minor); 3.78, dd, *J* 7, 11.5 Hz, H_B 14 (major); 3.63, dd, *J* 4, 7 Hz, H 13 (major); 3.57, dd, *J* 4, 7 Hz, H 13 (minor); 3.09, dd, *J* 2, 20 Hz, H 10*eq* (major); 3.05, dd, *J* 2, 20 Hz, H 10*eq* (minor); 2.73, dd, *J* 1, 20 Hz, H 10*ax* (minor); 2.72, dd, *J* 1, 20 Hz, H 10*ax* (major); 2.42, br td, *J* 2, 15 Hz, H 8*eq* (minor); 2.22, br td, *J* 2, 15 Hz, H 8*eq* (major); 1.98, dd, *J* 5, 15 Hz, H 8*ax* (major); 1.82, dd, *J* 5, 15 Hz, H 8*ax* (minor). *m/z* 400 (M, 5%), 382 (24), 364 (74), 333 (100).

Ketals (20) and (21)

The hydrochloride of daunorubicin (1) (27 mg) was hydrogenated over Adams catalyst (25 mg) in methanol as described earlier for preparation of glycoside (13). Workup led to non-glycosidic and glycosidic fractions. The former (4.5 mg, 25%) contained largely 5,7-bisdeoxy-13-dihydrodaunorubicinone (7) by t.l.c. The glycosidic fraction was hydrolysed in 0.2 M hydrochloric acid for 1 h at 90°. Filtration then gave largely 5-deoxy-13-dihydrodaunorubicinone (11) as a red solid (10.6 mg, 57%). This mixture of diastereoisomers was boiled with 2,2-dimethoxypropane (0.4 ml) and camphorsulfonic acid (0.5 mg) in dioxan (2 ml) for 15 min. The mixture was then cooled, diluted with water and extracted with dichloromethane. The extract was washed with water, dried and evaporated to a red solid. This was subjected to p.l.c. on silica gel in toluene/ethyl acetate (70 : 30). The major band, R_F 0.58, gave the (13*R*)-ketal (20) as a red solid (5.5 mg). δ 13.80, s, OH; 8.59, s, H 5; 8.00, d, *J* 8 Hz, H 1; 7.59, t, *J* 8 Hz, H 2; 7.06, d, *J* 8 Hz, H 3; 5.15, d, *J* 4 Hz, H 7; 4.19, q, *J* 6.5 Hz, H 13; 4.03, s, OMe; 2.99, dd,

J 1.5, 19.5 Hz, H 10*eq*; 2.53, d, *J* 19.5 Hz, H 10*ax*; 2.36, br d, *J* 14.5 Hz, H 8*eq*; 1.61, dd, *J* 5, 14.5 Hz, H 8*ax*; 1.54, 1.39, s, s, CMe_2; 1.31, d, *J* 6.5 Hz, 14-Me.

The minor band, R_F 0.50, gave the (13*S*)-ketal (21) as a red solid (2.6 mg). δ 13.84, s, OH; 8.60, s, H 5; 8.02, d, *J* 8 Hz, H 1; 7.60, t, *J* 8 Hz, H 2; 7.07, d, *J* 8 Hz, H 3; 5.11, br s, H 7; 4.13, q, *J* 6.5 Hz, H 13; 4.03, s, OMe; 3.02, dd, *J* 1, 19 Hz, H 10*eq*; 2.49, d, *J* 19 Hz, H 10*ax*; 2.18, ddd, *J* 1, 2, 14 Hz, H 8*eq*; 1.96, dd, *J* 5, 14 Hz, H 8*ax*; 1.45, 1.42, s, s, CMe_2; 1.33, d, *J* 6.5 Hz, 14-Me.

Hydrogenation of Carminomycin (25)

Method A

The hydrochloride of carminomycin (25) (50 mg) in methanol (20 ml) and dichloroacetic acid (200 mg) was hydrogenated while rapidly being stirred over Adams catalyst (8 mg) for 35 min at room temperature. The yellow filtrate was stirred in air at 0° with addition of saturated aqueous sodium hydrogen carbonate to give a purple suspension. This was extracted with ethyl acetate and the extract was washed with brine, dried and concentrated. The purple residue was column chromatographed on flash silica gel in chloroform/methanol/water (70 : 25 : 3). The major blue-purple band was recovered by evaporation, redissolving in chloroform/methanol and precipitation with ether several times to give 12-deoxycarminomycin (29) as a purple solid (26.8 mg, 59%). λ_{max} (log ε) 495 (3.65), 525 nm (3.57). λ_{max} (MeOH, qual., as the purple zwitterion) 526, 550sh nm. ν_{max} 3410, 1718, 1653, 1636 cm^{-1}. δ 8.08, s, H 12; 7.66, t, *J* 8 Hz, H 2; 7.50, d, *J* 8 Hz, H 1; 7.13, m, H 3; 5.55, br s, *J* 1 Hz, H 1′; 5.11, br s, H 7; 4.23, q, *J* 6.5 Hz, H 5′; 3.79, br d, *J* 3 Hz, H 4′; 3.58, br d, *J* 13 Hz, H 3′; 3.06, d, *J* 19.5 Hz, H 10*eq*; 2.87, d, *J* 19.5 Hz, H 10*ax*; 2.42, s, COMe; 2.27, d, *J* 15 Hz, H 8*eq*; 2.12, dd, *J* 5, 15 Hz, H 8*ax*; 2.05, dt, *J* 3, 13 Hz, H 2′*ax*; 1.88, dd, *J* 4, 13 Hz, H 2′*eq*; 1.31, d, *J* 6.5 Hz, Me′. *m/z* (electrospray) 499 (M+2H, 45%), 498 (M+H, 100), 369 (20).

