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STUDIES WITH ALKYLATING ESTERS—IV

THE METABOLISM OF PROPANE-1,3-DIMETHANESULPHONATE AND ITS RELEVANCE TO THE MODE OF ACTION OF MYLERAN

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Abstract—The metabolism of [^{14}C] and [^{35}S]propane-1,3-dimethanesulphonate (PDS) has been examined in the rat and the mouse. Both species excrete PDS unchanged together with methanesulphonic acid and the mercapturic acid S-(3-hydroxypropyl) cysteine-*N*-acetate. In addition, the mouse excretes propane-1,3-diol and the rat S-(3-hydroxypropyl) cysteine.

Both PDS and its homologue Myleran have comparable distributions in mouse tissues and share similar antispermatogenic and haemopoietic activities in rodents. The relevance of the "sulphur-stripping" ability of Myleran, to which its biological action has been attributed, is discussed in relation to these findings.

THE VARIATION in potency of the homologous series of methanesulphonoxyalkane diesters (I) as neutrophil suppressants has been explained by considerations of their chemical reactivity and physical properties.[1,2] However, recent investigations[3] into the anti-spermatogenic and anti-fertility properties of the lower homologues (I, $n=1$–4) suggest that there are fundamental differences between their modes of action rather than a more general effect reflecting a gradation of chemical reactivities or ability to penetrate cell membranes.

$$CH_3SO_2O—(CH_2)_n—OSO_2CH_3$$

I

Methylene dimethanesulphonate (I, $n=1$) produces spermatogonial destruction, rather than inhibition, and it has been suggested that this could occur by an action involving the intracellular release of formaldehyde.[4] Ethylene dimethanesulphonate (I, $n=2$) appears to exert its effect in the male rat by an anti-androgenic action[3] unaccompanied by the characteristic spermatogonial inhibition produced by Myleran (I, $n=4$). The inhibitory effect on spermatogonia of propane-1,3-dimethanesulphonate (PDS, I, $n=3$), together with its anti-fertility activity, indicates that this compound acts similarly to Myleran.

In considering the mode of action of Myleran, emphasis has been placed on its "sulphur-stripping" ability, that is the excission of sulphur from, for example, a peptide chain, leaving an abnormal amino acid or a reactive carbonium ion at the site of nucleophilic attack.[5] As PDS and Myleran effect rodent testes to similar extents, such reactions may also be important in the action of PDS. On this basis the

fate and reactivity of PDS have been examined in an attempt to elucidate whether such effects can be correlated with *in vivo* activity.

MATERIALS AND METHODS

Preparative methods

1,3-[^{14}C]*propane*-1,3-*dimethanesulphonate*. 1,3-[^{14}C]diethyl malonate (0·5 mc), diluted to 700 mg with inactive material in dry ether (30 ml), was added over 10 m to a stirred suspension of lithium aluminium hydride (1 g) in dry ether (150 ml) and refluxed for 2 hr. The cooled mixture was decomposed by dropwise addition of water (2 ml) and 2 N HCl (30 ml), ether extracted for 4 days and the dried ether extract reduced *in vacuo* to a brown oil. This crude 1,3-[^{14}C]propane-1,3-diol was dissolved in anhydrous pyridine (8 ml) and methanesulphonyl chloride (1·2 g) added dropwise at −5° over 2 hr. The solution was kept at 0° overnight, dissolved in water (10 ml), carefully acidified at −10° with 10 N HCl and extracted with chloroform (10 × 10 ml). The dried chloroform extract was reduced *in vacuo* to 10 ml and 60–80° petroleum ether added until cloudy. After 24 hr at 0°, 1,3-[^{14}C]propane-1,3-dimethanesulphonate separated as fine needles. Recrystallization from chloroform and 60–80° petroleum ether to constant specific activity gave 385 mg (37 per cent), 114 μc/mM. Melting point 45·5–46° (reported[2] 46·5°).

[^{35}S]*PDS and* [^{35}S]*Myleran*. These were prepared from 1,3-dibromobutane and 1,4-dibromobutane respectively, and [^{35}S]silver methanesulphonate by the method of Emmons and Ferris.[6] Both compounds had specific activity 4 mc/mM.

