THE MODE OF ANTIBACTERIAL ACTION OF SOME 'MASKED' FORMALDEHYDE COMPOUNDS

Michael J. GIDLEY, Jermy K. M. SANDERS*, Evelyn R. MYERS† and Michael C. ALLWOOD†

University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW and †*Regional Research and Development Laboratory, Central Pharmacy, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2QQ, England*

Received 20 March 1981

1. Introduction

Formaldehyde is well known as a powerful antibacterial agent but its irritancy and pungency have led to the development for clinical use of compounds containing 'masked' formaldehyde [1,2]. A promising recent example is taurolin, ((1) in fig.1) [3,4] which is also claimed to chemically inactivate bacterial endotoxins [5]. We propose here a molecular mechanism for the bactericidal action of taurolin based on the delivery of formaldehyde as a lethal methylene iminium ion, $R_2\overset{+}{N}{=}CH_2$. Such ions also account for the antibacterial activity of mixtures of amines or amino acids with sub-lethal concentrations of formaldehyde in general and may be relevant to the preservative properties of formaldehyde itself.

2. Materials and methods

Chemical reactions were followed by ^1H NMR spectroscopy (80 and 100 MHz). Bactericidal activities of mixed amine (35 mM) and formaldehyde (17 mM) solutions were tested against *Escherichia coli* (Manchester University bacterial culture collection, type 352) which was stored on tryptone soya agar, grown to stationary phase (18 h at 37°C) in tryptone soya broth, harvested by centrifugation, washed with distilled water (3 times) and the suspension adjusted to contain approx. 10^8 viable cells/ml (by absorbance at 500 nm). Approx. 10^7 viable cells were challenged by the test solution; after 6 min, 10% of the suspension was removed, serially diluted and plated out using the pour-plate method. Colonies of challenged and unchallenged bacteria were counted after 48 h incubation at 37°C. The percentage viability loss was determined using the amine alone as a control.

Taurolin and tauraltam ((1) and (3) in fig.1, resp.) were obtained from Geistlich Sons Ltd., Wolhusen, Switzerland.

3. Results and discussion

In an aqueous solution of taurolin the equilibrium shown in fig.1 is established [6]. This scheme implicated the non-isolable carbinolamine (2) as the major active species, the concentrations of tauraltam (3) and formaldehyde being insufficient to account for the observed bactericidal activity. To test this hypothesis other carbinolamines were generated in situ by mixing aqueous solutions of simple amines and formaldehyde: the results (fig.2a,b) may be summarised as follows:

(i) The aromatic amines imidazole (4) and pyrazole (5) readily formed carbinolamines but the resulting solutions were the least bactericidally active of those tested.

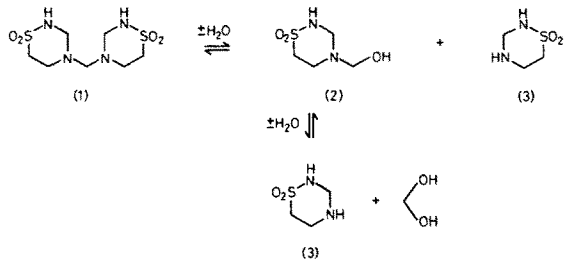

Fig.1. The state of taurolin (1) in aqueous solution. The distribution of the bridging methylene group is: taurolin 5%, carbinolamine (2) 53%, and formaldehyde 42%.

* To whom correspondence should be addressed

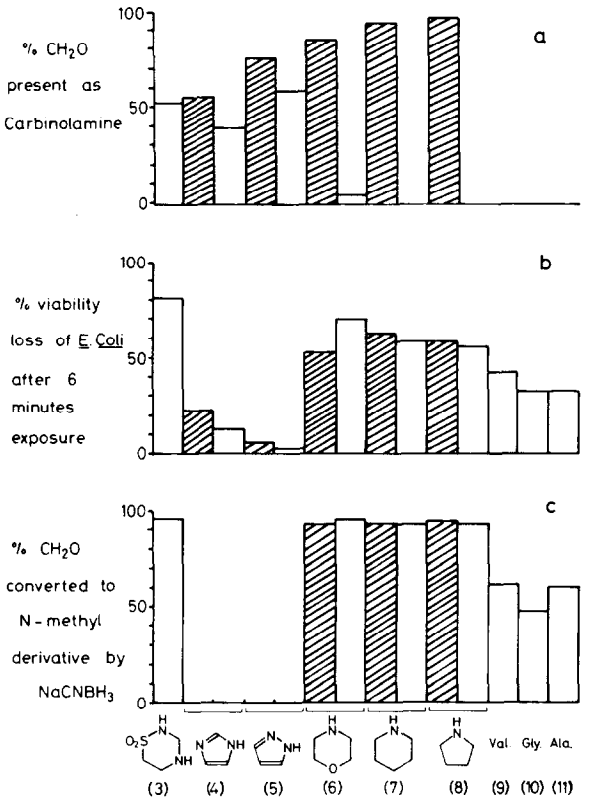

Fig.2. (b) The bactericidal activity of mixed amine (35 mM) and formaldehyde (17.5 mM) solutions compared with (a) the formation of carbinolamine and (c) the presence of iminium ions at neutral (□) and alkaline (▨) pH. The concentration of formaldehyde used caused no loss of viability on its own. Carbinolamine resonances appeared at 4.2–4.4 δ (5.5 δ for those derived from (4) and (5)) and *N*-methyl signals around 2.3–3.0 δ (3.7 δ for those derived from (4) and (5)).