A very polar minor purple band, difficult to elute and to obtain free from inorganic contaminant, was presumed to be the 13-dihydro species (26), as its zwitterion. On being heated with 0.2 M hydrochloric acid it gave an aglycone chromatographically indistinguishable from a minor product of hydrogenation of the phenylboronate (33). This product was inferred to be the 12-deoxy 13-dihydro compound (31).

Method B

Another hydrogenation of carminomycin hydrochloride (50 mg) but with added chloroacetic acid (200 mg) in place of dichloroacetic acid gave the same two glycosides together with a larger proportion of non-glycosidic material. The latter material was isolated as the early fraction eluted from a column of flash silica gel with chloroform/methanol/water (70 : 25 : 3), as a dark red solid (15 mg, 46%). Two components were separated from this solid by p.l.c. on oxalated silica in toluene/ethyl acetate (70 : 30). The component with R_F 0.40 gave *7,12-bisdeoxycarminomycinone* (27), red needles from acetone, m.p. dec. above 230° (Found: $M^{+\bullet}$, 352.0926. $C_{20}H_{16}O_6$ requires $M^{+\bullet}$, 352.0947). λ_{max} (log ε) 276sh (3.85), 335 (3.32), 500sh (3.77), 525 nm (3.82). ν_{max} 3490, 1690, 1650, 1625 cm^{-1}. δ 16.14, s, OH; 9.92, s, OH; 8.02, s, H 12; 7.61, t, *J* 8 Hz, H 2; 7.44, d, *J* 8 Hz, H 1; 7.09, d, *J* 8 Hz, H 3; 3.03–2.79, m, 7-CH_2, 10-CH_2; 2.37, s, COMe; 2.00–1.80, m, 8-CH_2. *m/z* 352 (M, 55%), 334 (52), 325 (25), 309 (100).

The component with R_F 0.13 gave *7,12-bisdeoxy-13-dihydrocarminomycinone* (28), red needles from acetone, m.p. 200–205° (dec.) (Found: $M^{+\bullet}$, 354.1082. $C_{20}H_{18}O_6$ requires $M^{+\bullet}$, 354.1103). λ_{max} (log ε) 274sh (3.90), 282sh (3.89), 340 (3.31), 494 (3.94), 520 nm (3.94). ν_{max} 3400, 1650, 1620, 1610, 1580 cm^{-1}. δ 16.10 (major), 16.08 (minor), s, s, OH, OH; 9.89, br s, OH; 7.94 (major), 7.93 (minor), s, s, H 12; 7.59, t, *J* 8 Hz, H 2; 7.40, d, *J* 8 Hz, H 1; 7.07, d, *J* 8 Hz, H 3; 3.84, q, *J* 6.5 Hz, H 13 (minor); 3.66, q, *J* 6.5 Hz, H 13 (major); 2.96–2.46, m, 7-CH_2, 10-CH_2; 2.16–2.00, m, 8-CH_2; 1.33, d, *J* 6.5 Hz, Me′ (major); 1.30, d, *J* 6.5 Hz, Me′ (minor). *m/z* 355 (M+H, 26%), 354 (M, 100), 309 (63).

12-Deoxycarminomycinone (30)

The hydrochloride of carminomycin (25) (40 mg) was heated in 0.2 M hydrochloric acid at 90° for 45 min. The precipitated solid was filtered off and dried to give carminomycinone (32) as a red solid (24 mg). It formed red needles from toluene, m.p. 225–228° (subl.) (lit.[23] 233–235°). The same product was obtained alternatively by stirring daunorubicinone (6) (33 mg) in dichloromethane (20 ml) with an excess of powdered anhydrous aluminium chloride for 16 h at room temperature under nitrogen. The purple mixture was then stirred with saturated aqueous oxalic acid solution for 30 min. Extraction with dichloromethane gave carminomycinone (32) as a red solid (32 mg). Without purification this solid (24 mg) in dichloromethane (10 ml) was stirred with phenylboronic acid (9 mg) and *p*-toluenesulfonic acid (1 mg) for 16 h at room temperature. The mixture was diluted with more dichloromethane and washed with saturated aqueous sodium hydrogen carbonate solution then water. Drying and evaporation gave *carminomycinone phenylboronate* (33) (29 mg, 98%), orange needles from ethyl acetate, m.p. 271–274° (Found: C, 66.2; H, 4.4. $C_{26}H_{19}BO_8$ requires C, 66.4; H, 4.1%). λ_{max} (log ε) 242 (4.25), 290 (3.63), 480sh (3.94), 490 (3.99), 506sh (3.85), 526 nm (3.80). ν_{max} 1723, 1600 cm^{-1}. δ 13.35, 12.86, 12.16, s, s, s, 3×OH; 7.86, dd, *J* 1, 7.5 Hz, H 1; 7.81, m, H 2′, H 6′; 7.68, dd, *J* 7.5, 8.5 Hz, H 2; 7.43, m, H 4′; 7.35–7.31, m, H 3, H 3′, H 5′; 5.82, dd, *J* 2.5, 3.0 Hz, H 7; 3.37, dd, *J* 1.5, 19 Hz, H 10*eq*; 3.27, d, *J* 19 Hz, H 10*ax*; 2.55, s, COMe; 2.37, ddd, *J* 1.5, 3, 14 Hz, H 8*eq*; 2.28, dd, *J* 2.5, 14 Hz, H 8*ax*. *m/z* 470 (M, 54%), 393 (25), 348 (84).

