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STUDIES WITH ALKYLATING ESTERS—III

THE METABOLISM AND FATE OF METHYLENE DIMETHANESULPHONATE

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Abstract—Methylene dimethanesulphonate (MDS, I) has been prepared labelled both with ^{35}S and ^{14}C and its distribution determined in the mouse. It is metabolised by rats and mice to *N*-formyl cysteine (III) and *N,N'*-diformyl cystine (IV). Through hydrolysis to formaldehyde it is also incorporated into the methyl group of methionine. The metabolism of formaldehyde has been re-investigated, corrected and shown to be similar to that of MDS.

INVESTIGATIONS of the anti-tumour potential of methanesulphonic diesters

$$CH_3SO_2O.(CH_2)_n.OSO_2CH_3$$

demonstrated that certain homologues (n=2 to 9) were active and also showed marked suppressant activity on bone marrow.[1, 2] Later studies showed that the simplest member, methylene dimethanesulphonate (MDS, n=1), besides suppressing bone marrow[3, 4] produced unusual antifertility effects in male rodents[3] and was a powerful inhibitor of certain transplantable rat tumours.[3, 5] Its short half-life in solution (22 min at 37° in water)[3] has led to the speculation that it may be active through a metabolite.[6] The fate of MDS in the rat and mouse has been investigated in an attempt to clarify this point.

MATERIALS AND METHODS

^{14}C-Methylene dimethanesulphonate (I)

^{14}C-iodoform (1·82 g) was prepared by sodium hypochlorite oxidation of 1,3-^{14}C-acetone (1·0 mc, diluted to 302 mg with inactive material), and reduced to ^{14}C-methylene di-iodide by sodium arsenite.[7] The ^{14}C-methylene di-iodide (0·8 g) and silver methane sulphonate (1·2 g) were dissolved in anhydrous acetonitrile (15 ml) and heated in a sealed ampoule at 120° for 6 hr. The solution was filtered and the filtrate evaporated *in vacuo*, dissolved in chloroform (15 ml), filtered and chilled. ^{14}C-methylene dimethanesulphonate separated as colourless plates, recrystallised to constant specific activity from benzene and 40–60° petroleum ether. M.p. 76–78° (reported m.p. 76–78°),[8] 0·256 g, s.a. 88 μc/mM (17% radiochemical yield from acetone).

^{35}S-Methylene dimethanesulphonate

This was prepared from methylene di-iodide and ^{35}S-silver methanesulphonate (2 mc) in 63 per cent yield. S.a. 4 mc/mM.

Thiazolidine-4-carboxylic acid (II)

A solution of formaldehyde (40%, 1·5 ml) was added to L-cysteine hydrochloride (2 g) in water (4 ml). Addition of pyridine (2·5 ml) and ethanol (5 ml) gave a dense precipitate of thiazolidine-4-carboxylic acid (TCA), recrystallised from aqueous ethanol. M.p. 200–201° (reported m.p. 196–197°)[9], 1·1 g. Using ^{14}C-formaldehyde or ^{35}S-cysteine hydrochloride, both ^{14}C- and ^{35}S- labels could be incorporated.

N-*formyl cysteine* (III) *and* N,N′-*diformyl cystine* (IV)

These were prepared by the methods of Mackenzie and Harris[10] and Du Vigneaud, Dorfmann and Loring[11] respectively.

Chromatography

Ascending paper chromatograms (Whatmans No. 1 for qualitative and No. 17 for quantitative work) were developed in either solvent A (*N*-butanol:glacial acetic acid: water 4:2:1) or solvent B (phenol:water 4:1) and metabolites detected either by spray reagents (ninhydrin or platinic acid[12]) or by scanning on a Packard Radiochromatogram Scanner. For gas–liquid chromatography, desalted urine samples were dried over phosphorus pentoxide, methylated with diazomethane and the methyl esters chromatographed on a Varian Aerograph Autoprep 705. The column was 5 ft × 1/8 in. o.d. packed with QF-1 on AW-DMCS Chromosorb W (80–100 mesh) with nitrogen carrier gas flow rate of 65 ml/min.

Isolation and identification of metabolites

Animal maintenance, antifertility testing and the collection of urine have been described previously.[13] All compounds were administered intraperitoneally to rats and mice in aqueous solution except for MDS which was given in 40% dimethyl sulphoxide in water. TCA was neutralised and administered as its sodium salt.

