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THE TOTAL SYNTHESIS OF 13(R)- AND 13(S)-DIHYDRO-4-DEMETHOXY-DAUNORUBICIN. REVISION OF STEREOCHEMISTRY OF THE MICROBIAL AND MAMMALIAN REDUCTION PRODUCT OF 4-DEMETHOXYDAUNORUBICIN

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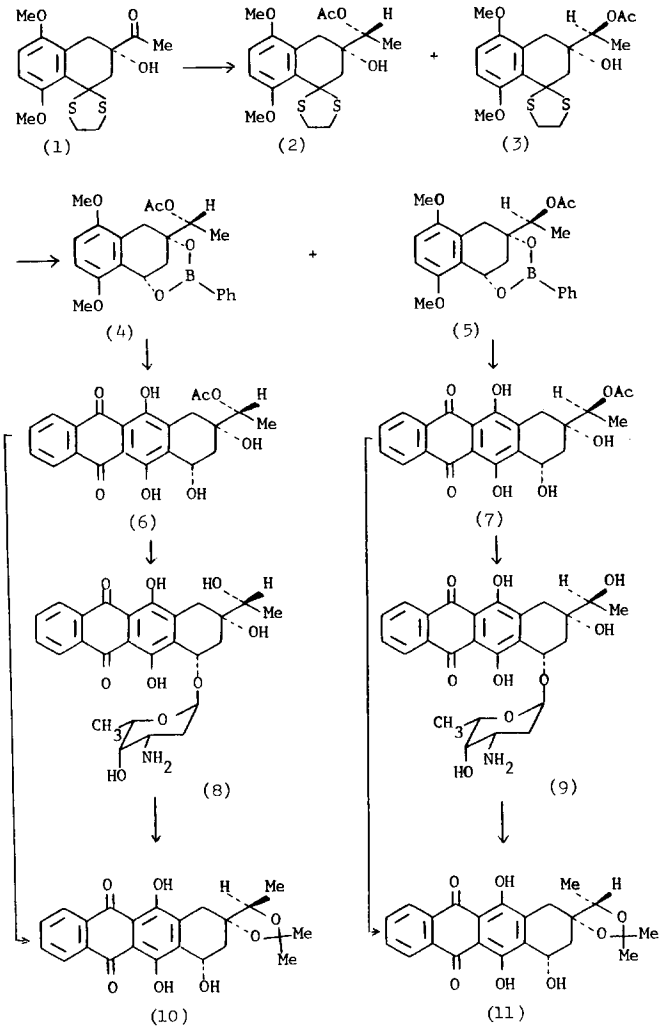
Summary: The properties of 13(S)-dihydro-4-demethoxydaunorubicin, rather than the 13(R)-isomer, correspond to those of the microbial and mammalian reduction product of 4-demethoxydaunorubicin.

The anthracyclines, daunorubicin (Daunomycin) and doxorubicin (Adriamycin), are widely used in the clinic for the treatment of human leukaemias and solid tumours.[1-6] However, understanding of their mode of action and associated toxicity in vivo is complicated by extensive metabolism. Studies in man[7] and animal tissues[8-11] have shown that one of the principal metabolic reactions involves reduction of the side chain carbonyl group to give the corresponding 13-dihydroanthracyclines. The finding that these metabolites have significant antitumour activity[12-13] has stimulated considerable interest in the preparation and biological properties of 13-dihydroanthracyclines. Their preparation has involved reduction of the parent compounds by microbial[14-15] and chemical methods[16] but the configuration at C-13 has not been defined. Recently, rubeomycins B and B_1, 13-dihydroanthracyclines obtained as fermentation products of a new actinomycete isolate, have been assigned R stereochemistry at C-13[17]: Cassinelli et al. reported stereoselective microbial reduction of 4-demethoxydaunorubicin (idarubicin), a synthetic anthracycline currently in clinical trial[18], to a product which they formulated as the 13(R)-dihydro derivative.[19] Furthermore, it was shown to be identical to the major metabolite, idarubicinol, obtained from the urine of patients treated with idarubicin; this may be responsible for some of the clinical activity.[20-21] In view of this, we wish to report the total synthesis of both 13(R)- and 13(S)-dihydro-4-demethoxydaunorubicin (8 and 9) and to compare their physical properties with those of the compound isolated by Cassinelli et al.

We have previously described the synthesis of 13(R)- and 13(S)-dihydro-4-demethoxyanthracyclinones (6 and 7)[22,25] through stereoselective hydride reductions of the ketone (1).[23] Unambiguous assignment of the side chain configuration was established by X-ray analysis[26] of the purified cis-benzeneboronates (4 and 5). Silver trifluoromethanesulphonate catalysed reaction of (6) with 1-chloro-2,3,6-trideoxy-4-O-p-nitrobenzoyl-3-trifluoroacetamido-L-lyxo-hexopyranose[24] followed by hydrolysis of protecting groups gave 13(R)-dihydro-4-demethoxydaunorubicin (8), isolated as the hydrochloride[25] [orange-red powder, mp 180-182°C; $[\alpha]_D^{20}$ +142° (0.05% in MeOH); retention time 33.76 min;[27] δ (CD_3SOCD_3) 1.16 (3H, d, J 6Hz), 1.17 (3H, d, J 6Hz), 1.70 (1H, dd, J 12 Hz and 3Hz), 1.90 (1H, dt, J 12 Hz and 3Hz), 2.03 (2H, m), 2.61 (1H,d, J 20Hz), 2.93 (1H, d, J 20Hz), 3.42 (1H, d, J 12Hz), 3.53 (1H, t, J 6Hz), 3.60 (1H, m),



