Sulphonium Salt Formation from the Reaction of Methionine with some Aziridine Alkylating Agents

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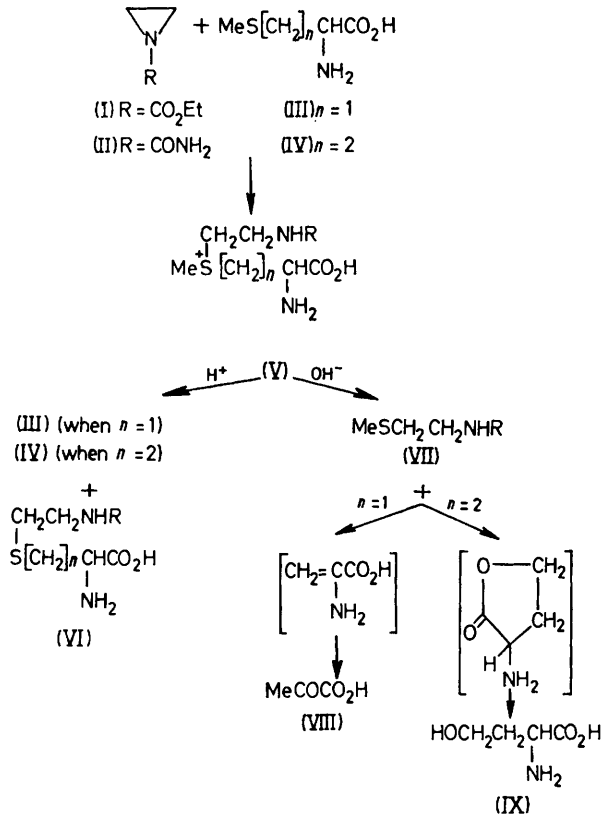
Summary Identification of degradation products confirms that the model aziridine compounds (I) and (II) alkylate methionine to produce stable sulphonium salts.

THE detailed mechanism of action[1] and metabolism[2] of aziridine-derived biological alkylating agents, which include many in current chemotherapeutic use, are unknown. Our studies of these compounds show that they react at pH 7·4

in dilute aqueous solution with methionine[3] to form sulphonium salts† of structure (V).



The aziridinyl ester (I) (10 mM) and methionine (IV) (5 mM) in 0·25M-phosphate buffer (1 l) for 16 h produce (V; R = CO_2Et, $n = 2$).‡ Since isolation of (V) proved difficult, it was characterised by its degradation products. Thus (V), extracted from Whatman 3 MM chromatograms, in constant boiling HCl over 24 h was converted into *S*-(2-aminoethyl)homocysteine[4] (VI; R = H, $n = 2$) as well as regenerating methionine (as in the acid decomposition of *S*-methylmethionine[5]). Base hydrolysis (5% NaOH at 90° for 16 h) of the reaction mixture containing (V), followed by ether extraction, gave 2-(methylthio)ethylamine (VII; R = H) isolated§ as a bis-$HgCl_2$ complex,[6] m.p. 147—149°. Similar treatment of the salt (V; R = $CONH_2$, $n = 2$) formed from the urea (II) and methionine produced, after chromatography of the ether extract on silica gel, 2-(methylthio)ethylurea (VII; R = $CONH_2$), m.p. 98—99°, identical with that prepared from (VII; R = H) and tetraisocyanatosilicon.[7] T.l.c. of the alkaline hydrolysates showed, in both cases, the presence of homoserine (IX) indicating that degradation of (V) accords with the intramolecular displacement of (VII) by the carboxylate anion.[5]

Acid hydrolysis of the corresponding *S*-methylcysteine-derived salt (V; R = CO_2Et, $n = 1$) produces *S*-(2-aminoethyl)cysteine (VI; R = H, $n = 1$).[4] Base elimination,[8] apart from yielding (VII), forms aminoacrylate, tautomeric with iminopyruvate which hydrolyses to pyruvic acid (VIII), isolated and identified as the 2,4-dinitrophenylhydrazone.

The sulphonium salts derived from [^{35}S]methionine and (I), (V; R = CO_2Et, $n = 2$), and (II), (V; R = $CONH_2$, $n = 2$), are quite stable [$t_{\frac{1}{2}}$ at pH 7·4 and 37° are 7 and 9 days respectively, decomposing to (VII; R = CO_2Et) and (VII; R = $CONH_2$), respectively and (IX)]. Examination of other aziridinyl alkylating agents, including triethylenemelamine (TEM) and bis-aziridinyl-*N*-methylaminophosphoramide (AM4), shows that analogous salts are formed with [^{35}S]methionine.† The production of similar complexes *in vivo* may be contributory towards their biological activity.

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† The specificity of sulphur alkylation at neutral pH was demonstrated by reaction of (I) and (II) with ethionine, methionine methyl ester, *N*-acetylmethionine, glycylmethionine, and *S*-methylcysteine; in all cases sulphonium salts were produced though no reaction occurred with the corresponding sulphoxides.

‡ The reaction is temperature and pH dependent; low yields at or above room temperature (2—10%) can be increased (to 60—70%) by freezing the reaction mixture. This will be discussed elsewhere.

§ All new compounds gave satisfactory analyses.

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[3] The potential aziridine-forming compound mustine [methylbis-(2-chloroethyl)amine] is reported to *S*-alkylate methionine though no products have been isolated (J. S. Fruton, W. S. Stein, and M. Bergmann, *J. Org. Chem.*, 1946, **11**, 559). Detection of homoserine as a degradation product suggests that aziridine itself alkylates methionyl units at pH 8·6 (W. A. Schroeder, J. R. Shelton, and B. Robberson, *Biochim. Biophys. Acta*, 1967, **147**, 590).
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[5] F. Ramirez, J. L. Finnan, and M. Carlson, *J. Org. Chem.*, 1973, **38**, 2597.
[6] Authentic 2-(methylthio)ethylamine (T. Wieland, E. F. Moller, and G. Dieckelmann, *Chem. Ber.*, 1952, **85**, 1035) formed an $HgCl_2$ complex, m.p. 152—153°, analysing for 1·6 $HgCl_2$.
[7] R. G. Neville and J. J. McGee, *Canad. J. Chem.*, 1963, **41**, 2123.
[8] J. J. Roberts and G. P. Warwick, *Biochem. Pharmacol.*, 1961, **6**, 205.