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THE STRUCTURE OF DAUNOMYCIN

R. H. Iwamoto, P. Lim and N. S. Bhacca

Division of Life Sciences, Stanford Research Institute

Menlo Park, California 94025 and

Department of Chemistry, Louisiana State University

Baton Rouge, Louisiana 70803

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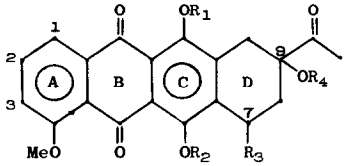
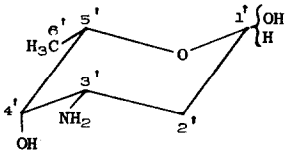
Daunomycin (1), 6, a bacterial metabolite, has shown interesting biological activity, especially as an antitumor agent. Mild acidic hydrolysis of daunomycin yields an aglycone, daunomycinone, 1, and the amino sugar daunosamine (2), 2. The structure of the sugar has been elucidated (2) and was confirmed by synthesis (3). With the exception of the placement of a methoxylgroup, the structure of daunomycinone (4) has also been elucidated.

Several other features of this interesting molecule, besides the position of the methoxyl group in ring A, remain to be established. These are: 1, the position of the glycosidic bond; 2, the configuration at the sugar anomeric carbon atom in the uncleaved molecule; and 3, the stereochemistry of the substituents on the saturated ring D.

We have carried out experiments that have established the position of the glycosidic bond, as well as the relative stereochemistry of the substituents at C-7 and C-9 on ring D. From the results of 220 MHz nmr spectra, we have also determined the stereochemical arrangement at the anomeric carbon atom of the sugar as well as the configuration about C-7.





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1. R_1, R_2, R_4 = H, R_3 = OH

3. R_1, R_2, R_3, R_4 = H

4. R_1, R_2 = CH_3, R_3 = OH, R_4 = H

5. R_1, R_2 = CH_3, R_4, R_3 } O CH_3 CH_3

Catalytic hydrogenolysis of daunomycin in dioxane employing $Pd/BaSO_4$ as the catalyst resulted in a good yield of 7-deoxydaunomycinone (4), 3, which is identical to the material obtained by similar treatment of daunomycinone. This result indicates that the sugar is coupled to the aglycone at C-7.

To ascertain the relative stereochemical arrangement of the C-7 and C-9 hydroxyl groups in ring D by preparation of a cyclic acetonide, it seemed advisable to block the phenolic hydroxyl groups to prevent the formation of more than one derivative. Treatment of daunomycin hydrochloride with methyl sulfate in the presence of potassium carbonate in boiling acetone gave a yellow oil that could be readily hydrolyzed by warm 0.2N HCl to crystalline dimethyl daunomycinone, 4, $C_{23}H_{22}O_8$,* m.p. 136-137°, (partial solidification and remelting at 151-155°). The nmr spectrum** of this compound is completely consistent with the proposed structure.

The relative cis-stereochemistry of the two hydroxyl groups on ring D was readily established by the successful conversion of dimethyl daunomycinone to an excellent yield of crystalline 7,9-acetonide. When 4 was treated with 2,2-dimethoxypropane (5) at room temperature in the presence of a catalytic amount of toluenesulfonic acid, an 80% yield of the acetonide was obtained. The yellow acetonide, 5, $C_{26}H_{26}O_8$, m.p. 177-178° exhibits two three-proton singlets in the nmr spectrum at 1.10 and 1.42 ppm, which are assigned to the gem-dimethyl groups of the acetonide. Hydrolysis of the acetonide was readily accomplished by 80% trifluoroacetic acid (7), which regenerates 4 in nearly quantitative yield.

The configuration about C-1', in daunomycin was established by nmr spectroscopy. Examination of the 220 MHz nmr spectra (attached spectrum) of daunomycin as pyridine-d_5 solutions clearly established that the anomeric proton, H_1' is equatorially oriented and that the sugar exists as the α-L form.

The resonance at 5.77 ppm was assigned to the anomeric proton.*** Double resonance experiments at 100 MHz and extrapolation to 220 MHz revealed that the C-1' proton, H_1', is spin coupled to the C-2' ax. and C-2' eq. protons with coupling constants of approximately 3.5 and 2.0 Hz, respectively. The magnitude of these couplings clearly indicate the C-1' proton is equatorially oriented.