The crude phenylboronate (33) was dissolved in ethyl acetate (15 ml) by warming. Adams catalyst (6 mg) and dichloroacetic acid (120 mg) were added and the mixture was hydrogenated for 2 h at room temperature. The filtrate was concentrated under reduced pressure. The residue was dissolved in tetrahydrofuran, and then saturated aqueous sodium hydrogen carbonate solution was added dropwise with stirring until a purple colour was established. The mixture was stirred for 5 min longer, then it was diluted with water and neutralized dropwise with 98% formic acid. It was extracted with ethyl acetate and the extract was washed with water, dried and evaporated to give 12-deoxycarminomycinone (30) as its phenylboronate (28 mg, 98%), which gave dark red clusters of microneedles from ethyl acetate/petrol, m.p. 222–224°. *m/z* 454 (M, 16%), 332 (100). This product was treated with pentane-2,4-diol and acetic acid in chloroform analogously to (9), to give *12-deoxycarminomycinone* (30), dark red needles from ethyl acetate, m.p. 191–193° (Found: $M^{+\bullet}$, 368.0896. $C_{20}H_{16}O_7$ requires $M^{+\bullet}$, 368.0896). λ_{max} (log ε) 262sh (4.24), 296sh (3.67), 310sh (3.49), 335 (3.37), 500 (3.90), 526 nm (3.87). ν_{max} 3420, 1711, 1654, 1629, 1612, 1586 cm^{-1}. δ 15.95, s, OH (D_2O-exchanged); 9.79, s, OH (D_2O-exchanged); 8.03, s, H 12; 7.64, t, *J* 8 Hz, H 2; 7.46, d, *J* 8 Hz, H 1; 7.12, d, *J* 8 Hz, H 3; 5.21, br d, *J* 5 Hz, H 7; 3.05, dd, *J* 1, 19.5 Hz, H 10*eq*; 2.83, br d, *J* 19.5 Hz, H 10*ax*; 2.41, s, COMe; 2.31 td, *J* 1, 14.5 Hz, H 8*eq*; 2.14, dd, *J* 5, 14.5 Hz, H 8*ax*. *m/z* 368 (M, 13%), 332 (86). This product (30) was also obtained by heating the glycoside (29) in 0.2 M hydrochloric acid, as for the foregoing hydrolysis of carminomycin (25).

12-Deoxy-13-dihydrodaunorubicin (40)

To a solution of the hydrochloride of daunorubicin (1) (50 mg) in methanol (40 ml) was added 0.1 M aqueous phosphate buffer (pH 7, 2 ml) and nitrogen was bubbled through the mixture, cooled in an ice bath. Addition of potassium borohydride (14.4 mg) and stirring for 30 min were followed by treatment with acetone (0.5 ml) and then dropwise addition of an excess of 1 M hydrobromic acid until the mixture turned red. It was then stirred at room temperature for 30 min, diluted with water and extracted repeatedly with dichloromethane. The organic extract was re-extracted with dilute hydrochloric acid and the combined aqueous acidic phases were then basified with solid sodium hydrogen carbonate and extracted with chloroform. This red extract was washed with a small volume of aqueous sodium hydrogen carbonate, dried and concentrated. After filtration, the concentrate was chilled in an ice bath. Methanolic hydrogen chloride (1 equiv.) was added, followed by an excess of ether. ^1H n.m.r. spectroscopy of the dark red precipitate (45.5 mg) (93%) showed this crude product to consist of (40) (a 1 : 1 diastereoisomeric mixture), (13) and (41) [76 : 15 : 9]. It was recrystallized from methanol/acetone to give *12-deoxy-13-dihydrodaunorubicin* (40) as its hydrochloride, dark red clumps of needles, m.p. 180–182° (Found: C, 59.0; H, 5.7; N, 2.8. $C_{27}H_{32}ClNO_9$ requires C, 59.0; H, 5.9;

N, 2.5%). λ_{max} (log ε) 283sh (3.98), 297sh (3.74), 325 (3.56), 486 (3.90), 520 nm (3.81). ν_{max} 3411, 1655, 1595 cm^{-1}. δ 8.04, s, H 12; 7.68, t, *J* 8 Hz, H 2; 7.56, d, *J* 8 Hz, H 1; 7.14, d, J 8 Hz, H 3; 5.58, br s, H 1′; 5.15, br s, H 7; 4.21, m, H 5′; 4.08, s, OMe; 3.77, br s, H 4′; 3.68, m, H 13; 3.50, br d, *J* 13 Hz, H 3′; 3.02, d, *J* 20 Hz, H 10*eq* (isomer A); 2.96, d, *J* 20 Hz, H 10*eq* (isomer B); 2.57, d, *J* 20 Hz, H 10*ax* (isomer A) (B); 2.47, d, *J* 20 Hz, H 10*ax* (isomer B) (A); 2.21, br d, *J* 15 Hz, H 8*eq*; 2.04, dt, *J* 3.5, 13 Hz, H2′*ax*; 1.94, dd, *J* 4.5, 15 Hz, H 8*ax* (isomer A) (B); 1.79, m, H 2′*eq*, H 8*ax* (isomer B) (A); 1.32, br d, *J* 6.5 Hz, Me′. *m/z* (electrospray) 514 (M + H, 48%), 386 (24), 385 (100).

