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GAS CHROMATOGRAPHIC RETENTION INDICES FOR ALL C$_1$- AND C$_2$-ALKYLATED BENZOTHIOPHENES AND THEIR DIOXIDES ON THREE DIFFERENT STATIONARY PHASES

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SUMMARY

The retention indices for all C$_1$- and C$_2$-alkylated benzothiophenes and their dioxides were determined on three stationary phases of different polarity: methylphenylsiloxane, polyethylene glycol and cyanopropylsiloxane. In many instances isomeric dioxides were better separated than the corresponding unoxidized benzothiophenes. The influence of the substitution pattern on the retention indices is discussed.

INTRODUCTION

Sulphur-containing compounds constitute an important part of petroleum products of various kinds. Interest in such compounds has increased markedly in the last few years following the realization of the deleterious effects that sulphur dioxide, formed by burning of, *e.g.*, organically bound sulphur, can have in the form of acid rain. A multitude of sulphur-containing compounds are known to occur in fossil fuels[1], one of the major classes of which are the polycyclic aromatic sulphur heterocycles (PASH).

The simplest representative of the PASHs is benzo[*b*]thiophene, corresponding to naphthalene among the polycyclic aromatic hydrocarbons (PAHs). Benzo[*c*]thiophene, possessing a quinoid structure and consequently being fairly reactive, is known only from synthesis. While alkylated benzothiophenes are minor components in some types of petroleum and petroleum-derived products, such as coal tar and carbon black, where mainly non-alkylated PASHs with larger ring systems are found[2,3], they abound in others, *e.g.*, in shale oils[2,4] and crude oils[5] where they, together with alkylated three-ring PASHs, make up the bulk of the PASHs.

The interest in determining individual alkylated isomers in complex mixtures has been spurred by the realization that in the same way that aromatic compounds, whose basic ring structures are isomeric, can display widely different degrees of carcinogenicity, so the alkylation of aromatic compounds can have an equally profound influence on their carcinogenicity. As little is known about this property of alkylated PASHs, a PAH example may serve well. The ability of monomethyl derivatives of benzo[*a*]pyrene (BaP) to initiate mouseskin tumours has been studied[6]; 1-, 3- and

TABLE I

COMPARISON OF THE NUMBER OF ALKYLATED DERIVATIVES OF NAPHTHALENE AND BENZOTHIOPHENE AS A FUNCTION OF THE TOTAL NUMBER OF CARBON ATOMS (x) IN THE SIDE-CHAINS

x	Naphthalenes	Benzothiophenes
1	2	6
2	12	21
3	30	62

11-methyl-BaP show higher activity than BaP itself, 4- and 12-methyl-BaP are about as effective as BaP and 2-, 5- and 6-methyl-BaP display a reduced ability in this respect; 7-, 8-, 9- and 10-methyl-BaP are inactive. The determination of individual alkyl isomers present in a complex mixture can thus be an important task.

Because of the asymmetry introduced into the molecule by the sulphur atom, there are many more isomers of the alkylated benzothiophenes possible than of the alkylated naphthalenes (the same is, of course, true for aromatic compounds containing other heteroatoms). Thus there are six methylbenzothiophenes but only two methylnaphthalenes. This is further illustrated by the data in Table I, which gives the number of C_1-, C_2- and C_3-alkylated benzothiophenes and naphthalenes theoretically possible (C_x denotes a total of x carbon atoms in all side-chains added together).

A fair amount of work has been carried out on the analysis of the dimethylnaphthalenes in crude oils[7], often for geochemical reasons, but no corresponding work is known for the more complex case of the benzothiophenes. The analytical challenge will be greater because of the larger number of isomers that need to be separated and because in most practical samples the benzothiophenes occur as minor constituents, obscured by the more abundant naphthalenes of the same degree of alkylation.

Traditionally, PASHs are determined by capillary gas chromatography (GC) on non-polar stationary phases such as SE-52 and DB-5[8], although more polar stationary phases have been shown to give enhanced selectivity for three-ring PASH isomers[9]. Superox 20M (a polyethylene glycol phase), alone or mixed with SE-52, was found to be superior to SE-52 because of increased interaction between the PASHs and the Superox phase[9]. For the considerably more polar benzothiophene dioxides, which contain the highly polarized SO_2 group, even more polar stationary phases might yield improved selectivity. Dioxides of PASHs do not seem to have been investigated by GC up to now.

In this work the retention data for all C_1- and C_2-benzothiophenes (with the exception of the 4-ethyl isomer) and their dioxides on three different stationary phases of various polarity were determined as a basis for the analysis of alkylated benzothiophenes in environmental and other samples.

