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ELECTRON-CAPTURE GAS CHROMATOGRAPHY OF SULPHONAMIDES AFTER EXTRACTIVE ALKYLATION

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SUMMARY

The rate of extractive alkylation of 24 structurally related sulphonamides has been studied with respect to the alkylating agent and organic solvent. The reaction rate is given by the time for quantitative alkylation calculated from the observed first-order rate constant with 0.1 *M* tetrabutylammonium (pH 10) as the aqueous phase. The highest reaction rates were obtained with methyl iodide and pentafluorobenzyl bromide as reagents and methylene chloride and methyl isobutyl ketone as organic solvents.

The minimum detectable concentration of the N^1-methyl derivatives with electron-capture detection was about 10^{-15} mole/sec.

INTRODUCTION

Many drugs contain the sulphonamide moiety and their gas chromatographic analysis is hampered by their acidic character, which causes adsorption in the gas chromatographic system.

Acylation[1] or alkylation[1–10] procedures have been used to improve the chromatographic properties of sulphonamides. Alkylation of secondary sulphonamides with diazomethane has been performed separately[2,4–6,10] or in combination with heptafluorobutyrylation of the 4-amino group in, *e.g.*, sulphamerazine[3,9]. Primary and secondary sulphonamides have been derivatized with dimethylformamide dimethylacetal[7,8] and with pentafluorobenzyl bromide by extractive alkylation[1].

The scope of this work was to study conditions for the extractive alkylation of secondary sulphonamides and to evaluate the gas chromatographic and electron-capture properties of the methyl derivatives.

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EXPERIMENTAL

Apparatus

Gas chromatography. A Varian 1400 gas chromatograph with a flame-ionization detector was used, with a glass column (150 × 0.2 cm I.D.) filled with 5% OV-17 on Gas Chrom Q (80–100 mesh). The column was operated at 250° and the injector and detector at 300°. The flow-rate of the carrier gas (nitrogen) was 30 ml/min.

A Hewlett-Packard 5710A gas chromatograph was used with a ^{63}Ni electron-capture detector operated in the constant-current mode. The glass column (120 × 0.2 cm I.D.) was filled with the same packing as above; the oven was kept at 272° and the injector and detector at 300° and 350°, respectively. Argon + 5% methane at a flow-rate of 40 ml/min was used as the carrier gas.

Mass spectrometry. The derivatives were identified in an LKB 9000 instrument after separation on a glass column filled with the same packing as above. The ionization energy was 70 eV.

Spectrophotometry. The photometric measurements were performed with a Zeiss PMQ II Spectralphotometer.

Reagents and chemicals

Methyl, ethyl and butyl iodide were obtained from E. Merck (Darmstadt, G.F.R.), propyl iodide from Kebo (Solna, Sweden), decyl iodide from Fluka (Buchs, Switzerland) and pentafluorobenzyl bromide from Pierce (Rockford, Ill., U.S.A.).

Chloroform, methylene chloride, 1-pentanol and methyl isobutyl ketone were of analytical-reagent grade.

Tetrabutylammonium hydrogen sulphate was obtained from Labkemi (Gothenburg, Sweden). It was neutralized with sodium hydroxide and a 0.1 *M* solution was prepared in 0.5 *M* carbonate buffer (pH 10).

Methods

(A) Extractive alkylation. A 2-ml volume of a solution of the sulphonamide (10^{-3} *M*) and the internal standard (griseofulvin, Mirex or 9-bromophenanthrene) in the organic solvent was mixed with an equal volume of 0.1 *M* tetrabutylammonium (pH 10). The alkylating agent was added to a concentration of 0.16 *M* and the mixture was shaken mechanically at 25°. Samples of 10 μl were withdrawn from the organic phase, mixed with 1 ml of ethyl acetate and extracted with 50 μl of phosphate buffer (pH 5.0) to quench the reaction. A 1–2-μl volume of the organic phase was taken for analysis by gas chromatography with electron-capture detection. The concentration of the alkylated sulphonamide was obtained from the ratio of the peak height to that of the internal standard.

(B) Distribution of sulphonamides as acids and ion pairs. The acid distribution was studied by equilibration of a solution of the sulphonamide (10^{-3} *M*) in carbonate buffer (pH 10) with an equal volume of organic solvent at 25° for 30 min.

