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GAS CHROMATOGRAPHIC AND GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC CHARACTERISATION OF SOME THIOSULPHONATES AND POLYMETHYLENE DIMETHANE THIOSULPHONATES

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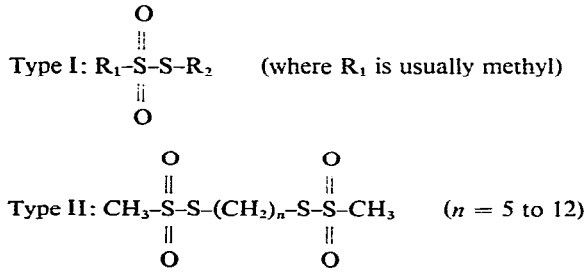
SUMMARY

The characterisation by gas chromatography (GC) and GC–mass spectrometry (MS) of thiosulphonates of the types $R_1 \cdot SO_2S \cdot R_2$ (type I) and $CH_3 \cdot SO_2S \cdot (CH_2)_n S \cdot SO_2CH_3$ (type II) is described. Type I thiosulphonates showed suitable GC–MS properties as the intact molecules. Type II could be characterised by GC–MS only after reduction to the dithiol; direct probe spectra were necessary to characterise all the intact type II compounds by MS.

INTRODUCTION

Thiosulphonates (sulphonate thioesters) have recently found application as thiol reagents in enzyme mechanism studies[1–3] and polymethylene dimethane thiosulphonates similarly are useful in cross-linking studies[4]. The thiosulphonates under discussion can be classified into two types:



Because these compounds are potentially useful protein thiol reagents, we required a convenient method for characterisation and assessment of purity. The use of gas chromatography–mass spectrometry (GC–MS) was suggested by a brief

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report[5] that several dialkyl thiosulphonates had been gas-chromatographed on Triton X-305 and that molecular ions were present in their mass spectra (direct probe). Similarly, the purification by preparative GC of the analogous polymethylene glycol dimethane sulphonates [CH_3–SO_2–O–$(CH_2)_n$–OSO_2–CH_3, n = 2 to 9] on Carbowax 20M has been reported[6]. The mass spectra of these latter types of sulphonates[6] and some of the type I thiosulphonates have been well documented[5,7].

In this paper we report on the characterisation by GC, MS and GC–MS of some type I and type II thiosulphonates and briefly describe the mass spectra of three new type I thiosulphonates and also the mass spectra of the type II series.

EXPERIMENTAL

The synthesis of and enzymic studies utilising the thiosulphonates will be published elsewhere. For GC and GC–MS, samples were dissolved in dry acetone at 5 mg/ml; fresh solutions were prepared for each analysis.

Reduction of type II compounds

Sodium borohydride, 10 μl of 10 mg/ml solution in methanol was added to 100 μl of 10 mg/ml methanol solutions of type II thiosulphonates. When the evolution of hydrogen had ceased (1 to 2 min), aliquots were assayed by GC or GC–MS. This procedure gave type II reduced (IIR) series.

Gas chromatography

Retention data were measured on a 2.1 m × 4 mm I.D. glass column packed with 3% OV-1 on Diatomite CQ, 100–120 mesh, fitted to a Pye 104 chromatograph with flame ionisation detector. The flow-rate of the nitrogen carrier gas was 40 ml/min, type I series were run at 100° column, injector 130°; type II at column temperatures between 220° and 290° (Table I), injector 280°; and type IIR at 130° to 240° (Table I), injector 150° to 250°.

MS and combined GC–MS

Spectra were obtained on a Kratos–AEI MS 30, to which a Pye 104 chromatograph was linked via a membrane separator. All GC–MS samples were run on a 1.5-m glass column packed with the OV-1 phase described above. The flow-rate of the helium carrier gas was 40 ml/min, injector 150°, column temperature 110° to 130° (to give retention times of 3 to 4 min) for type I series, and 150° to 225° for type IIR. MS conditions were source 130°, separator 180°, 24 eV ionising voltage (EI), 300 μA current. Confirmatory analyses of type I liquids were done by direct probe (gas) at 75°. Type II compounds could not all be run directly by GC–MS so the spectra of the "intact" compounds of this series were obtained by direct probe (solid) at temperatures between 120° and 250°. Spectra of probe samples were taken at 70 eV. All spectra were low resolution (1000).

