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Note

Pyrolysis gas chromatographic–mass spectrometric study of medicinal sulphonamides

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The identification of polymers[1], biological macromolecules[2] and microorganisms[3] by analytical pyrolysis gas chromatography (GC)[4] or pyrolysis mass spectrometry (MS)[5] is well documented. The application of these techniques to the study of lower molecular weight compounds[6,7] and in particular to antibacterial substances[8–10] has been limited. Our interest in the analysis of these compounds, especially in formulated products or in biological fluids, has led us to study the application of analytical pyrolysis in this field and here we report some of our results concerned with the identification of medicinal sulphonamides. Various methods have been developed for the identification of these compounds and chromatographic procedures include paper and thin-layer[11] chromatography, and high-pressure liquid[12] and ion-pair partition chromatography[13]. Although sulphonamides are generally too polar for direct GC and decompose during elution[14,15], several procedures are now available for derivatisation prior to analysis. These include methylation[16], permethylation and perethylation[17] for GC–MS studies and the use of perfluoracyl and pentafluorobenzyl compounds for electron capture detection of simple[18] or medicinal[19] sulphonamides. Methylated acetal derivatives have also recently been used for this purpose[20]. Degradation techniques are limited to hydrolysis and GC of the liberated amines[21] and to preparative pyrolysis and thin-layer chromatography for pyrimidine sulphonamides[22]. The TAS[23] (thermomicro and transfer-application-substance) procedure has also been applied to some sulphonamides[24].

EXPERIMENTAL

Preparation of samples

The samples (50–100 μg) were dissolved in 10–20 μl of a suitable solvent (usually methanol) and applied to a rotating wire by a microsyringe[25]. The solvent was evaporated using a hairdrier and the coated wires were stored under vacuum.

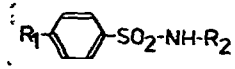
Apparatus and conditions

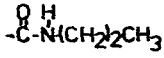
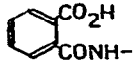
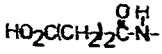
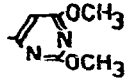
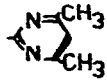
A Pye Curie point pyrolyser was used at a temperature of 770° maintained for 5 sec. A new wire and quartz tube were used for each sample. Chromatography was carried out on a Pye GCV gas chromatograph with 1.5 m × 3 mm I.D. dual glass columns packed with 2% KOH + 8% Carbowax 20M on Chromosorb W AW

DMCS, 100–120 mesh (Phase Separations, Queensferry, Great Britain). The temperature was programmed from 100° to 240° at 2°/min with a final hold of 10 min. The injection port was held at 275° and the detector oven heated to 350°. The air pressure was maintained at 0.5 kg/cm², the hydrogen at 1.3 kg/cm² and the nitrogen flow-rate was 50 ml/min. The data was collected using an Infotronics CRS 304-30 integrator. The identification of the pyrolysis fragments was achieved by means of a GC-linked VG Micromass 12B mass spectrometer operated with a trap current of 100 μA, an accelerating voltage of 4 kV and an ionisation energy of 22 eV.

TABLE I

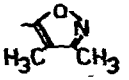
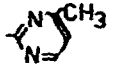
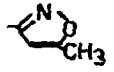
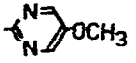
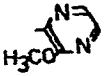
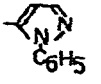
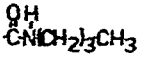
CHEMICAL STRUCTURE OF SULPHONAMIDES



No.	*Sulphonamide*	R_1	R_2	*Proprietary product*
1	Benzene sulphonamide	H	H	
2	Benzene sulphanilide	H		
3	Chlorpropamide	Cl		Diabinese (Pfizer)
4	*p*-Ethyl benzene sulphanilide	CH_3CH_2		
5	Phthalylsulphathiazole			Thalazole (May & Baker)
6	Succinylsulphathiazole			Sulphasuximide (MSD)
7	Sulphacetamide	H_2N		Eye drops
8	Sulphanilamide	H_2N	H	
9	Sulphadiazine	H_2N		Sulphatriad (May & Baker)
10	Sulphadimethoxine	H_2N		Madribon (Roche)
11	Sulphadimidine	H_2N		Sulphamezathine (ICI)

(Continued on p. 366)

TABLE I *(continued)*

No.	*Sulphonamide*	R_1	R_2	*Proprietary product*
12	Sulphafurazole	H_2N		Gantrisin (Roche)
13	Sulphaguanidine	H_2N		
14	Sulphamerazine	H_2N		Sulphatriad (May & Baker)
15	Sulphamethoxazole	H_2N		Gantanol, Bactrim (Roche) Septrin (Wellcome)
16	Sulphamethoxydiazine	H_2N		Durenate (Bayer)
17	Sulphamethoxypyridazine	H_2N		Midicel (Parke Davis) Lederkyn (*Lederle*)
18	Sulphametopyrazine	H_2N		Dalysep (Syntex) Kelfazine (Montedison)
19	Sulphaphenazole	H_2N		Orisulf (Ciba)
20	Sulphapyridine	H_2N		M & B 693 (May & Baker)
21	Sulphathiazole	H_2N		Sulphatriad (May & Baker)
22	Tolbutamide	CH_3		Rastinon (Hoechst)
23	*p*-Toluene sulphonamide	CH_3	-H	