The concentrations in the aqueous phase and in the organic phase, after re-extraction into carbonate buffer (pH 10), were determined photometrically.

The ion-pair distribution was studied as above with 0.1 *M* tetrabutylammonium in carbonate buffer (pH 10) as the aqueous phase.

(C) Minimum detectable concentrations. Methyl derivatives of the sulphon-

amides were prepared with concentrations in the milligrams per millilitre range. Dilutions in ethyl acetate were analysed by gas chromatography with electron-capture detection. The minimum detectable concentrations were calculated from the amount that gave a signal three times the noise[11].

RESULTS AND DISCUSSION

Identification of derivatives

The derivatives formed by the extractive alkylation procedure were identified by mass spectrometry. The mass spectrum of methylated sulphapyridine is presented in Fig. 1. The fragmentation pattern[12,13] indicates that the methylation occurs at the nitrogen in the sulphonamide group (N^1). Similar fragmentation patterns were obtained from the other sulphonamide derivatives.

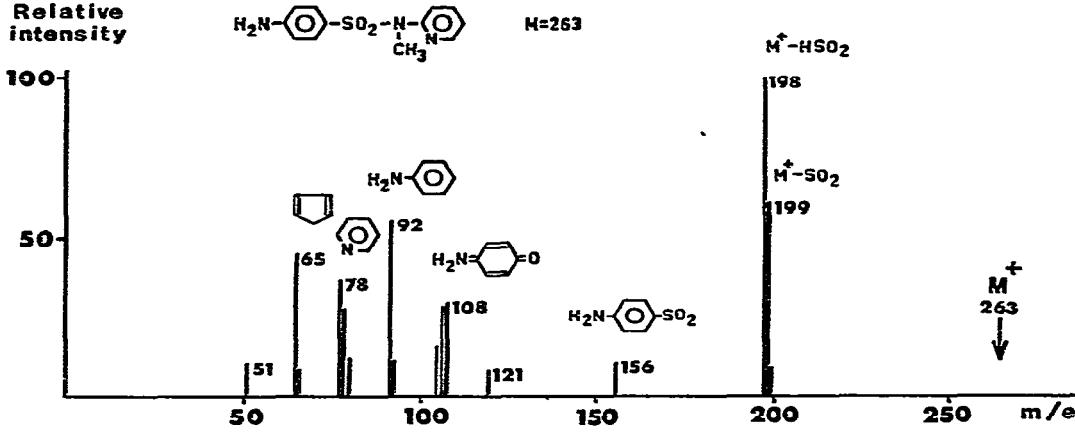


Fig. 1. Mass spectrum of methyl derivative of sulphapyridine. Ionization energy: 70 eV. Ions with less than 5% relative intensity and below *m/e* 50 have been omitted.

Alkylation rate

Model studies were performed with N-phenylbenzenesulphonamide. The yield of the extractive methylation was studied with the pure methyl derivative as reference. A recovery of 100 ± 3% was obtained, which indicates that the influence from side-reactions is negligible.

The studies of the reaction rate could, under this assumption, be based on the determination of the alkyl derivative formed. The measurements were made at suitable intervals during the first two half-lives of the sulphonamide and also after more than seven half-lives. A plot of $\ln[A]_\infty/([A]_\infty - [A]_t)$ *versus* time was made, where $[A]_\infty$ is the concentration of the alkylated product after more than seven half-lives of the sulphonamide and $[A]_t$ the concentration at time t. The rectilinear relationship obtained indicates that the alkylation is a pseudo-first-order process. The rate constant can be obtained from the slope of the line.

This method for the determination of the observed rate constant was used for all of the sulphonamides. A test of the quantitative conversion of the sulphon-

amide into alkyl derivatives was not performed in every instance, but the good linearity of the plots indicates that the N^1-alkylation process dominates.

Conditions for the extractive alkylation

The acid is extracted as an ion pair with a counter ion into the organic phase, where the alkylation takes place[1,14–16]. The extraction conditions and the properties of the organic phase and the alkylating agent influence the reaction.

Aqueous phase. Tetrabutylammonium (0.1 *M*) in carbonate buffer (pH 10) was used as the aqueous phase in all instances. With methylene chloride as the organic phase, the degree of extraction as tetrabutylammonium ion pairs exceeded 80% for all of the sulphonamides in Table III except compounds 4, 18 and 22, which gave degrees of extraction in the range 50–70%. Conditions giving a degree of extraction higher than 80% will have a limited influence on the time for quantitative alkylation. The acid distribution into methylene chloride was negligible at pH 10.