RESULTS

Gas chromatography

The retention times of type I, type II and type IIR compounds are summarised

TABLE I

RETENTION TIMES ON OV-1 OF ACYL THIOSULPHONATES, POLYMETHYLENE DIMETHANE THIOSULPHONATES AND DITHIOL PRODUCTS FROM REDUCTION OF POLYMETHYLENE THIOSULPHONATES

Compound	R_1	R_2	*n*	*Temperature (°C)*	*Retention time (min)*
Type I: R_1–SO_2S–R_2					
A	CH_3	CH_3		100°	3.0
B	CH_3	CH_2CH_3		100°	4.5
E	CH_3	CH_2CH_2F		100°	5.6
F	CH_3	CH_2CF_3		100°	1.8
C	CH_3	$CH(CH_3)_2$		100°	5.9
D	$(CH_3)_2CH$	$CH(CH_3)_2$		100°	9.4
Type II: $CH_3SO_2S(CH_2)_nSSO_2CH_3$					
			5	220°	9.3
			6	240°	6.4
			8	260°	5.9
			10	290°	3.75 (some decomposition)
			12	(decomposes, does not run)	
Type IIR: $HS(CH_2)_nSH$ *and* $S(CH_2)_nS$					
Dithiol			5	130°	2.1
Disulphide			5	130°	2.7
Dithiol			6	140°	2.55
Disulphide			6	140°	3.4
Disulphide			8	170°	6.4
Disulphide			10	170°	7.25
Disulphide			12	240°	2.1

in Table I. The longer chain homologues of the type II series showed a progressive decomposition such that for $n = 12$ the parent compound did not elute. Some decomposition is evident from the appearance in all type II samples of one or two rapidly eluting peaks (Fig. 1). GC–MS analysis showed that these peaks were the dithiol and

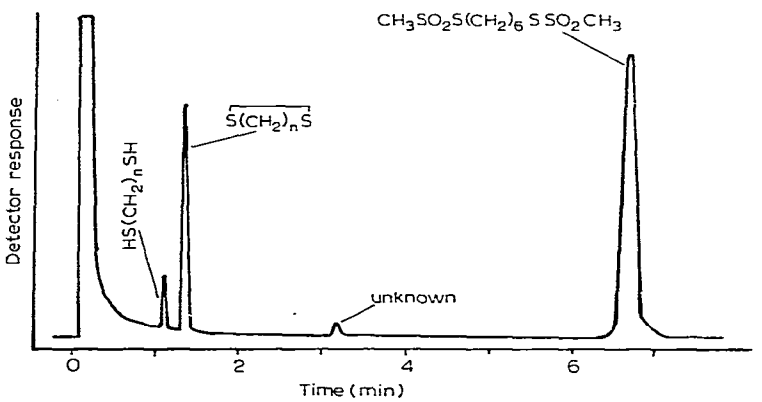

Fig. 1. GC trace of type II thiosulphonate $CH_3SO_2S(CH_2)_6S\ SO_2CH_3$ on OV-1 at 240° showing parent compound and thiol decomposition products.