EXPERIMENTAL

A Pye Unicam 4500 gas chromatograph equipped with a flame photometric detector was used throughout. The following columns were employed: 30 m × 0.25

mm I.D. DB-5 (J & W Scientific), film thickness 0.1 μm; 26 m × 0.20 mm I.D. Carbowax 20M (Hewlett-Packard); and 50 m × 0.33 mm I.D. CP Sil 88 (Chrompack), film thickness 0.22 μm. Hydrogen was employed as the carrier gas at a linear flow-rate of *ca.* 80 cm/s (measured at 60°C with a bubble flow meter at the detector). A Shimadzu C-R34 integrator or a Spectra-Physics Model SP 4100 computing integrator was used for retention time determinations. The temperature programme included an initial isothermal period of 2 min at 60°C, then programming at 4°C/min to a point just beyond the last peak. For the dioxides on CP Sil 88, the programme was 160°C for 2 min followed by a programming rate of 4°C/min.

The benzothiophenes were dissolved in hexane and the dioxides in ethyl acetate at a concentration of *ca.* 50 μg/ml. Between five and eight compounds were usually determined at a time. The total volume injected never exceeded 2 μl. Each compound was determined four to eight times; 96% of the compounds showed a standard deviation (σ) below 0.15 retention index units. In general, σ for the dioxides was higher than that for the unoxidized compounds.

The benzothiophenes were synthesized according to standard procedures through ring closure of an appropriately substituted α-(phenylthio)ketone or α-(phenylthio)acetal[10] with polyphosphoric acid (PPA). 2-Alkylated compounds were prepared through reaction of the anion of a suitable benzothiophene (generated with butyllithium) and an alkylating agent (dimethyl sulphate, ethyl *p*-methylbenzene sulphonate, *n*-propyl bromide). The crude products were purified by distillation and/or column chromatography on alumina with hexane as eluent.

$$(1)$$
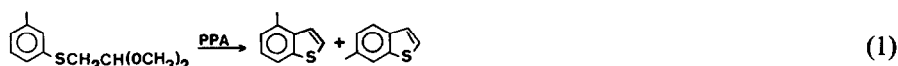
For *meta*-substituted (phenylthio)ketones, two isomeric benzothiophenes can be formed, a 4-alkyl and a 6-alkyl derivative (eqn. 1). In order to correlate the GC peaks of the products with the structures, an unambiguous synthesis of 4-methyl-benzothiophene was devised, outlined in eqn. 2. In the last step, lithium aluminium

$$(2)$$
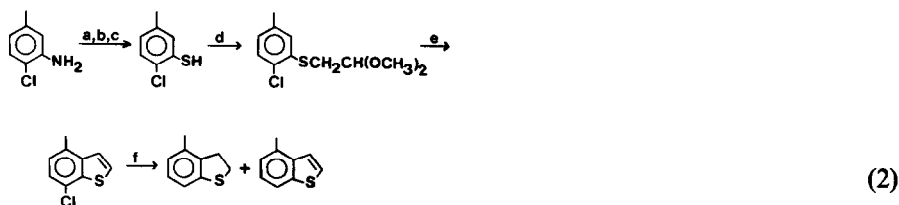
(a) NaNO$_2$, HCl

(b) KSC(=S)OC$_2$H$_5$

(c) NaOH

(d) ClCH$_2$CH(OCH$_3$)$_2$, KOH

(e) Polyphosphoric acid, toluene

(f) Na, tert-BuOH/THF

hydride failed to give any products, so sodium in *tert.*-butanol[11] had to be used. The main product surprisingly turned out to be 4-methyl-2,3-dihydrobenzothiophene

[identified by mass spectrometry (M^+ = 150) and NMR (multiplet at δ 3.15–3.30 ppm in CDCl$_3$)]. The products were separated by column chromatography on alumina. The minor, later eluting peak in the gas chromatogram of the products in eqn. 1 showed the same retention as 4-methylbenzothiophene from the reactions in eqn. 2. In other instances where the analogous isomerism may occur, the smaller peak was assigned to the 4-alkyl derivative, in agreement with this result and with steric considerations, which should favour the less crowded 6-substitution in the ring closure. This situation pertains to the following five pairs of compounds: 4-methyl- and 6-methyl-, 4-ethyl and 6-ethyl-, 2,4-dimethyl- and 2,6-dimethyl-, 3,4- and 3,6-dimethyl-, and 4,5- and 5,6-dimethylbenzothiophene.

No entries are given for 4-ethylbenzothiophene, because in all chromatograms of the product from the attempted synthesis of this compound (as a mixture with the 6-ethyl derivative), only one peak was found; also, in the oxidized fraction only one peak appeared. As the steric hindrance in the ring-closure reaction should favour 6-ethylbenzothiophene and as the GC data for the peak fit the expected data for the latter compound much better than for the former, probably no (or very little) of the 4-ethyl isomer is formed in the reaction.

The dioxides were synthesized by reaction at room temperature with *m*-chloroperbenzoic acid in chloroform for 30 min, followed by washing with solutions of sodium thiosulphate and sodium carbonate. They were purified by recrystallization from dilute ethanol and/or column chromatography on silica gel with benzene to elute non-polar substances followed by benzene–methanol (1:1) to elute the dioxides.