Alkylating agent. The rate of alkylation of sulphapyridine in methylene chloride with six different reagents is given in Table I. The rate is given as the calculated time for quantitative alkylation which is valid under the assumption that side-reactions are negligible.

The rate of alkylation decreases with increasing carbon chain length[17], ethylation requiring a reaction time about 30 times longer than that for methylation. The

TABLE I

RATE OF ALKYLATION OF SULPHAPYRIDINE

Conditions: see Methods, (A). Time for quantitative alkylation calculated as $7 \cdot 0.693 \cdot k_{obs}^{-1}$.

Alkylating reagent	*Calculated time for quantitative alkylation (min)*
Methyl iodide	15
Ethyl iodide	470
Propyl iodide	1400
Butyl iodide	1800
Decyl iodide	2800
Pentafluorobenzyl bromide	10

TABLE II

RATE OF METHYLATION OF SULPHAMETHOXAZOLE IN DIFFERENT ORGANIC SOLVENTS

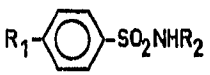
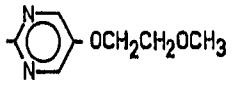
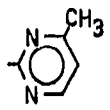
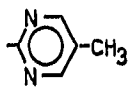
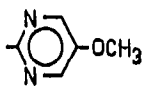
Reagent: methyl iodide [for further conditions, see Methods, (A)]. Calculation of time for quantitative methylation: see Table I.

Solvent	*Degree of extraction (%)*	*Calculated time for quantitative methylation (min)*
Methyl isobutyl ketone	63	20
Methylene chloride	>90	80
Chloroform	>90	130
1-Pentanol	>90	700

TABLE III

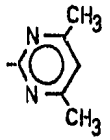
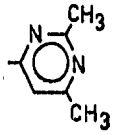
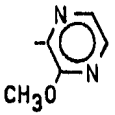
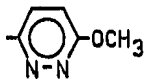
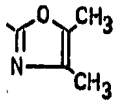
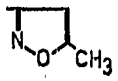
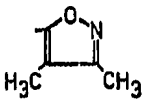
METHYLATION OF SULPHONAMIDES

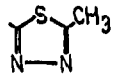
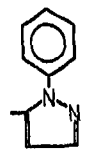
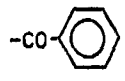
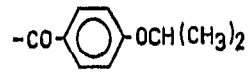
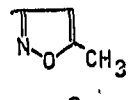
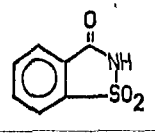
Reagent: methyl iodide. Organic solvent: methylene chloride [for further conditions, see Methods, (A)]. Calculation of time for quantitative methylation: see Table I.

No.	*Structure* 		*Generic name*	*Calculated time for quantitative methylation (min)*	*Retention relative to griseofulvin*	*Minimum detectable concentration (mole/sec $\times 10^{16}$)*
	R_1	R_2				
1	H		N-Phenylbenzenesulphonamide	40	0.09	6.4
2	H		Glymidine	60	0.53	3.7
3	H_2N		Sulphapyridine	15	0.47	8.0
4	H_2N		Sulphadiazine	70	0.66	4.2
5	H_2N		Sulphamerazine	40	0.69	3.9
6	H_2N		Isosulphamerazine	75	0.93	5.1
7	H_2N		Sulphamethoxydiazine	70	1.38	4.1

(Continued on p. 280)

TABLE III (*continued*)

No.	Structure		Generic name	Calculated time for quantitative methylation (min)	Retention relative to griseofulvin	Minimum detectable concentration (mole/sec $\times 10^{16}$)
	R_1	R_2				
8	H_2N		Sulphadimidine	30	0.71	3.5
9	H_2N		Sulphaisodimidine	150	0.50	3.6
10	H_2N		Sulphalene	550	0.69	2.2
11	H_2N		Sulphamethoxypyridazine	20	0.93	11
12	H_2N		Sulphamoxole	110	0.40	13
13	H_2N		Sulphisoxazole	250	0.42	2.6
14	H_2N		Sulphamethizole	55	0.98	2.9