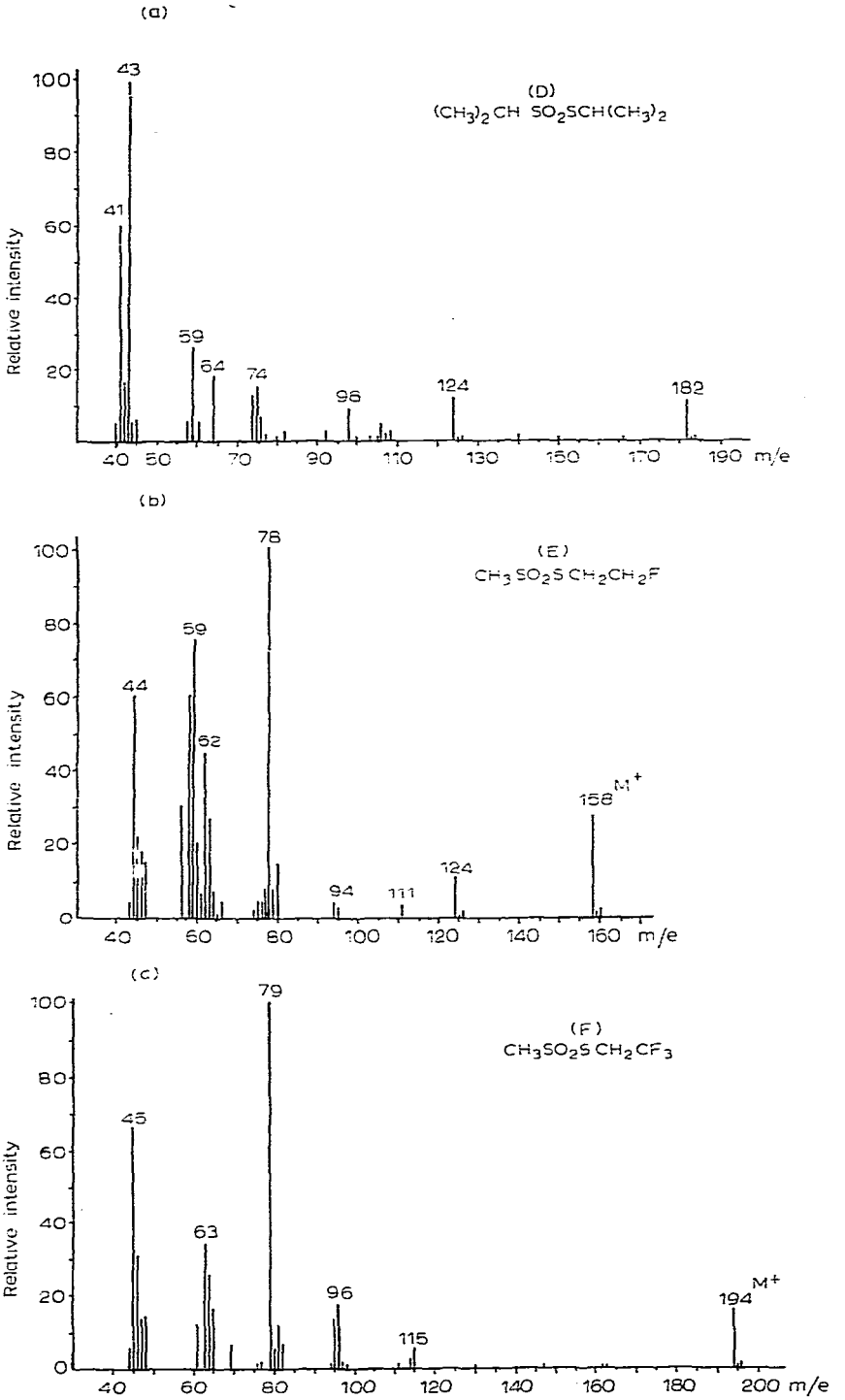

Fig. 2. Normalised mass spectra of type I thiosulphonates: isopropyl isopropane (D), monofluoroethyl methane (E) and trifluoroethyl methane (F) taken using GC–MS.

cyclic disulphide formed by (pyrolytic) decomposition. Confirmation of this identity was established by GC–MS of the type IIR series and this identification has been used in Table I.

GC–MS and MS

In the type I series, the mass spectra of A, B, and C (Table I), have been documented previously[7]. Thiosulphonates D, E and F are new compounds, and their normalised spectra obtained by GC–MS are given in Fig. 2 and briefly discussed below. There was agreement between the GC–MS and direct probe spectra, showing that there was no decomposition during chromatography.

Two representative spectra of type II (direct probe) and the corresponding type IIR (GC–MS) compounds $n = 6$ and 12, are given in Fig. 3. Both type II and IIR series gave characteristic fragmentation patterns; some aspects of these spectra are discussed below and shown in Fig. 5.

DISCUSSION

Many chemical and detailed mass spectral studies[5–7] have been described for dialkyl thiosulphonates (type I) and polymethylene glycol dimethanesulphonates (oxygen ester analogues of type II). However, a simple analytical procedure to characterise these compounds by GC and GC–MS is desirable for the routine assessment of identity and purity where these compounds are to be used in active site and inhibitor studies on enzymes.

