in either water or in dimethyl sulfoxide [8, 9], although they may do so within a membrane [9]. In contrast, analogue 1 does appear to adopt a predominant conformation in dimethyl sulfoxide solution.

Chemical shift values for analogue 1 (Table 1) are generally similar to those reported for either BN or GRP, but the values of $J_{SN}$ show a distinct minimum towards the centre of the three C-terminal residues and may indicate that these protons engage in intramolecular hydrogen bonds.

Sequential $d_{NN}$ NOEs were only observed for residues between position 2 and 5 and confirmed assignments. Only two longer range NOEs were observed, between a $\beta$-CH of Trp and a $\beta$-CH of $\sigma$-C (−3%) and between the amide proton of Leu and an aromatic proton (−3%), indicating some rigidity in the positioning of the side-chains.

On dilution with water, new signals appeared throughout the spectrum indicating the appearance of another conformation(s). This was most clearly seen in the amide resonance for Ac,c. A new signal at 8.78 p.p.m. appeared beside the original at 8.72 p.p.m., which did not change in position. At 328 K the two resonances remained separate, showing that the two conformations were not rapidly equilibrating.

Further studies are underway to better define this structure.

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Table 1. N.m.r. data

<table>
<thead>
<tr>
<th>Residue</th>
<th>$\sigma$-CH</th>
<th>NH</th>
<th>$J_{SN}$</th>
<th>$d_{NN}$</th>
<th>$d_{NN}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\sigma$ &lt; Glu</td>
<td>3.95</td>
<td>7.69</td>
<td>0.00</td>
<td>5.00</td>
<td>-</td>
</tr>
<tr>
<td>Trp</td>
<td>4.66</td>
<td>8.11</td>
<td>0.64</td>
<td>5.43</td>
<td>-3.0%</td>
</tr>
<tr>
<td>Ala</td>
<td>4.43</td>
<td>8.43</td>
<td>6.92</td>
<td>7.23</td>
<td>-2.0%</td>
</tr>
<tr>
<td>Val</td>
<td>3.81</td>
<td>7.98</td>
<td>5.86</td>
<td>6.03</td>
<td>-2.0%</td>
</tr>
<tr>
<td>Ac,c</td>
<td>-</td>
<td>8.73</td>
<td>-</td>
<td>4.67</td>
<td>-</td>
</tr>
<tr>
<td>$\sigma$-Phe</td>
<td>4.33</td>
<td>8.73</td>
<td>6.53</td>
<td>7.23</td>
<td>-3.0%</td>
</tr>
<tr>
<td>Leu</td>
<td>4.01</td>
<td>8.02</td>
<td>7.28</td>
<td>3.17</td>
<td>-</td>
</tr>
<tr>
<td>Met</td>
<td>4.18</td>
<td>7.79</td>
<td>8.27</td>
<td>3.33</td>
<td>-</td>
</tr>
</tbody>
</table>

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Effects of the pyridinamines octenidine and pirtenidine on yeast mitochondrial function

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The drugs octenidine hydrochloride [N,N'-4,10 decanediyldi-1/H/pyridinyl-4-ylidene]bis[-octanamide/dihydrochloride] and pirtenidine hydrochloride [N-4-octyl-4/H]-pyridinylidene]octanamide monohydrochloride are new drugs developed by Sterling Winthrop Research Institute as potential antimicrobial/antiplaque agents. The drugs (kindly provided by Sterling-Winthrop) have been shown to possess antifungal as well as antibacterial activity [1, 2]. We have investigated the effects of these drugs against mitochondrial function in the yeast Saccharomyces cerevisiae.

An effect of the drugs on yeast mitochondrial function was indicated by the differences in their minimal inhibitory concentrations (MICs) against S. cerevisiae N.C.Y.C. 239 growing on minimal media containing fermentable or non-fermentable carbon sources. With octenidine the MIC using glucose (fermentable) was 5 μg/ml and using sodium lactate (non-fermentable) was 1 μg/ml. With pirtenidine the MICs were respectively 20 and 4 μg/ml. MIC values were determined according to the broth dilution procedure of Shadomy & Espinel-Ingroff [3]. The greater sensitivity of the yeast to the drugs on the non-fermentable carbon source implies an effect on the assembly or functioning of the mitochondrial oxidative phosphorylation system.