4.21 (1H, q, J 6Hz), 4.81 (1H, d, J 6Hz, exch. D_2O), 4.92 (1H, m), 5.30 (1H, m), 5.48 (1H, d, J 6Hz, exch. D_2O), 7.95 (2H, m), 8.23 (2H, m)]. In an analogous manner (7) was converted to 13(S)-dihydro-4-demethoxydaunorubicin hydrochloride (9) [orange-red powder, mp 174-175°C; $[\alpha]_D^{20}$ +141° (0.05% in MeOH); retention time 35.38 min;[27] δ (CD_3SOCD_3) 1.16 (3H, d, J 6Hz), 1.17 (3H, d, J 6Hz), 1.69 (1H, dd, J 12Hz and 4Hz), 1.89 (2H, m), 2.23 (1H, d, J 15Hz), 2.75 (1H, d, J 18Hz), 2.83 (1H, d, J 18Hz), 3.34 (1H, d, J 12Hz), 3.55 (1H, t, J 6Hz), 3.60 (1H, m), 4.23 (1H, q, J 6Hz), 4.83 (1H, d, J 6Hz, exch. D_2O), 4.93 (1H, m), 5.28 (1H, s), 5.48 (1H, d, J 6Hz, exch. D_2O), 7.98 (2H, m), 8.27 (2H, m)]. Although the diastereoisomeric dihydroanthracyclines (8 and 9) exhibited similar melting points and optical rotations they could easily be distinguished by the chemical shift of C-10 protons in their ^1H n.m.r spectra, and of C-8 and C-10 carbons in their ^{13}C n.m.r spectra (table 1).

Using reverse phase h.p.l.c. Cassinelli et al. showed that the microbial reduction product corresponded to the less polar stereoisomer in the mixture obtained by chemical reduction of

4-demethoxydaunorubicin.[19] Using similar chromatographic conditions[27] we have found (9), the 13(S)-isomer, to be the less polar compound. The ^{1}H n.m.r. spectrum was not described but the ^{13}C n.m.r spectrum reported[19] closely resembled that of the 13(S)-isomer synthesised by us (table 1).

Table 1 - ^{13}C n.m.r. in D_2O-CD_3OD

Compound	Configuration	δ ppm	
		C-10	C-8
8	13(R)	31.8	34.7
9	13(S)	32.6	34.1
*		32.8	34.2

* chemical shifts reported by Cassinelli et al.[19]

The structural assignment by Cassinelli et al. was based on analysis of the ^{1}H n.m.r spectra of the corresponding 9,13-O-isopropylidene derivatives. Using the method described[19] we have prepared isopropylidene derivative (10), mp 231-232°C; $[\alpha]_D^{20}$ +97° (0.05% in dioxan) from glycoside (8) and isopropylidene derivative (11), mp 230-231°C; $[\alpha]_D^{20}$ +137° (0.045% in dioxan) from glycoside (9). Isopropylidene derivatives (10 and 11) were also obtained from the corresponding anthracyclinones (6 and 7), after saponification, ensuring that no inversion of configuration had occurred. In each case the ^{1}H n.m.r. data (table 2) corresponded well with that reported by Cassinelli et al., thus confirming their configurational assignment for these derivatives. However, whereas these workers reported that the faster moving compound on t.l.c.

Table 2 - ^{1}H n.m.r. of compounds (10) and (11)

Proton	δ ppm and multiplicity			
	10 [13(R)]	11 [13(S)]	13(R)*	13(S)*
Me	1.34, d, J 6Hz	1.37, d, J 6Hz		
Me	1.40, s	1.42, s		
Me	1.55, s	1.43, s		
H-8ax	1.69, dd, J 14.5, 5Hz	2.02, dd, J 14, 5Hz	1.68	2.02
H-8eq	2.43, dt, J 14.5, 2.5Hz	2.24, dt, J 14, 2.5Hz	2.42	2.18
H-10ax	2.69, d, J 19Hz	2.55, d, J 18.5Hz	2.68	2.51
H-10eq	3.15, dd, J 19, 2.5Hz	3.24, dd, J 18.5, 2.5Hz	3.13	3.19
H-13	4.20, q, J 6Hz	4.16, q, J 6Hz		
OH	4.34, d, J 10Hz	4.22, d, J 10Hz		
H-7	5.28, m	5.22, m		
ArH	7.84, m	7.84, m		
ArH	8.36, m	8.36, m		

* ^{1}H n.m.r. chemical shifts reported by Cassinelli et al.[19]

was the 13(R)-isomer (10), in our hands the faster moving compound corresponded to the 13(S)-isomer (11).[28] The observation of Cassinelli et al., that the isopropylidene compound derived from the microbial reduction product has the higher R_f is consistent with our data.

These studies confirm the structural assignment of the diastereoisomeric isopropylidene derivatives reported by Cassinelli et al., but our evidence indicates that the microbial reduction product and the main human metabolite of idarubicin correspond to 13(S)-dihydro-4-demethoxydaunorubicin (9), rather than the 13(R)-isomer as previously suggested.

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25. All new compounds were fully characterised by spectroscopic means (n.m.r., i.r., m.s., u.v.) and gave satisfactory elemental analysis (crystalline compounds).
26. We are indebted to Dr J Daly, Hoffmann-La Roche, Basle for carrying out the X-ray analysis.
27. Column: 25 cm x 0.4 cm id, Lichrosorb RP 18 (5 μ). Mobile phase A: CH_3CN/aqueous solution of KH_2PO_4 (10 g/l), adjusted to pH 2.67 with 0.5 M citric acid, 10/90 by volume. Mobile phase B: CH_3CN. Elution: for 40 min (80% A + 20% B) then for 20 min (40% A + 60% B). Flow rate: 0.6 ml/min. Detection: 254 nm.
28. T.l.c analysis used Merck pre-coated plates, silica gel 60F -254, eluted with CH_2Cl_2.

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