Thiosulphonates of the type I series all showed good GC and GC–MS properties (Table I, Fig. 2) and can be characterised readily. The short-chain members, $n=5$ and 6, of type II series gave main peaks on GC due to the intact molecule, and two fast-eluting peaks, whereas $n=8$ to 12 compounds showed a progressive loss of GC mobility of the parent compound and one main fast eluting peak. These peaks were shown to be due to a "double de-methanesulphonylation", presumably by pyrolysis on injection, to produce the corresponding open chain disulphides and cyclic dithiols.

This essentially reductive process was intentionally carried out using the facile reduction[8] of thioesters with borohydride, and resulted in products having the same GC retention times and mass spectra as the decomposition products. A full GC characterisation of the type II series is thus possible after reduction to type IIR. For $n=5$, 6 and 8, the first eluting peak is the straight chain dimercaptan, $HS(CH_2)_nSH$, the second peak the cyclic dithiol $\overline{S–R–S}$, the re-oxidised form. For $n=10$ and 12, only the cyclic dithiol form was observed, and after prolonged standing the shorter chain dimercaptans slowly oxidised to the cyclic form, indicating that cyclisation probably is independent of the GC process.

The normalised spectra of the three thiosulphonates, isopropyl isopropane (D), monofluoroethyl methane (E) and trifluoroethyl methane (F) are given in Fig. 2. Fragmentation patterns are similar to those of other thiosulphonates[7]. The major peaks were the molecular ions, and the cleavage of the S–S bond (with H transfer in E) to give a peak at *m/e* 115 $[SCH_2CF_3]^+$ in F, and base peaks at *m/e* 78 $[SCHCH_2F]^+$ for E and *m/e* 79 $[SO_2CH_3]^+$ for F. The base peak in D is *m/e* 43 $[CH(CH_3)_2]^+$. These and other fragments are outlined in Fig. 4a and b.

Intact type II compounds did not give molecular ions, the first major fragment

occurs at $M^+ - 79$ by loss of CH_3SO_2, similar to the oxygen analogues[6]. However, unlike the latter, the corresponding transsulphonylation does not occur except perhaps for $n=8$ where *m/e* 191 may be the thio analogue $[CH_3SO_2H \cdot S \cdot SO_2\ CH_3]^+$ of the protonated methane sulphonic acid anhydride previously observed[6]. The replacement of oxygen by sulphur presumably promotes charge retention on the thiol fragment such that the principal high-molecular-weight fragments arise by the loss of methane sulphonyl and methane thiosulphonate groups (Fig. 5), and no base peak at 79 is observed. In the absence of high resolution measurements, the identities of the lower mass fragments were assumed to be similar to those in the type IIR series discussed below, in which the oxygens are absent. Facile loss of both methane sulphonyl groups gives a group of peaks (H)S–R–S(H); this, and the resulting fragment series C_nH_{2n-1} and $C_nH_{2n-1}S$, are therefore similar to the type IIR spectra.

