AN INVESTIGATION OF THE POSSIBLE MODE OF ACTION OF NEOTHRAMYCIN, A PYRROLO {2,1-C}{1,4}BENZODIAZEPINE ANTITUMOUR AGENT

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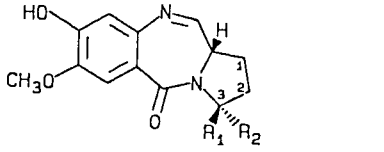
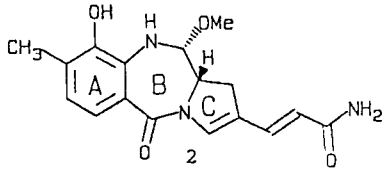
Neothramycins A and B (1) are members of the pyrrolo[2,1-c][1,4]benzodiazepine (PBD) group of antitumour antibiotics which includes anthramycin (2) and tomaymycin (3) (Hurley et al 1984). The PBDs inhibit the replication and transcription of DNA by forming a covalent adduct in the minor groove of DNA through alkylation of the 2-amino group of a guanine residue, reaction occurring at the C11 position. However, neothramycin differs from anthramycin and tomaymycin in that it exists in the N10-C11 imine form and has a second potentially electrophilic site at C3. Furthermore, it has been reported to react with guanosine in DMSO/water over 24 hours to form predominantly the C3-adduct (Maruyama et al 1981).



Neothramycin A: R_1=H, R_2=OH.
Neothramycin B: R_1=OH, R_2=H.

1



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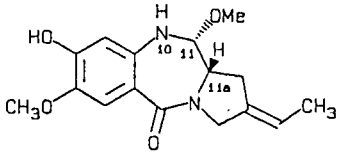

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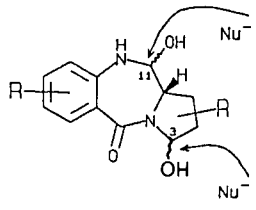

Figure 1

NMR and HPLC assay procedures involving thiophenol as a model nucleophile have been developed to assess the electrophilicity of PBDs (Morris et al 1989). The objective of this investigation was to determine which centre (C3 or C11) is the more reactive towards thiophenol (Fig.1).

Initial reversed phase HPLC studies showed that butylneothramycin A (BNA) reacted too rapidly with thiophenol to obtain kinetic data, although it indicated that BNA was more reactive than anthramycin or tomaymycin. The NMR of BNA in d_6-DMSO showed that the stock sample was mainly the N10-C11 imine form with a small amount of the C11(S)-carbinolamine present. Reaction with thiophenol occurred immediately to give two C11-adducts [C11(R) and C11(S)]. Hydrolysis of BNA in d_6-DMSO was monitored after addition of D_2O. The imine moiety partially hydrolysed to a mixture of C3(R)- and C3(S)-carbinolamines in the C11(S)-carbinolamine form. After subsequent addition of thiophenol, the residual imine reacted immediately but the C11-carbinolamine species reacted more slowly to give C11(S)- and C11(R)-adducts, with the C11(S)-species being the favoured form. No reaction with thiophenol was observed at the C3 position.

In contrast to the reported reaction of guanosine at the C3-position of neothramycin, the results of this investigation show that under similar conditions thiophenol reacts at C11 which supports currently accepted theories on the mode of action of the PBDs.

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Hurley, L.H., Thurston D.E. (1984) Pharm. Res. 1: 52-59
Maruyama, I.N. et al (1981) Biochem. Biophys. Res. Com. 98: 970-975
Morris S.J. et al (1989) J. Pharm. Pharmacol. 41: 107P