12-Deoxydaunorubicin (22)

Method A

A solution of the hydrochloride of daunorubicin (1) (27.7 mg) in methanol (2 ml) containing trimethyl orthoformate (1 ml) and camphorsulfonic acid (2.5 mg) was left to stand at room temperature for 23 h. The resulting solution, containing the ketal (44) (δ 3.50, 3.48, s, s, 2×OMe; 1.52, s, 14-Me), was then diluted with methanol (20 ml) and 0.1 M aqueous phosphate buffer pH 7 (1 ml), and a stream of nitrogen was bubbled through the mixture in an ice bath. Potassium borohydride (5.4 mg) was added and, after stirring for 20 min, the yellow mixture was treated with acetone (1 ml) followed dropwise by 1 M hydrobromic acid, until it gave a red solution. This was allowed to warm to room temperature and to stand for 60 min. Distilled water was then added and the mixture was extracted with dichloromethane to remove aglycone. The aqueous phase was basified with solid sodium hydrogen carbonate and it was then extracted with dichloromethane. The red extract was washed with a little water, dried and concentrated. The concentrate was filtered and then chilled to 0°. Methanolic hydrogen chloride (1 equiv.) in methanol was added followed by precipitation with ether. Filtration then gave a dark red solid (25.4 mg). ^{1}H n.m.r. spectroscopy showed this was a mixture of (22) (71%), (34) (8%) and (12) (4%). The major product *12-deoxydaunorubicin* (22) as its hydrochloride was purified by several recrystallizations from methanol/acetone, to give red-black cubes, m.p. 182–184° (Found: C, 59.0; H, 5.6; N, 2.3. $C_{27}H_{30}ClNO_9$ requires C, 59.2; H, 5.5; N, 2.6%). λ_{max} (log ε) 263sh (4.13), 270sh (4.07), 308sh (3.48), 492 (3.97), 520 nm (3.94). ν_{max} 3416, 1711, 1654, 1636, 1599 cm^{-1}. δ 8.05, s, H 12; 7.68, t, *J* 8 Hz, H 2; 7.65, d, *J* 8 Hz, H 1; 7.14, d, *J* 8 Hz, H 3; 5.57, d, *J* 4 Hz, H 1′; 5.12, m, H 7; 4.21, q, *J* 6.7 Hz, H 5′; 4.06, s, OMe; 3.77, br s, H 4′; 3.55, ddd, *J* 3, 5, 13 Hz, H 3′; 3.01, d, *J* 19.5 Hz, H 10*eq*; 2.83, d, *J* 19.5 Hz, H 10*ax*; 2.40, s, COMe; 2.24, br d, *J* 15 Hz, H 8*eq*; 2.10, dd, *J* 5, 15 Hz, H 8*ax*; 2.03, dt, *J* 4, 13 Hz, H 2′*ax*; 1.82, dd, *J* 5, 13 Hz, H 2′*eq*; 1.30, d, *J* 6.5 Hz, Me′. *m/z* (electrospray) 512 (M+H, 28%), 384 (24), 383 (100).

Method B

A mixture of the hydrochloride of daunorubicin (1) (104 mg), propane-1,3-diol (2 ml), trimethyl orthoformate (0.5 ml) and camphorsulfonic acid (5 mg) was allowed to stand at room temperature for 16 h, and then it was diluted with methanol (50 ml) and 0.1 M aqueous phosphate buffer (pH 7) (5 ml). A stream of nitrogen was bubbled through the mixture in an ice bath and potassium borohydride (60 mg total) was added in three equal portions over 1.3 h. The resulting dark yellow mixture was then treated dropwise with 1 M methanolic hydrogen chloride until it gave a red solution. This was stirred for 10 min at room temperature, diluted with water and washed with dichloromethane. The aqueous phase was basified with solid sodium hydrogen carbonate and extracted with dichloromethane to give a red extract. This was re-extracted with 0.1 M hydrochloric acid. This acidic phase was allowed to stand for 16 h at room temperature, whereupon the glycosidic hydrochloride fraction (85 mg), consisting of (22), together with minor proportions of (34) and (12), was recovered as in *A*. Recrystallization from methanol/acetone gave (22), m.p. 182–184°, identical with the product from *A*.

5,12-Bisdeoxydaunorubicin (34)