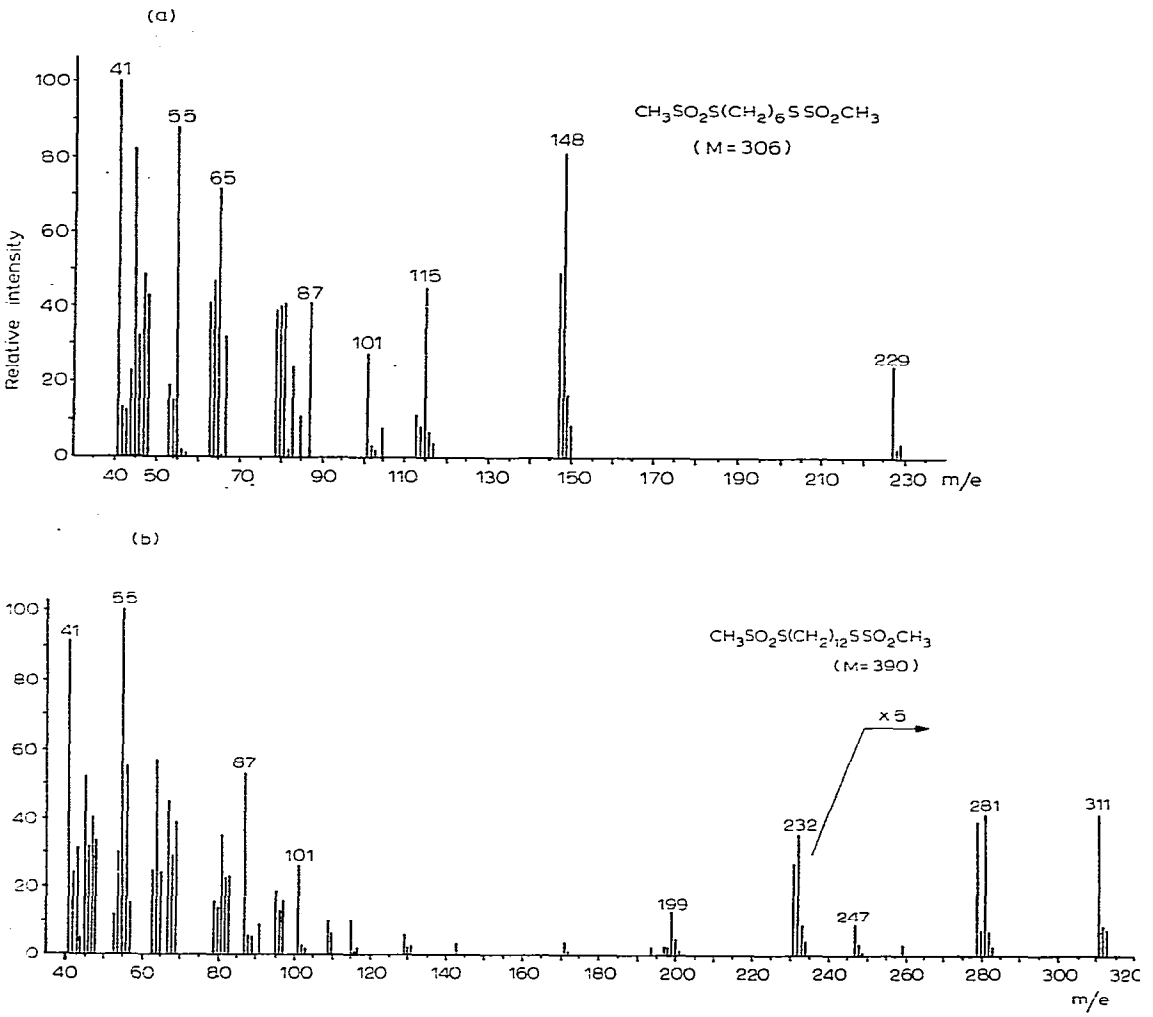

Fig. 3.