1,3-[^{14}C]*propane*-1,3-*diol*. This was prepared by base hydrolysis of 1,3-[^{14}C]PDS.

S-(3-*hydroxypropyl*) *cysteine*. This was synthesized from L-cysteine HCl and 3-bromopropanol analogous to the synthesis of S-(2-hydroxyethyl) cysteine by Nachtomi *et al.*[7] Precipitation from water with acetone gave the compound as a white powder (37 per cent), melting point 211° (decomp.). (Found: C, 43·42; H, 7·96; S, 19·26%. $C_6H_{13}NSO_3$ requires: C, 43·61; H, 7·93; S, 19·40%.) Peroxide oxidation[8] gave *S*-(3-*hydroxypropyl*) *cysteine*-*S*-*oxide*, precipitated from water with methanol as a white powder in 45 per cent yield, melting point 149° (decomp.). (Found: C, 37·56; H, 6·43; N, 7·13; S, 16·53%. $C_6H_{13}NSO_4$ requires: C, 36·93; H, 6·71; N, 7·18; S, 16·43%.)

S-(3-*hydroxypropyl*) *cysteine*-N-*acetate*. Acetylation of S-(3-hydroxypropyl) cysteine with acetic anhydride at room temperature for 2 days gave the mercapturic acid as a brown oil, homogenous by both paper and thin-layer chromatography. It was characterised as the dicyclohexylamine salt, white crystals from acetone and methanol, melting point 179–181° (decomp.). (Found: C, 59·38; H, 9·20; N, 6·79; S, 7·96%. $C_{20}H_{38}N_2SO_4$ requires: C, 59·68; H, 9·51; N, 6·96; S, 7·68%.)

Chromatography

Ascending chromatography was performed on Whatman no. 1 and no. 17 papers in solvent 1 (*n*-butanol–glacial acetic acid–water, 4:2:1). Thin layer chromatograms were of silica gel G (250 μ), activated at 110° prior to use, and developed either in solvent 1 or solvent 2 (100% chloroform). Analysis of the chromatograms was carried out either on a BTL paper strip scanner or a Packard model 7201 Radiochromatogram Scanner. R_f values are given in Table 1.

TABLE 1. R_f VALUES FOR METABOLITES OF PDS*

	Paper†		Thin-layer‡	
PDS	—	(0·85)	—	(0·57)
Methanesulphonic acid	0·22	(0·22)		
S-(3-hydroxypropyl) cysteine	0·42	(0·42)	0·31	(0·31)
S-(3-hydroxypropyl) cysteine-S-oxide	—	(0·19)	—	(0·31)
S-(3-hydroxypropyl) cysteine-*N*-acetate	0·74	(0·75)	0·48	(0·46)
Propane-1, 3-diol	0·36	(0·37)	0·31	(0·31)

* Figures in parentheses refer to authentic samples.
† Whatman No. 1 paper, solvent 1.
‡ Silica gel G plates, solvent 1.

Animals and administration of compounds

Rats were male Wistars (about 250 g) and mice were males of an RF strain (about 25 g). Male Dutch rabbits weighed 1·8–2·3 kg. Animals were maintained in metabolic cages, with access to water and pellet food *ad lib.*, allowing separate collection of urine, faeces and, when required, expired gases. Myleran and PDS were administered in 40% dimethyl sulphoxide in water and propane-1,3-diol as an aqueous solution.

Radioactive assay

Techniques for the measurement of radioactivity in expired gases, urine, faeces, blood and tissues have been previously described.[8,9]

Isolation and identification of metabolites

Urine was chromatographed on Whatman no. 17 papers and the radioactive areas eluted with water.

(a) S-(3-*hydroxypropyl*) *cysteine.* Oxidation or acetylation changed the chromatographic mobility of this metabolite similar to those of authentic compounds. It was further characterised by gas chromatography of the methyl ester with a Varian Aerograph Autoprep 705. On a 5ft × $\frac{1}{8}$ in. column of 5% SE-30 on 80–100 mesh AW DMCS Chromosorb W, S-(3-hydroxypropyl) cysteine methyl ester had a retention time of 1·4 min (110° column, nitrogen carrier gas flow rate 63 ml/min).