(ii) The aliphatic amines piperidine (7) and pyrrolidine (8) formed carbinolamines with formaldehyde at high pH but not at neutral pH (when they are protonated). However, the antibacterial activity was essentially pH independent, and very much higher than that of formaldehyde alone.

(iii) Amino acids (9–11) gave antibacterial solutions with formaldehyde but showed no detectable carbinolamine formation.

These results may be readily explained if the active antibacterial agent is the methylene iminium ion, $R_2\overset{+}{N}{=}CH_2$, formed by dehydration of the carbinolamine. In the aromatic amines this process can only occur at the expense of aromaticity (fig.3a) and is therefore not favoured. In the aliphatic series, small

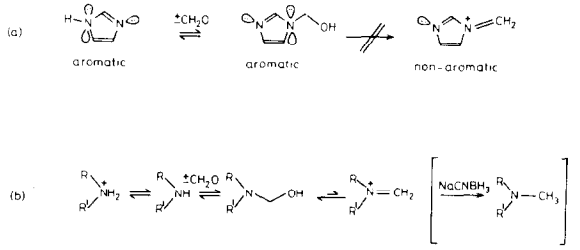

Fig.3. (a) The reaction of imidazole with formaldehyde. The lone pair which is part of the aromatic sextet is preserved upon carbinolamine formation but would have to be used in methylene iminium ion production. (b) Partial equilibrium in mixid amine/formaldehyde solutions (for a more complete description see [8]) and the trapping of iminium ions by sodium cyanoborohydride.

concentrations of such ions are likely even in the apparent absence of carbinolamine. Iminium ions would be readily attacked by water (to regenerate the carbinolamine), by amines (to give an aminal RR $NCH_2NR''R'''$ related to taurolin) or by nucleophiles in bacteria leading to their eventual loss of viability.

The presence of methylene iminium ions was tested for by the addition of sodium cyanoborohydride ($NaCNBH_3$) which traps the ion as the correspondingly *N*-methyl compound whilst only slowly reducing formaldehyde to methanol (fig.3b). The reaction was followed by ^{1}H NMR spectroscopy. The results (fig.2c) show that the bactericidally more active solutions were almost completely converted to the *N*-methyl derivative; however, solutions containing imidazole or pyrazole were unchanged (except for the slow reduction of formaldehyde to methanol) thus showing the expected reluctant formation of the corresponding iminium ion (fig.3a). The essentially irreversible hydride reaction removes iminium ions from the equilibrium and drives the reaction towards the *N*-methyl compound even when no carbinolamine is detectable (fig.3b).

The correlation (fig.2b,c) between bacericidal activity and the ability of the solutions to be reduced by sodium cyanoborohydride provides strong evidence that these 'masked' formaldehyde solutions do in fact operate via methylene iminium ions, i.e. the ions are trapped in vitro by cyanoborohydride in a comparable manner to their in vivo trapping by nucleophilic species in bacteria.

The overall activity of taurolin is greater than that observed in other mixed secondary amine/formalde-

hyde solutions (taurolin being equivalent in aqueous solution to a 2:1 mixture of tauraltam and formaldehyde (fig.1)) due to the bactericidal properties of tauraltam. This activity has also been shown to arise from methylene iminium ion production in the $N–CH_2–N$ portion of the molecule:

(i) Taurinamide ($H_2NCH_2CH_2SO_2NH_2$) is non-bactericidal.

(ii) Addition of ^{13}C-labelled formaldehyde to an aqueous solution of tauraltam showed (by ^{13}C–NMR) incorporation of the label into the $N–CH_2–N$ position.

This indicates that ring opening and reclosing, presumably via an intermediate methylene iminium ion, is an easy reaction, although the equilibrium is overwhelmingly in favour of the cyclic compound. Taurolin therefore exerts its antibacterial action by providing more than one potential methylene iminium ion per molecule.

In conclusion, we have shown that the bactericidal activity of formaldehyde can be substantially enhanced if it is delivered as a methylene iminium ion. These ions can be produced in mixed aliphatic amine and formaldehyde solutions as well as from 'masked' formaldehyde compounds.

Acknowledgement

We thank the SRC for studentships for M. J. G and E. R. M.

References

[1] Haler, D. (1963) Nature 198, 400–401.
[2] Block, B. P. (1967) Clin. Trials J. 4, 629–632.
[3] Browne, M. K., Mackenzie, M. and Doyle, P. J. (1978) Surg. Gynaecol. Obstet. 146, 721–724.
[4] Browne, M. K. (1979) Brit. Med. J. 2, 1004–1005.
[5] Pfirrman, R. W. and Leslie, G. B. (1979) J. Appl. Bacteriol. 46, 97–102.
[6] Myers, E., Allwood, M. C., Gidley, M. J. and Sanders, J. K. M. (1980) J. Appl. Bacteriol. 48, 89–96.
[7] Borch, R. F., Bernstein, M. D. and Durst, H. D. (1971) J. Am. Chem. Soc. 93, 2897–2904.
[8] Kallen, R. G., Viale, R. O. and Smith, L. K. (1972) J. Am. Chem. Soc. 94, 576–584.