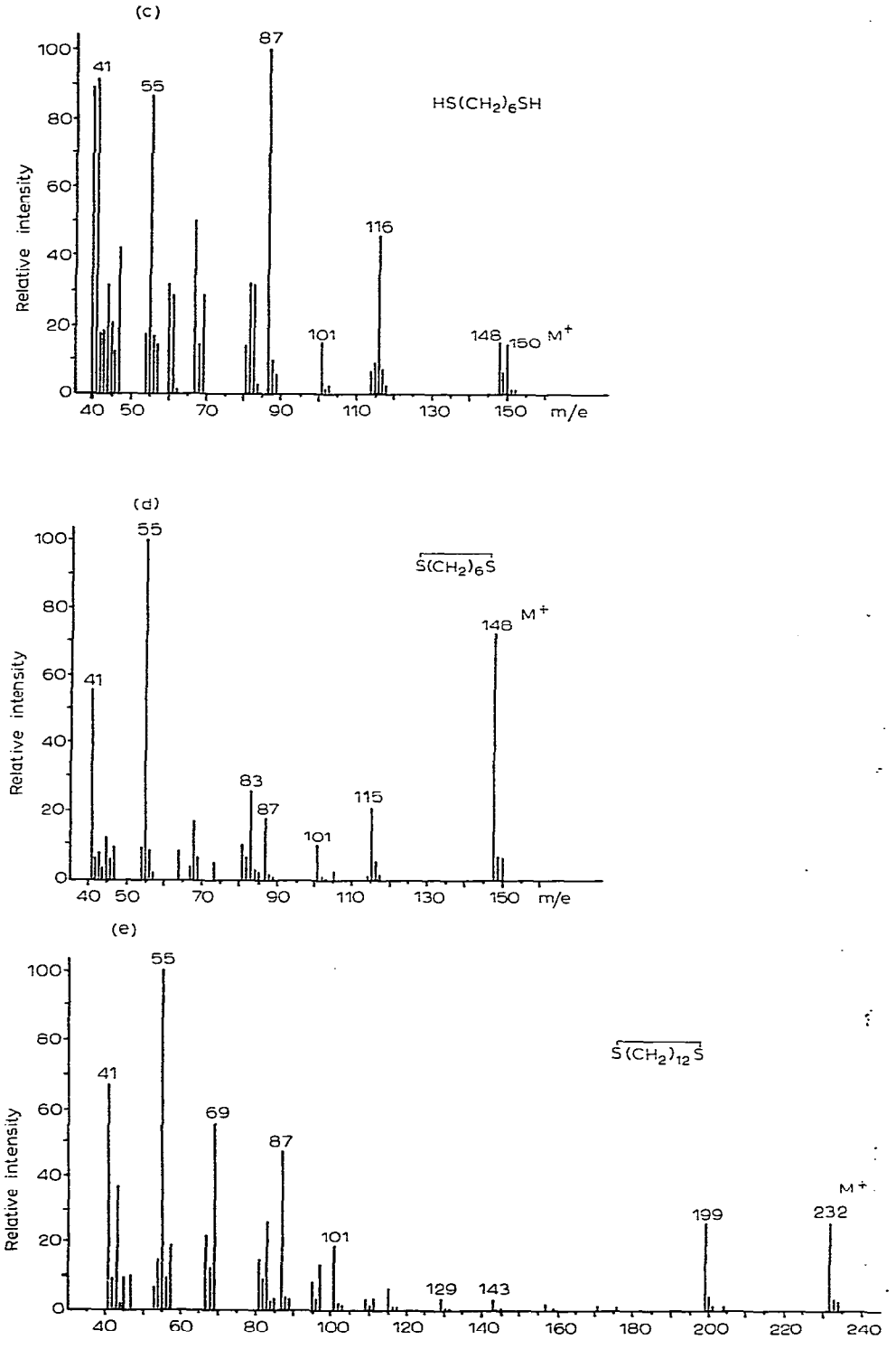

Fig. 3. Normalised mass spectra of type II and type IIR thiosulphonates; (a) and (b) by direct probe, (c), (d) and (e) by GC–MS.

