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ELECTRON CAPTURE GAS CHROMATOGRAPHY OF SULPHONAMIDES

EFFECTS OF STRUCTURE AND TEMPERATURE ON DETECTOR RESPONSE

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SUMMARY

The electron capture response of twenty-three sulphonamide derivatives has been determined. The detector response was measured after derivatization of the simple sulphonamides with different alkyl and acyl reagents in order to evaluate the structural requirements for a high response towards the electron capture detector. An electron capture response of the order of 10^{-15} mole/sec was obtained with derivatives with two functional groups, *e.g.*, phenyl or carbonyl, in conjugation with the sulphonamide group. The response decreased some 100 times with only one such group present. Sulphonamide derivatives with a high electron capture response showed a decreased response, or were insensitive, towards an increased detector temperature.

INTRODUCTION

The response of the electron capture detector (ECD) to some sulphonamides with alkyl and aryl substituents was recently discussed[1]. It was concluded that the sulphonamide group has electron capturing properties, which can be strongly amplified by conjugation with phenyl groups. Several sulphonamide drugs have two aromatic rings in conjugation with the sulphonamide group and have ECD response values in the range $2 \cdot 10^{-16}$–$13 \cdot 10^{-16}$ mole/sec after N-methylation[2]. Derivatization of the acidic sulphonamide hydrogen was mandatory for gas chromatography (GC). The application to low concentration analysis was demonstrated with sulphapyridine and its N^4-acetyl metabolite extracted from serum samples[1].

In the choice of derivatization procedure for compounds with the sulphonamide group, consideration has to be given to stability, chromatographic properties and ECD response of the derivative formed. Besides alkylation[2–4], acylation with perfluoroacyl reagents[4] has been employed to improve the chromatographic properties of sulphonamides. The ECD response is often temperature dependent and by

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appropriate choice of detector temperature it may be possible to increase the selectivity of an analytical procedure by the depression of the response of extraneous material compared to that from the substance of interest[5].

Therefore, for the analysis of low concentrations of sulphonamides, it is important not only to elucidate the structural requirements for a high ECD response, but also to establish its temperature dependence. In the present study, the ECD response as well as its temperature dependence has been studied for five sulphonamide model compounds after alkylation and acylation.

EXPERIMENTAL

Gas chromatography

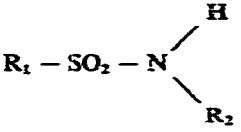
A Hewlett-Packard 5710 A gas chromatograph with a constant current ECD was used. The glass column (120 × 0.2 cm I.D.) was filled with 5% OV-17 on Gas-Chrom Q (80–100 mesh). Argon with 5% methane was used as carrier gas with a flow-rate of 30 ml/min.

Reagents and chemicals

The sulphonamides shown in Table I were used as model compounds. They were prepared as described previously[4]. Diphenyl sulphone was purchased from EGA-Chemie (Steinheim bei Heidenheim, G.F.R.).

TABLE I

STRUCTURE OF SULPHONAMIDES



No.	*Name*	R_1	R_2
I	Benzenesulphonamide	C_6H_5	H
II	N-Ethylbenzenesulphonamide	C_6H_5	C_2H_5
III	N-Phenylbenzenesulphonamide	C_6H_5	C_6H_5
IV	N-Phenylmethanesulphonamide	CH_3	C_6H_5
V	N-Decylmethanesulphonamide	CH_3	$C_{10}H_{12}$

Methods

Preparation of derivatives. Acylation and alkylation of the sulphonamide model compounds were done as described earlier[4]. Methylation of N-decylmethanesulphonamide was performed with methyl iodide and 20% potassium hydroxide in methanol.

A quantitative derivatization was controlled by GC with flame ionization detection, and the identity of the derivative formed was confirmed by GC–mass spectrometry.

ECD. Solutions of the derivatives were prepared and diluted with *n*-heptane to a concentration which on injection of a 1-μl volume gave a peak height correspond-

ing to at least ten times the background noise. The injected amounts were within the linear range of the detector in all cases. The minimum detectable concentration was calculated from the amount of derivative that gave a signal three times the average noise.

Temperature dependence of the electron capture response. The temperature dependences of the ECD response of the sulphonamide derivatives were determined using detector temperatures in the range 150–350°. In a few cases lower temperatures could be used. The temperature stability was ascertained by an overnight equilibration between each setting. Besides the minimum detectable concentration, the temperature dependence is illustrated by plotting $\ln A/n \times T^{3/2}$ *versus* $1/T$ (ref. 5) where A is the peak area in mm² (attenuation 1, chart speed 10 mm/min), n the number of moles injected and T the temperature in °K.