Method A

A solution of the hydrochloride of daunorubicin (1) (26 mg) in methanol (1.5 ml) containing trimethyl orthoformate (0.5 ml) and camphorsulfonic acid (2.5 mg) was left to stand at room temperature for 23 h. Lithium carbonate (4 mg) was added with stirring for 10 min. Dry tetrahydrofuran (15 ml) was then added and the mixture was stirred in an ice bath, after nitrogen was bubbled through it. Lithium borohydride solution (70 µl of 2 M in tetrahydrofuran) was then added by syringe. After 10 min another aliquot (70 µl) was added and, after then stirring for 45 min, a pale lemon-coloured mixture was obtained. This was treated with acetone (0.5 ml), followed dropwise by 1 M hydrobromic acid, until it gave a more strongly yellow-coloured mixture. After stirring at room temperature for 45 min this was diluted with distilled water and extracted twice with dichloromethane. The organic extract was re-extracted with 0.1 M hydrochloric acid and the combined aqueous fractions were then basified with solid sodium hydrogen carbonate, followed by final extraction into dichloromethane. The yellow organic extract was washed with a little water, dried and concentrated. The concentrate was filtered and chilled to 0° prior to addition of methanolic hydrogen chloride (1 equiv.), followed by precipitation with diethyl ether. This gave an orange solid on centrifuging and drying (21 mg) (86%). ^{1}H n.m.r. spectroscopy showed the crude product to contain a small proportion (1 : 7) of 5-deoxydaunorubicin (12), which was removed by repeated recrystallization. Alternatively, the presence of this contaminant was avoidable by interposing repeated rapid extraction of the first dichloromethane solution of (34) and (12) with 0.025 M aqueous sodium hydroxide, until the blue colour of the extract diminished, then continuing workup as before; this gave (34) (66%). From either procedure, the product was purified by recrystallization from methanol/acetone to give orange platelets of *5,12-bisdeoxydaunorubicin* (34) as its hydrochloride, m.p. 172–175° (Found: C, 61.1; H, 6.0; N, 2.6. $C_{27}H_{30}ClNO_8$ requires C, 61.0; H, 5.7; N, 2.6%). λ_{max} (log ε) 263sh (4.16), 295sh (3.95), 330 (3.50), 448 nm (3.71). ν_{max} 3420, 1710, 1660, 1610 cm^{-1}. δ 9.05, s, H 5; 8.58, s, H 12; 7.66, m, H 1, H 2; 7.09, m, H 3; 5.59, br d, *J* 3 Hz, H 1′; 5.13, br s, H 7; 4.22, q, *J* 6.5 Hz, H 5′; 4.10, s, OMe; 3.71, br s, H 4′; 3.53, br d, *J* 13 Hz, H 3′; 3.04, d, *J* 19.5 Hz, H 10*eq*; 2.93, d, *J* 19.5 Hz, H 10*ax*; 2.40, s, COMe; 2.25, br d, *J* 15 Hz, H 8*eq*; 2.14, dd, *J* 5, 15 Hz, H 8*ax*; 2.05, dt, *J* 3, 13 Hz, H 2′*ax*; 1.84, dd, *J* 4, 13 Hz, H 2′*eq*; 1.31, d, *J* 6.5 Hz, Me′. *m/z* (f.a.b.) 518 (M+Na, 18%), 496 (M+H, 5), 331 (30), 307 (35).

Method B

A mixture of the hydrochloride of daunorubicin (1) (100 mg), propane-1,3-diol (1 g), trimethyl orthoformate (0.5 ml) and camphorsulfonic acid (10 mg) was stirred at room temperature for 16 h. Methanol (6 ml) was then added to the red gel, followed by lithium carbonate (8 mg) and stirring was continued for 1 h. The mixture was diluted with tetrahydrofuran (60 ml) and then degassed under nitrogen. It was then cooled in an ice bath and lithium borohydride (780 µl of 2 M in tetrahydrofuran, total) was added in three equal portions over 1.5 h. Then acetone (2 ml) was added, followed dropwise by 1 M hydrobromic acid until it turned yellow. The resulting mixture was allowed to stand at room temperature for 30 min, diluted with water and basified with solid sodium hydrogen carbonate, prior to being extracted with dichloromethane. The extract was re-extracted into 0.1 M hydrochloric acid. The resulting acidic solution was left to stand at room temperature for 16 h, when the glycoside was recovered as its hydrochloride (65 mg, 69%), as in *A*. Recrystallization from methanol/acetone gave (34), identical with the product from *A*.

12-Deoxy-13-dihydrodoxorubicin (45)

To a solution of the hydrochloride of doxorubicin (2) (25 mg) in methanol (25 ml) was added 0.1 M aqueous phosphate buffer (pH 7, 1.25 ml) and nitrogen was bubbled through the mixture, cooled in an ice bath. Addition of potassium borohydride (7 mg) and stirring for 25 min gave a clear yellow solution. This was quenched with acetone (0.5 ml) then dropwise with 1 M hydrobromic acid until it gave a red solution, which was warmed to room temperature. Water was then added and the mixture was extracted with a little dichloromethane to remove non-glycosidic by-product. The aqueous phase was basified by addition of solid sodium hydrogen carbonate and extracted with chloroform until the aqueous phase was colourless (typically 10 extractions). The extract

was washed with a little water, dried, concentrated and filtered. Addition of methanolic hydrogen chloride (1 equiv.) to the filtrate at 0° followed by an excess of diethyl ether gave the product, recovered as a dark red solid (23.2 mg) (95%). It was recrystallized from methanol/acetone to give *12-deoxy-13-dihydrodoxorubicin* (45), a 1 : 1 mixture of diastereoisomers, as its hydrochloride. This was isolated as a black powder, m.p. 195–199° (dec.) (Found: C, 57.4; H, 5.5; N, 2.7. $C_{27}H_{32}ClNO_{10}$ requires C, 57.3; H, 5.7; N, 2.5%). λ_{max} (log ε) 283sh (3.95), 296sh (3.71), 308sh (3.45), 490 nm (3.92). ν_{max} 3422, 1654, 1636, 1598 cm^{-1}. δ 8.03, s, H 12; 7.68, t, *J* 8 Hz, H 2; 7.55, d, *J* 8 Hz, H 1; 7.14, d, *J* 8 Hz, H 3; 5.57, br s, H 1′; 5.13, br s, H 7; 4.23, br q, *J* 6.5 Hz, H 5′; 4.07, s, OMe; 3.99–3.92, m, 14-CH_2; 3.76, br s, H 4′; 3.68–3.60, m, H 13; 3.51, br d, *J* 12 Hz, H 3′; 3.08–2.96, m, H 10*eq*; 2.76–2.62, m, H 10*ax*; 2.49, br d, *J* 15 Hz, H 8*eq* (isomer A); 2.22, br d, *J* 15 Hz, H 8*eq* (isomer B); 2.10–1.78, m, H 8*ax*, H 2′; 1.33, d, *J* 6.5 Hz, Me′. *m/z* (electrospray) 530 (M+H, 42%).