(b) S-(3-*hydroxypropyl*) *cysteine*-N-*acetate.* Acid hydrolysis (5 N HCl at 95° for 1 hr) or treatment with acylase (pH 7·4 at 37° for 3 hr) converted the mercapturic acid to S-(3-hydroxypropyl) cysteine which was identified as above.

(c) *Propane*-1,3-*diol.* The eluates corresponding to this metabolite were each taken to dryness *in vacuo* and inactive diol (about 25–30 mg) added. Reaction of the mixture with phenyl *iso*-cyanate gave propane-1,3-diol *bis*-phenylurethane, recrystallised from aqueous methanol, melting point 140–141°. This was chromatographed on silica gel G plates in solvent 2 and scanned for activity. In this solvent system the *bis*-phenylurethane had an R_f of 0·48.

(d) *Methanesulphonic acid.* This was identified both by paper chromatography and by gas chromatography of the methyl ester as previously described.[4]

In vitro *reactivity of PDS*

(a) PDS and cysteine ethyl ester HCl were treated with sodium ethoxide in ethanol according to the method of Parham and Wilbur.[10] The reaction mixture was concentrated *in vacuo*, filtered, dissolved in water and saponified with 5 N sodium hydroxide. Chromatography showed the presence of S-(3-hydroxypropyl) cysteine and S,S'-*bis*-(cysteinyl) propane.[11] The latter was also isolated[10] as the ethyl ester HCl, colourless plates from ethanol and ether (45 per cent yield), melting point 158–160°. (Found: C, 37·90; H, 6·66; N, 7·00; S, 15·25%; $C_{13}H_{28}N_2S_2O_4Cl_2$ requires: C, 37·96; H, 6·86; N, 6·81; S, 15·59%.)

(b) Hydrolysis in phosphate buffer at 37° or by refluxing in 50% aqueous acetone[2] produced only propane-1,3-diol, isolated as the *bis*-phenylurethane derivative in 90 per cent yield.

RESULTS AND DISCUSSION

During the 24 hr following intraperitoneal administration of [^{35}S]PDS (50 mg/kg), rats and mice excreted similar amounts of unchanged compound (35 and 30 per cent respectively). Over the following 3 days a further 10 per cent of the dose was excreted in the urine, the only metabolite at all times being [^{35}S]methanesulphonic acid (MSA, II) formed either by hydrolysis of the ester or during its detoxification. Administration of [^{35}S]MSA (50 mg/kg) produced only low levels of tissue radioactivity in the mouse,

TABLE 2. COMPARATIVE DISTRIBUTION OF RADIOACTIVE LABEL IN MOUSE TISSUES AFTER ADMINISTRATION OF [^{35}S]PDS AND [^{35}S]MYLERAN

Tissue	Hours after administration					
	1		3		24	
Bone*	7·4	(3·1)	3·3	(2·0)	0·1	(0·1)
Brain	0·9	(2·0)	0·5	(1·3)	—	—
Fat	0·5	(0·9)	0·4	(0·3)	—	—
Heart	2·0	(2·2)	1·1	(1·3)	0·1	(0·1)
Large intestine†	1·9	(1·9)	1·1	(1·4)	0·1	—
Small intestine†	1·5	(2·1)	1·2	(1·3)	—	—
Kidney	1·6	(2·4)	1·3	(1·6)	0·1	(0·1)
Liver	1·1	(1·7)	0·3	(1·0)	0·2	—
Lung	2·3	(3·3)	1·5	(1·7)	—	—
Muscle‡	1·1	(1·4)	0·7	(0·7)	0·1	—
Skin	0·8	(1·4)	0·6	(0·7)	0·1	—
Spleen	2·8	(4·4)	2·4	(2·1)	0·8	(0·3)
Stomach†	3·9	(4·7)	2·3	(2·9)	0·4	(0·2)
Testis	2·1	(1·6)	1·2	(1·4)	0·1	(0·1)
Cauda epididymis	0·9	(2·4)	1·2	(1·6)	0·3	(0·1)
Plasma	3·2	(1·9)	0·7	(0·7)	—	—
Whole blood	5·1	(2·8)	1·1	(0·8)	—	—
Residue of animal	2·2	(2·7)	1·3	(0·8)	0·1	(0·1)

Results are expressed as percentage administered dose/g wet weight of tissue after intraperitoneal doses of PDS (50 mg/kg) and Myleran (35 mg/kg). Values for Myleran are in parentheses.