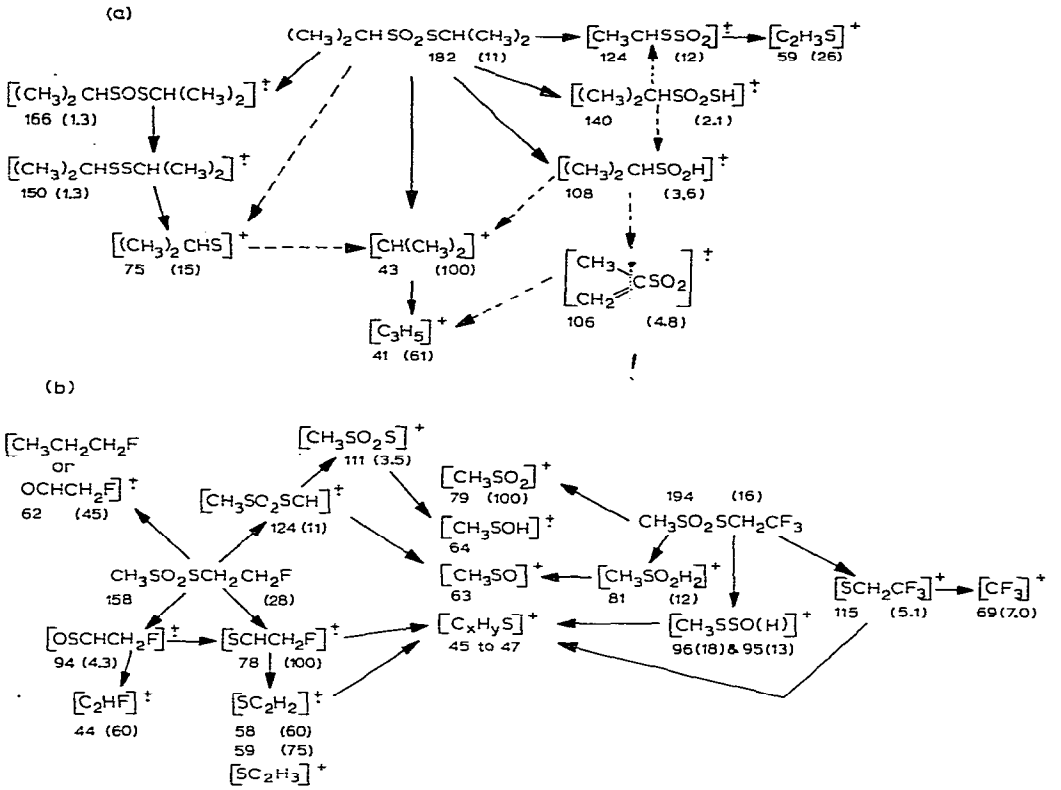

Fig. 4a and b. Fragmentations of isopropyl isopropane (D), monofluoroethyl methane (E) and trifluoroethyl methane (F) thiosulphonates; *m/e* values and relative intensities (in brackets) are shown for the major fragments.

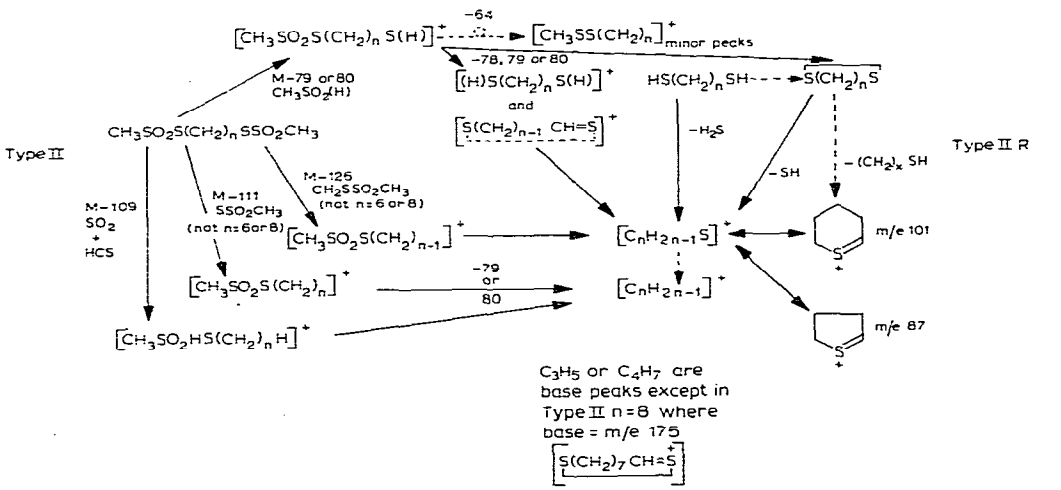

Fig. 5. Generalised fragmentation patterns of $CH_3SO_2S(CH_2)_nS\ SO_2CH_3$ (type II) and reduction products $HS\ (CH_2)_nSH$ and $\overline{S(CH_2)_nS}$ (type IIR) thiosulphonates.

Type IIR series gave prominent molecular ions on GC–MS, with the disulphides tending to lose 2H (possibly due to some formation of the cyclic form in the MS source) and H_2S, whereas the cyclic dithiols lose SH. The main series of fragments were (H)S–R–S(H), C_nH_{2n-1} and $C_nH_{2n-1}S$, with the sulphur containing ions of higher relative intensity in the disulphide series. A fragment at *m/e* 101 in both series may be due to the formation of the cyclic ion C_5H_9S (Fig. 5) by expulsion of $(CH_2)_{n-4}SH$ from the molecular ion (*cf.* alkyl tetrahydro thiophenes[9]). The spectra of the four carbon[10] and five carbon[11] cyclic dithiols have been described; base peaks at *m/e* 55 or 69 are common to all these cyclic dithiols.

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