12-Deoxydoxorubicin (23)

The hydrochloride of doxorubicin (2) (26 mg) was dissolved in methanol by heating in a large volume of the solvent then concentrating to 1 ml. This solution was treated with 0.1 M methanolic hydrogen bromide (0.75 ml) and trimethyl orthoformate (0.5 ml) for 64 h at room temperature. The mixture was then diluted with water and basified by addition of sodium hydrogen carbonate. Extraction with chloroform, drying and evaporation gave a red-orange solid comprising largely the dimethyl ketal (46) (δ 3.98, s, H 14; 3.56, 3.53, s, s, 2×OCH_3). Without purification, this was dissolved in methanol (15 ml), and then 0.1 M aqueous phosphate buffer (pH 7; 0.75 ml) was added prior to a stream of nitrogen being bubbled through the mixture in an ice bath. Potassium borohydride (4.8 mg) was then added with stirring for 30 min to give a yellow solution. This was quenched with acetone (0.5 ml), followed dropwise by 1 M hydrobromic acid until the colour turned sharply red. The resulting solution was allowed to warm to room temperature over 15 min. The mixture was then diluted with water and, after adding an excess of solid sodium hydrogen carbonate, it was then extracted with chloroform. The extract was dried and evaporated under reduced pressure to give a dark red residue, which was dissolved in 0.25 M hydrobromic acid (2 ml) and acetone (2 ml) and allowed to stand overnight at room temperature. It was then diluted with water and extracted with dichloromethane to remove aglycone. The aqueous phase was then basified with solid sodium hydrogen carbonate and extracted with chloroform. The extract was washed with a little water, dried and concentrated under reduced pressure. The concentrate was filtered and the filtrate was chilled in ice prior to reaction with methanolic hydrogen chloride (1 equiv.), followed by precipitation with ether. This gave the product (23) as a dark red solid (16 mg) (63%). ^{1}H n.m.r. spectroscopy showed the crude product was a mixture of (23), (15) and (35) (83 : 15 : 12). The major product *12-deoxydoxorubicin* (23) as its hydrochloride was purified by several crystallizations from methanol/acetone to give black microplates, m.p. 192–196° (dec.) (Found: C, 57.3; H, 5.3; N, 2.3. $C_{27}H_{30}ClNO_{10}$ requires C, 57.5; H, 5.4; N, 2.5%). λ_{max} (log ε) 275sh (4.15), 297sh (3.83), 325 (3.72), 490 (3.95), 515 nm (3.92). ν_{max} 3419, 1724, 1658, 1635, 1598 cm^{-1}. δ 8.06, s, H 12; 7.69, t, *J* 8 Hz, H 2; 7.56, d, *J* 8 Hz, H 1; 7.15, d, *J* 8 Hz, H 3; 5.57, br d, *J* 3.5 Hz, H 1′; 5.14, br s, H 7; 4.78, 4.74, ABq, *J* 17 Hz, 14-CH_2; 4.20, q, *J* 6.5 Hz, H 5′; 4.08, s, OMe; 3.77, br s, H 4′; 3.55, br d, *J* 13 Hz, H 3′; 3.08, br d, *J* 19.5 Hz, H 10*eq*; 2.89, d, *J* 19.5 Hz, H 10*ax*; 2.30, d, *J* 15 Hz, H 8*eq*; 2.15, dd, *J* 5, 15 Hz, H 8*ax*; 2.03, dt, *J* 3.5, 13 Hz, H 2′*ax*; 1.83, dd, *J* 5, 13 Hz, H 2′*eq*; 1.30, d, *J* 6.5 Hz, Me′. *m/z* (electrospray) 529 (M+2H, 20%), 528 (M+H, 57), 512 (30), 399 (100).

5,12-Bisdeoxydoxorubicin (35)