RESULTS AND DISCUSSION

ECD response of N-alkylated sulphonamides

N-Methyl derivatives (Table IIa). One of the structural requirements for a high ECD response for the sulphonamides was revealed after the determination of the response of the N-methylated compounds. After introduction of two phenyl groups to the sulphonamide moiety, as in compound 5, the ECD response was in the order of 10^{-16} mole/sec. The response of compound 5 was of the same order of magnitude as found among bacteriostatic sulphonamide drugs, which have a common structural feature[2]. A poor response (10^{-14} mole/sec) was shown with only alkyl substituents or at most one phenyl ring attached to the sulphonamide group. About 0.1 ng can be detected on column for these derivatives (number of plates, $N = 1600$, retention time, $t_R = 5$ min).

It was also shown that the nitrogen atom as such only slightly influenced the re-

TABLE II

ELECTRON CAPTURE RESPONSE FOR N-ALKYLATED SULPHONAMIDES

No.	R_1	R_2	*Minimum detectable concentration (mole/sec × 10^{16}) at (detector temp.)*	
			150°	*350°*
(a) N-Methyl derivatives				
1	C_6H_5	C_2H_5	5000	200
2	CH_3	$C_{10}H_{21}$	2000*	—
3	C_6H_5	CH_3	25000	250
4	CH_3	C_6H_5	5000	200
5	C_6H_5	C_6H_5	1.2	12
(b) N-Pentafluorobenzyl derivatives				
6	C_6H_5	C_2H_5	0.18	3.0
7	C_6H_5	C_6H_5	0.14	0.6
8	CH_3	$C_{10}H_{21}$	0.5	2.1
9	C_6H_5	$C_6F_5CH_2$	0.10	0.6
10	CH_3	C_6H_5	0.11	3.5

* 270°.

sponse, since diphenyl sulphone showed a minimum detectable concentration of $4 \cdot 10^{-15}$ mole/sec (detector temperature 150°), similar to that of compound 5.

N-Pentafluorobenzyl derivatives (Table IIb). Introduction of a pentafluorobenzyl group into compounds without electron capturing properties will yield derivatives with minimum detectable concentrations in the 10^{-16} mole/sec range[6]. A response of the same magnitude for pentafluorobenzylated sulphonamides was found irrespective of other substituents in the molecule. Introduction of a second pentafluorobenzyl group did not increase the response further (compound 9). A derivatization of a compound having inherent electron capturing properties with pentafluorobenzyl bromide and similar reagents would be detrimental to the selectivity of the method owing to an increase in background signal from the reagent itself and reagent generated products in the analytical region of the chromatogram.

Electron capture response of N-acylated sulphonamides

N-Acetyl derivatives (Table III). Acetylation does not usually confer electron capturing properties upon a molecule, and therefore acetyl derivatives are considered to be more or less transparent towards the detector. After N-acetylation a minimum detectable concentration in the 10^{-16} mole/sec range was found for three of the model sulphonamides (compounds 11, 12 and 14). Besides the acetyl group, these three sulphonamides have a phenyl group attached to the sulphonamide moiety.

N-Trifluoroacetyl and N-heptafluorobutyryl derivatives (Table IIIb and c). The detector response after perfluoroacylation was similar to that of the acyl derivatives. Perfluoroacylation of the aliphatic sulphonamide gave derivatives with about the

TABLE III

ELECTRON CAPTURE RESPONSE FOR N-ACYLATED SULPHONAMIDES

No.	R_1	R_2	*Minimum detectable concentration (mole/sec × 10^{16}) at detector temp.*	
			150°	*350°*
(a) N-Acetyl derivatives				
11	C_6H_5	C_2H_5	6.4	6.2
12	C_6H_5	C_6H_5	0.7	2.3
13	CH_3	$C_{10}H_{21}$	230	33
14	CH_3	C_6H_5	2	0.2
(b) N-Trifluoroacetyl derivatives				
15	C_6H_5	C_2H_5	15	11
16	C_6H_5	C_6H_5	0.4	40
17	CH_3	$C_{10}H_{21}$	1.1	7.4
(c) N-Heptafluorobutyryl derivatives				
18	C_6H_5	C_2H_5	6.0	6.8
19	C_6H_5	C_6H_5	0.4	40
20	CH_3	$C_{10}H_{21}$	0.26	26
(d) N-Pentafluorobenzoyl derivatives				
21	C_6H_5	C_2H_5	0.1	1.0
22	C_6H_5	C_6H_5	0.1	2.1
23	CH_3	C_6H_{21}	0.26	1.3

same sensitivity (compounds 17 and 20). Introduction of the strong electrophore heptafluorobutyryl improved the response compared to that of the trifluoroacetyl derivatives but only to a minor extent (Table IIIb and c).

Previous studies on the ECD response of perfluoroacylated amines and alcohols[7] and phenols[8] showed responses 100 times higher for the heptafluorobutyryl derivatives compared to the trifluoroacetyl derivatives. On the other hand, a trifluoroacetylated aliphatic carbamate[9] showed a response in the same range as heptafluorobutyrylated amine derivatives. It is also known that bis(trifluoroacetyl) derivatives of primary amines give high response values[10]. This indicates that trifluoroacetylated sulphonamides capture electrons more like a trifluoroacetylated amide than the corresponding derivative from an amine or an alcohol.

