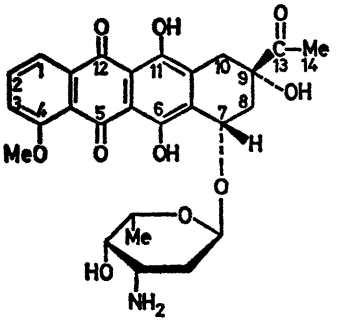
Acetyl Proton Exchange in Daunomycin

By DON R. PHILLIPS* and GORDON C. K. ROBERTS

(*Biochemistry Department, La Trobe University, Bundoora, Victoria* 3083, *Australia, and National Institute for Medical Research, The Ridgeway, Mill Hill, London* NW7 1AA)

Summary ^{1}H N.m.r. studies have shown that the acetyl methyl protons of daunomycin (**1**) exchange with deuterium with unexpectedly short half-lives of 26 h at 25 °C and only 29 min at 55 °C.

DAUNOMYCIN (**1**) is a cytotoxic antibiotic widely used in the treatment of leukaemia.[1,2] The general mode of action is thought to be due to its ability to intercalate into DNA.[2] However, the detailed mode of action is the subject of intensive study. In the course of ^{1}H n.m.r. studies of the interaction of daunomycin with oligonucleotides, we noted



(1)

that the incubation of a solution of daunomycin in D_2O phosphate buffer led to a progressive decrease in the intensity of the acetyl methyl proton resonance (relative to that of the methoxy protons). There was no other change in the spectrum with time, and comparison with incubations in H_2O, together with the fact that the full intensity of the resonance could be recovered by a subsequent incubation in H_2O, showed clearly that the decrease in intensity was due to exchange of the methyl protons with solvent deuterium.

The rate of exchange was determined at several temperatures; in each case simple first-order kinetics were seen, and the observed rate constants are given in the Table. The

TABLE. Rate constants for the exchange of the acetyl methyl protons of daunomycin (D_2O, 10 mM phosphate, 150 mM NaCl, pD 6·8)

Temperature/°C	$10^5k/s^{-1}$
25	0·73
34	2·2
45	15·0
55	40·0

calculated activation energy is 46 kJ mol^{-1}. The exchange is presumably the result of an enolisation of the acetyl group,[3] though it is very much faster than the reported rates of enolisation of acetone[3] (2×10^{-8}—7×10^{-7} s^{-1} at 25 °C) or monohydroxyacetone[4] (1-hydroxypropan-2-one) (1×10^{-7} s^{-1} at 30 °C). In the crystal[5] the C(9)-OH of daunomycin (**1**) is approximately *trans* to the carbonyl of the acetyl group, and forms a hydrogen bond to the glycosidic oxygen on C(7). This restricted orientation of the hydroxy group may contribute to the enhanced rate of exchange seen in daunomycin, since the lone pair of electrons of the C(9)-O will be directed towards the acetyl methyl protons.

These observations have important practical consequences for the use of [^{3}H]daunomycin both *in vitro* and *in vivo*. 'Generally' tritiated daunomycin contains tritium in the acetyl group, and loss of radioactivity from this position has been observed *in vitro*.[6] The half-life of the acetyl protons under the conditions used here is 26 h at 25 °C, 9 h at 34 °C, and only 29 min at 55 °C. While the magnitude of the tritium isotope effect is not known, these figures suggest that loss of tritium from [^{3}H]daunomycin to water might be very significant in equilibrium dialysis experiments, tissue distribution of the drug, pharmacokinetics, *etc.*

If the concentration of daunomycin is estimated from its radioactivity, an error could be introduced in assuming the existence of only rapidly exchanging, 'labile' protons (hydroxy and amino) and 'stable' protons, since the acetyl methyl protons exchange at intermediate rates. In the absence of a detailed distribution pattern of the labelling, we have assumed that the radioactivity of daunomycin (after

exchange of the 'labile' sites) is due to labelling of (a) H-1, H-2, and H-3 (exchange of the aromatic protons is usually favoured by Wilzbach's method [7] which was used to prepare tritiated daunomycin[1]), (b) the acetyl methyl protons, and (c) 1·2 sites on the aglycone (this figure is based on the known distribution ratio of 2·57 between the aglycone and the amino sugar,[1] and allows for the acid catalysed exchange of all acetyl methyl radioactivity under the acid conditions used in the hydrolysis procedure[1]). The maximum decrease of radioactivity arising from tritium exchange of the acetyl methyl protons is $(3/7{\cdot}2) \times 100 = 42\%$. Unless careful controls are used to allow for this time dependent exchange of the acetyl methyl radioactivity (in the time interval of one day at ambient temperatures) a serious underestimate of daunomycin concentration could arise. It should also be noted that the exchanged counts will be in water, and will therefore distribute like free daunomycin in most equilibrium dialysis experiments.

This work was supported by a grant from the Australian Research Grants Committee. We thank Farmitalia, Milan, for the gift of daunomycin.

(*Received, 20th December* 1978; *Com.* 1361.)

[1] J. Bernard, R. P. M. Boiron, and U. J. R. Maral, 'Rubidomcin,' Springer-Verlag, Berlin, 1969.
[2] A. DiMarco, F. Arcamone, and F. Zunino, 'Mechanism of Action of Antitumour Agents,' in 'Antibiotics,' vol. III, eds. J. W. Corcoran and F. E. Hahn, Springer-Verlag, Berlin, 1975.
[3] H. M. Dawson and E. Spivey, *J. Chem. Soc.*, 1930, 2180; J. Hine, 'Physical Organic Chemistry,' Section 5-2d, McGraw-Hill, New York, 1962; *Accounts Chem. Res.*, 1978, **11**, 1; J. Hine, J. C. Kaufmann, and M. S. Cholod, *J. Amer. Chem. Soc.*, 1972, **94**, 4590; R. P. Bell, 'The Proton in Chemistry,' 2nd edn., Chapman and Hall, London, 1973; R. P. Bell and O. M. Lidwell, *Proc. Roy. Soc.*, 1940, **A176**, 88.
[4] S. J. Reynolds, D. W. Yates, and U. Pogson, *Biochem. J.*, 1971, **122**, 285.
[5] S. Neidle and G. Taylor, *Biochim. Biophys. Acta*, 1977, **479**, 450.
[6] F. Arcamone, personal communication.
[7] E. A. Evans, 'Tritium and its Compounds,' Butterworth, London, 1966, p. 107.