The hydrochloride of doxorubicin (2) (50 mg) was dissolved in methanol (30 ml) by gentle reflux, and then the solution was concentrated under reduced pressure to a small volume (2 ml). Methanolic hydrogen bromide (0.1 M, 1.5 ml) and trimethyl orthoformate (1.5 ml) were added and the mixture was stirred at room temperature for 3 days. It was then treated with lithium carbonate (5.5 mg) and diluted with tetrahydrofuran (35 ml), and a stream of nitrogen was bubbled through it at ice-bath temperature. Lithium borohydride solution (140 μl of 2 M in tetrahydrofuran) was then added, followed by further aliquots (2×140 μl) after 1 and 2 h. After 2.5 h the pale lemon solution was treated with acetone (0.5 ml) and then, dropwise, with 1 M hydrobromic acid until the colour turned sharply orange. The resulting solution was stirred at room temperature (15 min). Water was added and the mixture was then basified with solid sodium hydrogen carbonate. Extraction with dichloromethane gave an orange solution which was washed with a little water, dried and evaporated. The resulting solid was dissolved in acetone (4 ml) and hydrobromic acid (0.25 M, 4 ml) and was left to stand at room temperature for 66 h. The mixture was diluted with water, and aglycones were removed by dichloromethane extraction. Further extraction with dichloromethane, following addition of solid sodium hydrogen carbonate, then gave the glycosidic fraction. The latter fraction was washed with a little water, dried and concentrated. The residue was re-dissolved in dichloromethane/methanol, filtered and chilled. Methanolic hydrogen chloride solution (1 equiv.) at 0° was then added, followed by an excess of ether. The precipitate was recovered by centrifugation and drying to give *5,12-bisdeoxydoxorubicin* (35) as its hydrochloride, a dark orange solid (29 mg, 61%). Crystallization from methanol/acetone gave dark orange platelets, m.p. 180–181° (Found: C, 59.1; H, 5.4; N, 2.4. $C_{27}H_{30}ClNO_9$ requires C, 59.2; H, 5.5; N, 2.5%). λ_{max} (log ε) 293sh (3.91), 338 (3.42), 455 nm (3.79). ν_{max} 3404, 1723, 1662, 1614 cm^{-1}. δ 9.06, s, H 5; 8.58, s, H 12; 7.64, m, H 1, H 2; 7.07, m, H 3; 5.58, br d, *J* 3.5 Hz, H 1′; 5.13, br s, H 7; 4.79, 4.75, ABq, *J* 17 Hz, 14-CH_2; 4.19, q, *J* 6.5 Hz, H 5′; 4.08, s, OMe; 3.76, br d, *J* 3 Hz, H 4′; 3.53, m, H 3′; 3.11, dd, *J* 4, 19.5 Hz, H 10*eq*; 2.92, dd, *J* 5, 19.5 Hz, H 10*ax*; 2.29, br d, *J* 15 Hz, H 8*eq*; 2.15, dd, *J* 5, 15 Hz, H 8*ax*; 2.04, dt, *J* 3.5, 13 Hz, H 2′*ax*; 1.84, dd, *J* 4.5, 13 Hz, H 2′*eq*; 1.31, d, *J* 6.5 Hz, Me′. *m/z* (electrospray) 512 (M+H, 84%), 383 (57). *m/z* (f.a.b.) 534 (M+Na, 8%).

4,5,12-Trisdeoxydaunorubicin (36)

To a solution of the hydrochloride of idarubicin (37) (25 mg) in methanol (2.5 ml) was added trimethyl orthoformate (1.25 ml) and camphorsulfonic acid (2.5 mg). The mixture was allowed to stand at room temperature for 16 h. It was then cooled in ice, tetrahydrofuran (25 ml) was added and nitrogen was bubbled through. A solution of lithium borohydride in tetrahydrofuran (72 μl of 2 M) was then added by syringe followed, after 30 and then 70 min, by two further aliquots (2×72 μl). After 110 min, the pink solution was treated with acetone (0.5 ml) then dropwise with 1 M hydrobromic acid until it gave a yellow solution, which was stirred at room temperature for 30 min. It was then diluted with water and extracted twice with dichloromethane. The extract was re-extracted with 0.1 M hydrochloric acid. The combined aqueous phases were basified with solid sodium hydrogen carbonate and finally extracted with dichloromethane, and the yellow extract was washed with water, dried and concentrated. Addition of methanolic hydrogen chloride (1 equiv.) at 0° and an excess of ether precipitated the hydrochloride of *4,5,12-trisdeoxydaunorubicin* (36) (14.6 mg, 62%). Crystallization from methanol/acetone gave orange platelets, m.p. 160–162° (dec.) (Found: C, 58.9; H, 5.6; N, 2.5. $C_{26}H_{28}ClNO_7 \cdot 1.5\ H_2O$ requires C, 59.0; H, 5.9; N, 2.6%). λ_{max} (log ε) 273 (4.11), 284 (4.13), 295 (4.12), 408 nm (3.54). ν_{max} 3421, 1707, 1664, 1616 cm^{-1}. δ 8.66, 8.63, s, s, H 5, H 12; 8.01, m, H 1, H 4; 7.64, m, H 2, H 3; 5.50, br d, *J* 3.5 Hz, H 1′; 5.05, br s, H 7; 4.11, q, *J* 6.5 Hz, H 5′; 3.71, br s, H 4′; 3.46, br td, *J* 2.5, 13 Hz, H 3′; 2.99, br d, *J* 19.5 Hz, H 10*eq*; 2.81, d, *J* 19.5 Hz, H 10*ax*; 2.42, s, COMe; 2.16, br d, *J* 15 Hz, H 8*eq*; 2.03, dd, *J* 5, 13 Hz, H 8*ax*; 1.95, dt, *J* 4, 13 Hz, H 2′*ax*; 1.75, dd, *J* 4.5, 13 Hz, H 2′*eq*; 1.22, d, *J* 6.5 Hz, Me′. *m/z* (f.a.b.) 488 (M+Na, 15%), 466 (M+H, 35), 327 (31).

12-Deoxydaunorubicinone (49), 5,12-Bisdeoxydaunorubicinone (50) and 4,5,12-Trisdeoxydaunorubicinone (51)

12-Deoxydaunorubicin (22), 5,12-bisdeoxydaunorubicin (34) and 4,5,12-trisdeoxydaunorubicin (36) were hydrolysed separately in 0.2 M hydrochloric acid for 1 h on a steam bath. The precipitated solids were respectively recrystallized from acetone.