N-Pentafluorobenzoyl derivatives (Table IIId). The response of these derivatives was similar to the corresponding pentafluorobenzyl derivatives (Table IIb). The response values were somewhat higher than for the perfluoroacylated compounds above, although the difference was less than has been observed for amine derivatives[7,11].

The temperature dependence of the ECD response

The attachment of electrons to molecules has been classified into three different mechanisms[5] which have different temperature dependences. The mechanisms can be determined when the electron capture coefficient K' is plotted *versus* $1/T$ in a modified Arrhenius plot. The temperature dependence of the response of six alkyl and acyl derivatives of N-ethylbenzenesulphonamide using a similar plot is shown in Fig. 1.

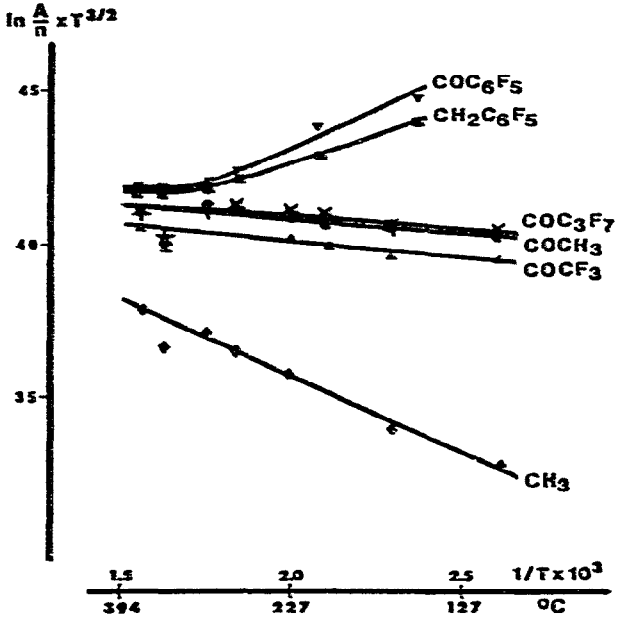


Fig. 1. Temperature dependence of the ECD response for N-ethylbenzenesulphonamide derivatives.

From the figure and also from the response values in Tables II and III it is obvious that acylated N-ethylbenzenesulphonamides have a response independent of the detection temperature. On the other hand, N-ethyl-N-methylbenzenesulphonamide had a 25-fold higher response on increasing the detector temperature from 150 to 350°. The behaviour of the sulphonamides above is typical for a dissociative electron capture mechanism[5]. The temperature dependence of the response of the N-pentafluorobenzyl derivative was similar to that of the N-pentafluorobenzoyl derivative.

From these results it is obvious that a high sensitivity can be gained by appropriate choice of detector temperature. An increased selectivity of an analytical procedure can also be obtained if detection of a compound is made at optimum temperature while the response of extraneous material is depressed at this temperature.

From Table III it can be seen that most acylated sulphonamides have their highest response at low detector temperature and that the response decreased 5–20 times in going from 150 to 350°.

Compound 13 (N-acetyl-N-decylmethanesulphonamide) and the N-methylated sulphonamides with fairly low response (compounds 1–3) were the only derivatives which increased their response with increasing detector temperature. The gain was about 100 times for compound 3 over the temperature range studied.

Choice of derivatization procedure

Based on the discussions above the following recommendations can be made concerning the choice of derivative for sulphonamides.

N-Methylation is the derivatization procedure of choice for those sulphonamides which have inherent electron capturing properties. The derivatives have an excellent stability, good GC properties and an ECD response in the range 10^{-16} mole/sec. This makes possible submicrogram analysis from biological samples. Methylation procedures will leave a minimum of disturbing components in the chromatograms, as derivatization of extraneous material usually gives transparent products. Owing to the high sensitivity and good selectivity of the procedure, it is possible to perform the derivatization directly with the biological sample present, *e.g.*, by extractive alkylation[1,11,12].

TABLE IV

RELATIVE RETENTION OF N-ETHYLBENZENESULPHONAMIDE DERIVATIVES

Column: 5% OV-17.

Compound	*Relative retention*
N-Ethylbenzenesulphonamide (8 min, 166°)	1.00
Derivative	
Heptafluorobutyryl	0.28
Trifluoroacetyl	0.36
Methyl	0.80
Acetyl	1.2
Pentafluorobenzoyl	2.5
Pentafluorobenzyl	3.8

For sulphonamides with only one group in conjugation with the sulphonamide moiety, the conjugation must be increased, *e.g.*, by the introduction of an acetyl or a phenyl group.

Too long a retention of a derivative is inconvenient as a higher column bleed may occur. Although there was a ten-fold difference in retention between the derivatives (Table IV), formation of a heptafluorobutyryl derivative will generally offer little advantage over methylation or acetylation in the analysis of low concentrations of sulphonamides by GC–ECD.

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