Both octenidine and pirtenidine inhibit oxygen uptake in exponentially growing cultures of S. cerevisiae N.C.Y.C. 239 (measured with a Clark oxygen electrode using the procedure of Morgan & Whittaker [4]). Octenidine hydrochloride (0.3 μg/ml) and pirtenidine hydrochloride (2 μg/ml) reduced the control oxygen uptake rate of 1.8×10⁻⁷ pmol O₂/h per cell by 91% and 47%, respectively.

The effects of the drugs on the synthesis of mitochondrial cytochromes was examined using oxidized-reduced difference spectra in the visible light range. Octenidine hydrochloride (0.3 μg/ml) brought about a reduction in the total cytochrome content of a culture grown to late exponential phase and a diminution in the levels of cytochromes aa₃ and 1990
Induction of apoptosis (programmed cell death) in tumour cell lines by widely diverging stimuli

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Introduction

Ultrastructural studies to date suggest that most, if not all, cell death in higher animals may be categorized into two distinct morphological patterns [1]. The most widely recognized is characterized by marked swelling of mitochondria followed by dissolution of internal and plasma membranes, typically termed necrosis [2]. The second pattern is characterized by condensation of the cell with maintenance of organelle integrity and the formation of surface protuberances that separate as membrane-bounded vesicles. This process was originally called shrinkage necrosis but was subsequently renamed apoptosis [3]. Previous reports have identified a variety of agents which induce cell death either via apoptosis or necrosis. For example, glucocorticoid treatment of thymocytes [4], the effect of thyroxine in tadpole tail during metamorphosis [5] and the withdrawal of adrenocorticotropic hormone from the adrenal gland [3] all induce cell death by

\[ \text{b relative to cytochrome c} \]

The latter effect would be expected if octenidine acted either as an inhibitor of mitochondrial protein synthesis or as a petite mutant. In contrast, pirtenidine showed no evidence of an effect on cytochrome synthesis.

The possibility that octenidine might act to induce the formation of petite mutants in \textit{S. cerevisiae} was investigated. \textit{Petite} mutants have suffered loss or gross deletion of their mitochondrial DNA [5] and are consequently incapable of mitochondrial protein synthesis. Fig. 1(a) shows that octenidine does increase the level of \textit{petite} mutants in a culture in a dose-dependent fashion. The level of \textit{petite} mutants observed, however, was insufficient to account for the diminution of respiration induced by this drug. The potent \textit{peteie} mutagen ethidium bromide gives 100% \textit{petite} mutants after 24 h incubation at 10 \( \mu \text{g/ml} \). Rather surprisingly octenidine diminishes the \textit{petite} mutagenic effect of ethidium bromide in a dose-dependent manner (Fig. 1a), so much so that, at an octenidine concentration of 0.5 \( \mu \text{g/ml} \) the mutant level achieved is hardly affected by the presence or absence of ethidium bromide. Fig. 1(b) shows that octenidine, added at various times during the course of ethidium bromide mutagenesis, appeared to cause immediate inhibition of mutagenesis. In contrast to these observations, pirtenidine neither induced \textit{petite} mutation nor antagonized ethidium bromide induction of \textit{petite} mutants.

It seems likely from these observations that pirtenidine has a direct effect on the mitochondrial electron transport system. It is probable that octenidine also shows this effect. In addition, possibly because of its bifunctional nature, octenidine interferes with mitochondrial assembly causing \textit{petite} mutation and diminished cytochrome synthesis. It is possible that the inhibitory effect of octenidine on ethidium bromide mutagenesis might relate to the requirement for ATP for efficient mutagenesis by ethidium bromide [7].

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