RESULTS AND DISCUSSION

The sulphonamides used in this investigation are recorded in Table I together with the proprietary and ethical names for the various formulated products. The pyrolysis of those sulphonamides recorded in Table II was found to be characterised by fission about the labile *sulphonamido group* and yielded a simple pyrogram in each case. This consisted of a short retention time gas composed solely of sulphur dioxide followed by two intense peaks due to aniline and a heterocyclic amine. These identifications were confirmed by retention time comparisons and by MS. Aniline was found to be common to all fragmentations and served as an internal reference. The heterocyclic amine was produced by cleavage at the sulphonamido group and characterised the sulphonamide under test. This is illustrated for the pyrimidine sulphonamides in

TABLE II

RETENTION INDICES (ANILINE = 1.00) OF SULPHONAMIDES WITH SIMPLE FRAGMENTATION

Sulphonamide	*Retention index*
Sulphapyridine	1.26
Sulphadiazine	1.30
Sulphamerazine	1.33
Sulphadimidine	1.47
Sulphamethoxydiazine	1.85
Sulphaphenazole	3.16

Table II, and in Fig. 1. This data demonstrates the variation in retention time which enables the identification of a specific sulphonamide to be achieved.

MS fragmentation of sulphonamides[26,27] is characterised by the presence of diarylamines derived via the extrusion of sulphur dioxide from the molecular ion. Simple sulphonamides (2 and 23 in Table I) have been found to undergo analogous secondary reactions and small amounts of biphenyl, carbazole and diphenylamine were detected on pyrolysis of benzene sulphonamide, in addition to the major components benzene and aniline. The results of methoxy-substituted sulphonamides again produced characteristic pyrograms. In this series however, only sulphamethoxydiazine underwent the simple decomposition noted previously to yield sulphur dioxide, aniline and 2-amino-5-methoxy-pyrimidine. The remaining sulphonamides (Table III) yielded secondary products resulting from *trans*-methylation reactions involving aniline and the methoxyheterocyclic. Thus sulphametopyrazine and sulphamethoxypyridazine yielded N-methylaniline and sulphadimethoxine, which has two methoxy substituents, in addition yielded N,N-dimethylaniline. This activity may be accounted for by the known tendency for the methoxyl groups positioned α to a π-deficient ring nitrogen atom to undergo thermally-initiated free-radical intermolecular rearrangements resulting in methyl migration[28,29].

In sulphacetamide an acetyl group replaces a heterocyclic substituent. This change increases the incidence of secondary reactions and pyrolysis of the hydrated sodium salt yields several products, among these acetanilide is an intense peak and is unique to sulphacetamide and so may be used as the diagnostic fragmentation product. Smaller amounts of carbon dioxide, sulphur dioxide, acetonitrile, acetic acid, benzene and acetophenone as well as aniline were detected.

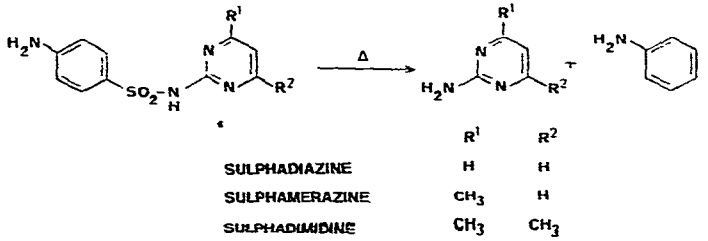


Fig. 1. Pyrolytic composition of pyrimidine sulphonamides.

TABLE III

RELATIVE AREAS OF AMINES OBTAINED FROM METHOXYSULPHONAMIDES SHOWING TRANS-METHYLATION

Sulphonamide	*Relative areas*			*Retention index R_2NH_2 (aniline = 1.00)*
	Me_2NPh	*MeNHPh*	*R_2NH_2*	
Sulphametopyrazine	—	0.07	0.23	2.48
Sulphamethoxypyridazine	—	0.18	—	—
Sulphadimethoxine	0.63	0.94	2.75	0.24

Formulated sulphonamides may also be studied by this method as it has been found that the excipients in tablets, *e.g.* magnesium stearate, lactose and starch, do not interfere either with the fragmentation pathways or with the overall appearance of the pyrogram. The technique is particularly useful for formulated mixtures. Fig. 2 records data obtained from Sulphatriad which contains a mixture of three sulphonamides and illustrates the clear identification of the components.

The technique has also been applied to the quantitative analysis of these drugs and also to the detection of sulphonamides and metabolites in urine. These results will be communicated at a later date[30].

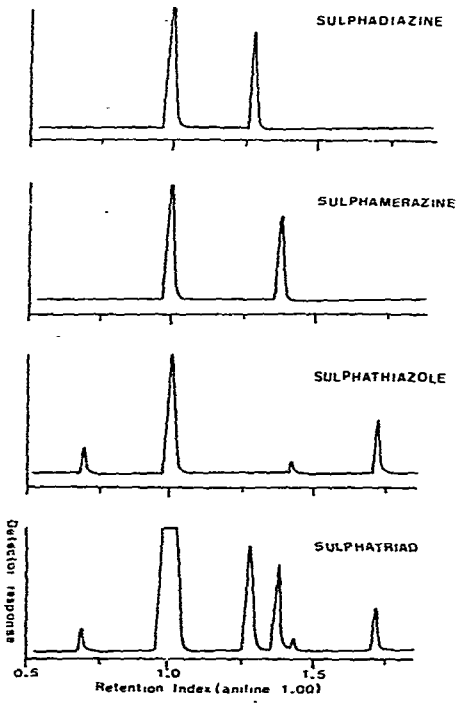


Fig. 2. Pyrogram of formulated mixture.

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