Additional information was also obtained from the spectra. The resonance at 5.41 ppm was attributed to the benzylic proton at C-7 of the aglycone, which exhibits spin couplings to C-8 ax. and C-8 eq. protons of 5.0 and 1.5 Hz, respectively. The size of the coupling constants demonstrate that the proton at C-7 is pseudoequatorially oriented. It was also

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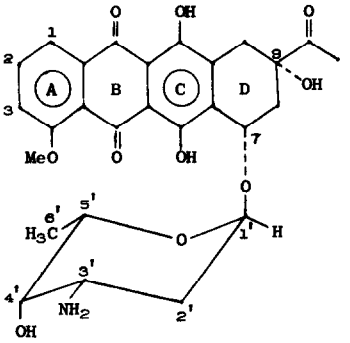
* All new compounds reported gave satifactory elemental analyses, m.p.s are uncorrected.

** Recorded on Varian A60A spectrometer, unless otherwise specified. $CDCl_3$ used as the solvent; TMS was used as the internal standard.

*** See, for example, spectra Nos. 640, 641, and 682, Varian Catalog Nmr Spectra.

observed that by varying the solvents from pyridine-d_5 to acetone-d_6, the coupling between C-8 ax. and C-7 proton in the spectrum of daunomycin changes from 5.0 to 3 Hz. Furthermore, after slight warming, the acetone-d_6 spectrum exhibits two sets of resonances for the amino sugar as well as the methoxyl protons. These observations not only indicate a conformational mobility of the sugar but serve to locate the position of the methoxyl group at C-4. For if the methoxyl group were situated at C-1, it would be too distantly removed to detect the changes occurring at C-1' and/or C-7. A more detailed account of the conformational mobility of daunomycin will be presented in a separate communication.

The structure and relative stereochemistry of daunomycin depending upon the conformation of ring D can be represented by the following figure.****



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It is possible that the benzylic position (C-7) is altered during acidic hydrolysis of daunomycin and dimethyl daunomycin.

The circular dichroism curves of daunomycin and daunomycinone are very similar in the region of 280 nm. Removal of the hydroxyl group at C-7 to give 7-deoxydaunomycinone, which exhibited a very weak dichroic effect, and implies that the large effect shown in the CD curves of daunomycinone and daunomycin is largely contributed by the substituent at C-7.

Furthermore it was observed that in the formation of the glycoside between daunomycinone and acetobromoglucose, only one compound could be detected. If daunomycinone were appreciably racemized during its preparation from daunomycin, then a diastereomeric pair of glycosides would be obtained and would therefore be separable as was seen in an allied case (6).

**** Since the conformation of ring D is not known, the structure which is epimeric at both C-7 and C-9 could also be written.

If complete epimerization occurred, then the CD curve would be opposite to that found in daunomycin. The CD curve of 7-(tetra-O-acetyl-β-D-glucopyranosyloxy)daunomycinone was found to be similar in all important respects to that of daunomycin.

Further work is in progress to confirm the position of the methoxyl group as well as the conformation of the D ring.

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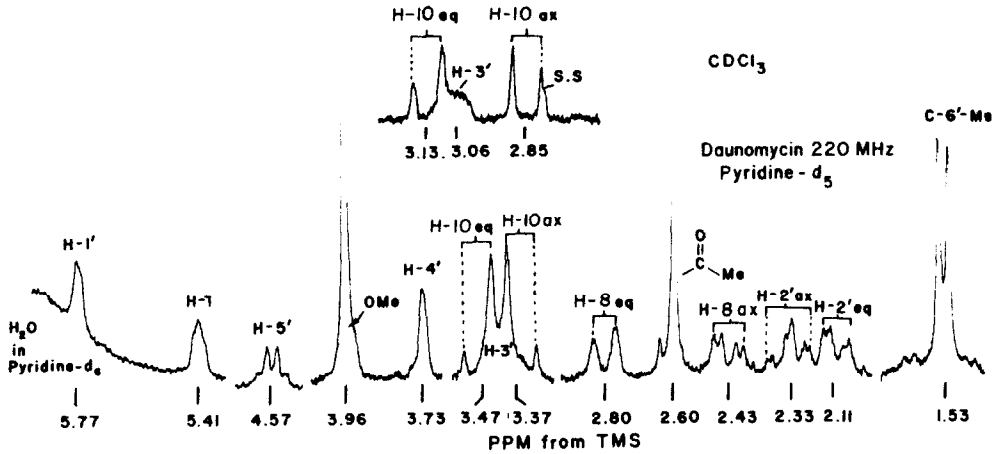

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