(a) *Methanesulphonic acid.* This was identified by paper chromatography in both solvent systems and confirmed by GLC of its methyl ester against authentic methyl methanesulphonate (Aldrich Chemical Co.).

(b) *Methionine.* The area corresponding to methionine was eluted from No. 17 papers (Solvent B) with water and reduced *in vacuo*. Oxidation of the concentrate gave methionine sulphoxide, identified by the method of Neely.[14]

(c) N-*formyl cysteine.* This was identified by chromatography in both solvent systems and by GLC of its methyl ester (Table 2).

(d) N,N′-*diformyl cystine.* The amounts of this compound varied though identification was achieved by paper chromatography in both solvent systems. When eluted from quantitative chromatograms, *N*-formyl cysteine readily dimerised in air to *N,N′*-diformyl cystine.

Tissue distribution

Tissue samples were digested in perchloric acid and hydrogen peroxide and counted in an IDL Tritomat liquid scintillation counter with phosphor solutions as previously described.[13]

TABLE 1. DISTRIBUTION OF RADIOACTIVITY IN MOUSE TISSUES OF ^{35}S-MSA AND ^{35}S-MDS

Tissue	Hours after administration of ^{35}S-MSA			Hours after administration of ^{35}S-MDS		
	1	3	24	1	3	24
Bone*	0·61	0·22	—	5·6	1·5	—
Brain	0·15	0·07	0·01	0·41	0·67	—
Cauda epididymis	0·85	0·87	0·18	1·4	0·14	—
Fat	0·24	0·03	0·05	0·45	0·86	0·19
Heart	0·29	0·12	—	1·6	0·69	—
Large intestine†	0·34	0·12	0·01	0·02	0·86	0·12
Small intestine†	0·43	0·20	0·01	1·4	0·31	—
Kidney	3·6	2·6	—	0·94	0·56	—
Liver	0·31	0·36	—	0·16	0·25	—
Lung	0·81	0·12	—	1·9	0·56	—
Muscle‡	0·51	0·07	—	0·91	0·64	—
Skin	0·66	0·35	0·33	1·2	0·63	0·02
Spleen	0·61	—	—	4·3	1·7	—
Stomach‡	0·58	0·19	—	2·3	0·83	—
Testis	0·32	0·09	—	1·4	1·4	0·32
Whole blood	3·5	—	—	4·2	1·0	—
Plasma	8·3	0·40	—	2·1	0·4	—
Residue of animal	1·3	0·30	0·02	1·75	0·93	0·10

Results are expressed as per cent administered dose per g of wet tissue from an intraperitoneal dose of 0·22 mM/kg.
* Whole femur. † Include contents. ‡ Gastrocnemius.

TABLE 2. CHROMATOGRAPHY OF METHYLENE DIMETHANESULPHONATE AND FORMALDEHYDE METABOLITES*

Compound		Paper chromatography (R_f)		GLC†	
		Solvent A	Solvent B	95°	110°
MDS	I	— (0·82)	— (0·85)	—	—
MSA		0·22 (0·22)	0·40 (0·40)	2·0 (2·0)	1·2 (1·2)
Methionine		0·46 (0·47)	0·70 (0·70)	—	—
N-Formyl cysteine	III	0·32 (0·32)	0·52 (0·51)	3·0 (3·0)	1·7 (1·7)
N,N' Diformyl cystine	IV	0·20 (0·18)	0·30 (0·30)	—	—
Thiazolidine-4-carboxylic acid	II	— (0·40)	— (0·68)	— (4·2)	— (2·2)

* Mobilities of synthetic compounds are shown in parentheses.
† Retention times of the methyl esters (in minutes).