12-Deoxydaunorubicinone (49) gave dark red needles, m.p. 189–191° (Found: $M^{+\bullet}$, 382.1045. $C_{21}H_{18}O_7$ requires $M^{+\bullet}$, 382.1052). λ_{max} (log ε) 272 (3.88), 320 (3.40), 490 (3.88), 510 nm (3.88). ν_{max} 1710, 1655, 1630, 1590 cm^{-1}. δ 14.90, s, OH; 8.01, s, H 12; 7.65, t, *J* 8 Hz,

H 2; 7.51, d, *J* 8 Hz, H 1; 7.09, d, *J* 8 Hz, H 3; 5.22, d, *J* 5 Hz, H 7; 4.07, s, OMe; 3.04, dd, *J* 2, 19.5 Hz, H 10*eq*; 2,81, dd, *J* 1, 19.5 Hz, H 10*ax*; 2.41, s, COMe; 2.31, td, *J* 2, 14.5 Hz, H 8*eq*; 2.10, dd, *J* 5, 14.5 Hz, H 8*ax*. *m/z* 382 (M, 15%), 346 (62).

5,12-Bisdeoxydaunorubicinone (50) gave orange microneedles, m.p. 144–146° (Found: $M^{+\bullet}$, 366.1099. $C_{21}H_{18}O_6$ requires $M^{+\bullet}$, 366.1103). λ_{max} (log ε) 260sh (4.07), 280sh (3.97), 296 (3.88), 323 (3.51), 440 nm (3.68). ν_{max} 3434, 1709, 1665, 1615 cm^{-1}. δ 9.02, s, H 5; 8.48, s, H 12; 7.61, m, H 1, H 2; 7.01, m, H 3; 5.22, br d, *J* 4.5 Hz, H 7; 4.07, s, OMe; 3.08, dd, *J* 2, 19.5 Hz, H 10*eq*; 2.85, br d, *J* 19.5 Hz, H 10*ax*; 2.42, s, COMe; 2.30, td, *J* 1.5, 14.5 Hz, H 8*eq*; 2.10, dd, *J* 5, 14.5 Hz, H 8*ax*. *m/z* 366 (M, 44%), 330 (88), 305 (100).

4,5,12-Trisdeoxydaunorubicinone (51) gave yellow needles, m.p. 135–137° (dec.). λ_{max} (log ε) 260sh (4.37), 270 (4.63), 296 (4.28), 380 (3.70), 412 nm (3.72). ν_{max} 3425, 1711, 1664, 1617 cm^{-1}. δ 8.64, 8.61, s, s, H 5, H 12; 8.07, m, H 1, H 4; 7.71, m, H 2, H 3; 5.22, td, *J* 1.5, 5 Hz, H 7; 3.09, dd, *J* 2.5, 20 Hz, H 10*eq*; 2.87, dd, *J* 1.5, 20 Hz, H 10*ax*; 2.42, s, COMe; 2.31, td, *J* 2.5, 15 Hz, H 8*eq*; 2.11, dd, *J* 5, 15 Hz, H 8*ax*; 4.50, 3.70, br s, br s, 2×OH (both D_2O-exchanged). *m/z* 336 (M, 2.4%), 316 (26), 301 (40), 300 (79), 285 (100).

Isolation of Anthrone Glycosides (53) and (54)

The hydrochloride of carminomycin (25) (5 mg) was hydrogenated in methanol (4 ml) over Adams catalyst (1.8 mg) and dichloroacetic acid (20 mg) for 40 min at room temperature. Analytical t.l.c. then indicated the formation of a pale yellow, non-fluorescent product, of polarity consistent with a glycoside. The mixture was filtered through Celite under nitrogen and the filtrate was concentrated under vacuum, then diluted with ether to give a precipitate recovered by centrifugation. The solid was re-dissolved in methanol, then the mixture was treated with an excess of hydrogen chloride in methanol and re-precipitated with an excess of ether to give the anthrone (54) as its hydrochloride, a tan solid (2.5 mg). δ (CD_3OD) 7.55, dd, *J* 7.5, 8.5 Hz, H 2; 7.03, br d, *J* 7.5 Hz, H 1; 6.86, br d, *J* 8.5 Hz, H 3; 5.47, br d, *J* 4 Hz, H 1′; 5.17, dd, *J* 3, 5 Hz, H 7; 4.27, br q, *J* 6.5 Hz, H 5′; 4.26, br s, anthrone CH_2; 3.65, br s, H 4′; 3.60, m, H 3′; 3.10, d, *J* 18 Hz, H 10*eq*; 3.05, br d, *J* 18 Hz, H 10*ax*; 2.33, s, COMe; 2.29, br d, *J* 14 Hz, H 8*eq*; 2.18, dd, *J* 5, 14 Hz, H 8*ax*; 2.02, dt, *J* 4, 13 Hz, H 2′*ax*; 1.88, dd, *J* 5, 13 Hz, H 2′*eq*; 1.28, d, *J* 6.5 Hz, Me′.

The hydrochloride of daunorubicin (1) (5 mg) was hydrogenated in methanol (2 ml) and chloroacetic acid (20 mg) over Adams catalyst (1 mg) for 20 min at room temperature. The mixture was filtered under nitrogen and then concentrated under reduced pressure. Dilution of the yellow concentrate with ether gave a tan solid recovered by centrifugation. The hydrochloride was prepared in the usual way to give the anthrone (53) as a tan solid (2 mg). Partial δ (CD_3OD) 7.89, d, *J* 8 Hz, H 1; 7.47, t, *J* 8 Hz, H 2; 7.30, d, *J* 8 Hz, H 3; 4.04, s, anthrone CH_2; 3.99, s, OMe; 2.29, s, COMe; 1.28, d, *J* 6.5 Hz, Me′.

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