15	H_2N		Sulphamethoxazole	80	0.40	2.5
16	H_2N		Sulphaphenazole	270	1.71	1.9
17	H_2N		Sulphathiazole	30	0.49	7.6
18	H_2N	$-COCH_3$	Sulphacetamide	1300	0.16	3.2
19	H_2N		Sulphabenzamide	15000	0.83	5.7
20	H_2N		Sulphaproxyline	50	0.69	7.6
21	CH_3CONH		N^4-Acetylsulphapyridine	50	1.16	12
22	CH_3CONH		N^4-Acetylsulphadiazine	100	1.69	6.7
23	CH_3CONH		N^4-Acetylsulphamethoxazole	90	0.91	3.1
24			Saccharin	2700	0.03	2.8 [16]

rate of alkylation with pentafluorobenzyl bromide was of the same order as that with methyl iodide. Alkylation studies on sulphamethoxazole and sulphaisodimidine gave similar results.

Organic solvent. The rate of methylation of sulphamethoxazole in four organic solvents is given in Table II. The highest rate of methylation is obtained in methyl isobutyl ketone, while the rate is more than 30 times lower in pentanol.

Methylation of sulphonamides

The rates of methylation of sulphonamides with methyl iodide in methylene chloride are given in Table III. The following observations can be made:

(1) the calculated time for quantitative methylation is less than 100 min for most of the sulphonamides;

(2) sulphonamides with a 2-substituent in the heterocyclic ring (compounds 10, 13 and 16) are methylated at a lower rate than the unsubstituted compounds;

(3) acetylated sulphonamides (compounds 21, 22 and 23) are methylated more slowly than the parent compounds (3, 4 and 15).

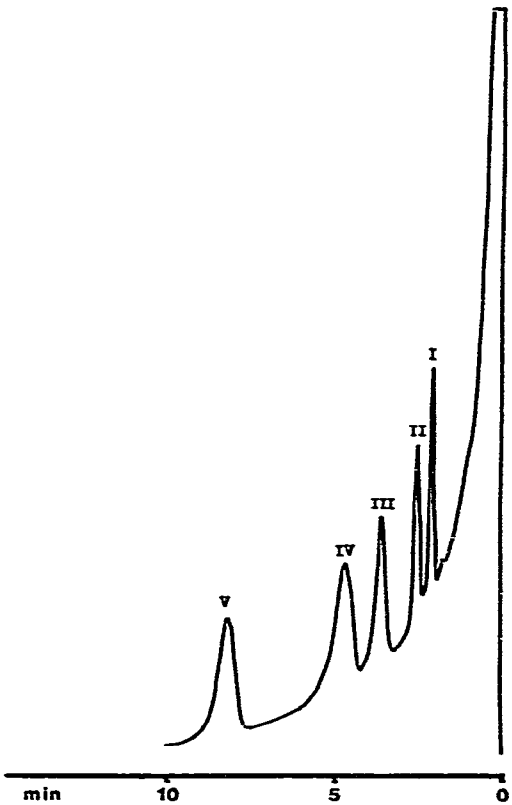


Fig. 2. Gas chromatogram of methylated sulphonamides. Sample: sulphamethoxazole 1 ng (I), sulphaisodimidine 1 ng (II), sulphadimidine 1 ng (III), sulphamethoxypyridazine 3.5 ng (IV) and sulphaphenazole 2.5 ng (V) in ethyl acetate. Stationary phase: 5% OV-17.

Electron-capture gas chromatography of methylated sulphonamides

The methylated sulphonamides have good gas chromatographic properties; a chromatogram is shown in Fig. 2. Quantitation can be effected with good precision, a 100-pg sample being determined with a standard deviation of 3–5% ($n = 10$).

The following conclusions can be drawn regarding the relationship between structure and retention:

(1) sulphonamides with an amino group in the 4-position have considerably longer retentions than the unsubstituted compounds (compare compounds 1 and 3, and 2 and 7);

(2) acetylation of the 4-amino group increases the retention by a factor of about two (see compounds 21 and 3, 22 and 4, and 23 and 15);

(3) 4′-substitution gives longer retention than 3′-substitution (see compounds 5 and 6).

The methylated sulphonamides have excellent electron-capturing properties giving minimum detectable concentrations of the order $2 \cdot 10^{-16}$–$13 \cdot 10^{-16}$ mole/sec.

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