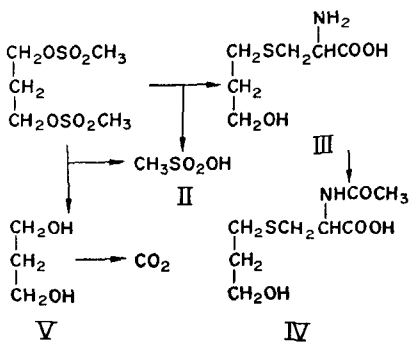
* Whole femur.

† Including contents.

‡ Gastrocnemius.

excretion being almost quantitative within 6 hr.[4] Consequently tissue radioactivity of animals dosed with [^{35}S]PDS is primarily due to the ester itself, the results (Table 2) reflecting its similar distribution to that of [^{35}S]Myleran. The more rapid excretion of radioactive label from Myleran-dosed animals probably results from its shorter half-life[12] giving lower tissue levels 24 hr from administration than those from PDS-dosed animals.

Rats given [^{14}C]PDS (50 mg/kg, intraperitoneally) excreted S-(3-hydroxypropyl) cysteine (III) and S-(3-hydroxypropyl) cysteine-*N*-acetate (IV) as urinary metabolites. Mice, however, apart from producing a small amount of the mercapturic acid (IV), gave the hydrolysis product of PDS, propane-1,3-diol (V), so that PDS is the first member of the series of dimethanesulphonates (I) in which a species difference has been shown to occur (Fig. 1). 1,3-[^{14}C]propane-1,3-diol is extensively degraded



by rats to carbon dioxide (40 per cent in 24 hr after intraperitoneal administration of 17 mg/kg) whereas a similar molar dose of [^{14}C]PDS (50 mg/kg) produces only 3 per cent of the dose as carbon dioxide. Corresponding values for the mouse are 53 and 15 per cent. These results suggest that the carbon dioxide produced from PDS comes mainly from extensive degradation of the diol.* Production of propane-1,3-diol from PDS appears to be a specific function of mouse tissues as the half-life of PDS, both in plasma and physiological saline at 37°, is approximately 50 hr.

In vitro results[11,14] have shown the relative ease with which Myleran reacts with a number of thiol-containing compounds to produce tetrahydrothiophene and we have applied the reaction[10] with cysteine ethyl ester to PDS. PDS reacts readily under basic conditions though the cyclic excision compound, propylene sulphide, could not be detected. No 2-methyl-2,4-carbethoxy thiazolidine, indicative of such a reaction,[10] could be isolated; the only detectable products in the hydrolysed reaction mixture being S-(3-hydroxypropyl) cysteine and 1,3-*bis*-(S-cysteinyl) propane. Hence PDS reacts under these conditions as does Myleran though there is no evidence of the S-alkyl-cysteine intermediate cyclising and "sulphur-stripping".

PDS and Myleran are both detoxified by conjugation with cysteinyl units, the

* Although it has been reported[13] that propane-1,3-diol is not excreted unchanged from oral administration to the rabbit, we have shown that it is detectable in rabbit urine from doses ranging from 0·6 mg to 1 g/kg. Oral administration of [^{14}C]PDS (5 mg/kg) produced only the cysteine-conjugated metabolites (III) and (IV) similar to the rat.

product from Myleran readily cyclising to give tetrahydrothiophene[5,11] which is excreted mainly as 3-hydroxy tetrahydrothiophene sulphone.[5] The product from PDS, however, does not cyclise and appears as the conjugated urinary metabolite. As "sulphur-stripping" reactions do not occur *in vitro* or *in vivo* with PDS, yet its distribution and pharmacological effects[2,3] closely resemble Myleran, we suggest that such a reaction may not be important[14] to the biological activity of either compound.

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