RESULTS AND DISCUSSION

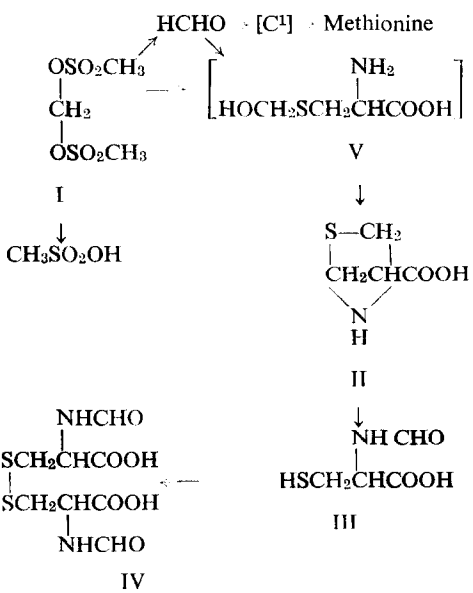
Alkylation of nucleophiles by methanesulphonic esters occurs with liberation of methanesulphonic acid (MSA); MDS readily hydrolyses yielding formaldehyde and MSA. Reports that MSA is excreted by rodents almost quantitatively and unchanged[15] were substantiated for the mouse by administration of ^{35}S-MSA. For ^{35}S-MDS, therefore, excretion of MSA should be an indication of the extent of hydrolysis and detoxification. Further, the comparative tissue label from ^{35}S-MSA and ^{35}S-MDS should be indicative of the distribution due to the ester itself. On this basis (Table 1), the distribution of MDS appears remarkably selective for certain tissues. Femur and spleen 1 hr from injection possess a significantly greater level of radioactivity than other tissues which remain appreciable 3 hr after dosing. Comparison with the

distribution of radioactivity from ^{35}S-MSA suggests that a major proportion of label from ^{35}S-MDS-treated animals must have reached tissues as intact drug. Considering its short half-life, the distribution of MDS must have an important bearing on its pharmacological action if the effects result from reaction of the intact ester at particular tissue sites. When distribution is rapid, as appears to be for MDS, then considerations of half-lives may be of lesser importance in determining biological activity.

The metabolism of MDS may be expected to resemble that of the halogenated hydrocarbons since both methylene dibromide and bromochloromethane, when incubated with rat tissues also yield formaldehyde.[16] In the rat, Neely[14] reported that ^{14}C-formaldehyde (i.p.) produced methionine and serine whilst another metabolite was inferred to be thiazolidine-4-carboxylic acid (TCA, II).

We have re-investigated the metabolism of formaldehyde in both rat and mouse and, while confirming major incorporation into the two amino acids, TCA could not be detected. The inferred metabolite was isolated and identified as *N*-formyl cysteine (III) together with traces of *N,N'*-diformyl cystine (IV). Three urinary metabolites from rats and mice receiving ^{14}C-MDS have also been identified as methionine, *N*-formyl cysteine and *N,N'*-diformyl cystine. Serine was not detected, possibly due to the low specific activity of the labelled ester used. Not unexpectedly, the metabolism of MDS and formaldehyde show close resemblance.

As TCA is formed by reaction of either MDS or formaldehyde with cysteine, its metabolism was investigated in the event that it was a transient intermediate in the metabolism of both compounds. Although excreted in part unchanged by the rat, its main metabolite is *N*-formyl cysteine. No labelled methionine could be detected in urine indicating that conversion of TCA to this amino acid does not occur *in vivo*, supporting previous findings that TCA is not a dietary substitute in methionine deficient rats.[17] ^{14}C-TCA was not extensively degraded to carbon dioxide in the rat



(18 per cent expired in 24 hr from administration) indicating that the 80 per cent conversion of formaldehyde to carbon dioxide[14] occurred mainly by a route not involving TCA or *N*-formyl cysteine. At similar dose levels (15 mg/kg/i.p.), both MDS and formaldehyde were degraded by the rat to carbon dioxide (45 and 38 per cent respectively in 24 hr) with low urinary excretion of radioactive label (8 and 7 per cent). A high proportion of label from both compounds must therefore pass into a one-carbon pool.

Formaldehyde reacts with the thiol group of cysteine to yield S-methylol cysteine (V) which cyclises to produce TCA.[9] If MDS alkylates cysteine units *in vivo* in a manner analogous to the homologue ethylene dimethanesulphonate,[13] S-methylol cysteine would be formed as from formaldehyde (Fig. 1). Any distinction, therefore, between the *in vivo* action of both compounds on chemical grounds is difficult to establish.

S,S′-methylene-bis(cysteine-*N*-acetate) is formed only to a small extent in equimolar mixtures of cysteine-*N*-acetate and methylene dibromide, and does not appear to be formed at all in comparable reaction with either MDS or formaldehyde. Hence MDS, like formaldehyde appears to act in a monofunctional manner as would be expected on a chemical basis. Perhaps the wide tissue distribution of ^{35}S-MDS (Table 1) could represent an efficient form of 'formaldehyde' distribution, although the latter has no effect on male rat fertility. Methylene diacetate, like MDS, hydrolyses to formaldehyde but shows little indication of the biological effect at 500 mg/kg whereas MDS produces characteristic sterilant effects at one dose of 15 mg/kg[3] and even at 2 mg/kg daily by mouth.[18]

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