RESULTS AND DISCUSSION

In the GC analysis of real samples, there are in principle two different ways to circumvent the obscuring effect of the much more abundant PAHs on the PASHs: either to use a procedure that contains a step to separate the two types of compounds, or to employ a sulphur-selective detection, commonly flame photometric detection (FPD). Several methods have been proposed for the separation of PASHs from other aromatics[12–14], but none could be made to work satisfactorily when tested for this work. Therefore, FPD remained the choice for detecting sulphur-containing samples and was used exclusively here.

Retention data are preferentially expressed in the form of retention indices (RI). The RI system is based on comparison of the retention times of the compounds of interest with those of calibration standards which, by definition of the original Kováts retention index scale, are the homologous *n*-alkanes. The choice of FPD means that a new series of calibration standards containing a sulphur atom had to be found. The problem is similar to that caused with electron-capture detection, which is widely used for the detection of compounds containing electronegative substituents, typically halogen atoms. In that case standards containing halogen atoms had to be used; the homologous series of the *n*-alkyl trichloroacetates was adopted and has been widely accepted[15].

In the analysis of PAHs, problems were encountered when RI were calculated on the basis of *n*-alkanes[16,17], which led Lee and co-workers to substitute a series of aromatic compounds (benzene, naphthalene, phenanthrene, chrysene and picene) for the *n*-alkanes. This reduced the dependence of RI on factors such as film thickness,

temperature fluctuation and wear of the column, leading to much more reproducible values. The wear of the column with time can have a profound influence on the RI if aromatic compounds are analysed against n-alkane standards. A case involving PCB analyses illustrates this[18]; the problem was solved by using PCBs as (aromatic) standards rather than n-alkanes, as the retention volumes of PCBs relative to each other remained constant.

In agreement with this approach, the following PASHs were selected as RI standards for use with the flame photometric detector: thiophene, benzothiophene, dibenzothiophene (I), benzonaphtho[2,1-d]thiophene (II) and benzophenanthro-[2,1-d]thiophene (III). These compounds were (arbitrarily) assigned the exact RI values of 100, 200, 300, 400 and 500, respectively. However, thiophene and III were not needed in this work. The width of the intervals between consecutive standards will be considerably larger with a limited number of aromatic standards rather than with the more numerous n-alkanes (*ca.* 5.5 times larger on DB-5), but experimentally this does not seem to lead to poorer precision or accuracy[17]. Under the present conditions, one unit corresponds to between 9 and 10 s, depending on the column and interval.

(I)   (II)   (III)
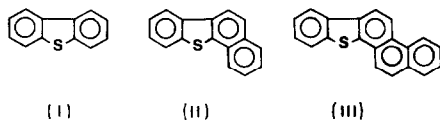
The RI values were calculated according to the equation commonly used for temperature-programmed analyses[16,19]:

$$RI = 100 \cdot \frac{t_{R_x} - t_{R_z}}{t_{R_{z+1}} - t_{R_z}} + 100z$$

where t_R is retention time, x is the compound of interest and z and $z + 1$ are the number of aromatic rings in the standard thiophenes eluting immediately prior to and after the compound, respectively. All compounds eluted during the programming.

Lee and co-workers[16,17] published the RI values of a large number of polycyclic aromatic compounds including some PASHs and nitrogen-containing aromatic compounds. In order to increase the usefulness of the body of data and to facilitate comparisons, the experimental conditions in this work were kept as close as possible to those in their revised work[17]. This includes a 2 min initial isothermal period followed by programming at 4°C/min. Instrumental limitations determined the present starting temperature of 60°C rather than 40°C as in the published work. Further, the choice of our starting temperature was also dictated by the recommended minimum working temperatures of 60 and 55°C, respectively, for two of our stationary phases, Carbowax and CP Sil 88. The higher starting temperature will have some influence on the RI of the faster eluting of the two-ring compounds but hardly any on those of the three-ring and higher PASH.

In Table II the RI values determined on the three stationary phases for all C_1- and C_2- and 2-propylbenzothiophene (except for 4-ethylbenzothiophene, see Exper-

TABLE II

RETENTION INDICES OF ALKYLATED BENZOTHIOPHENES AND BENZOTHIOPHENE DIOXIDES ON DB-5, CARBOWAX 20M AND CP SIL 88

Compound	Benzothiophenes			Dioxides		
	DB-5	Carbowax 20M	CP Sil 88	DB-5	Carbowax 20M	CP Sil 88
Benzothio-phene	200.00	200.00	200.00	276.77	339.06	364.64
2-Methyl	217.97	207.82	208.30	284.44	325.91	346.10
3-Methyl	220.59	213.01	214.30	303.22	355.84	389.16
4-Methyl	220.53	214.15	214.90	302.26*	354.72	384.34
5-Methyl	219.49	212.88	212.85	299.82*	353.24	379.25
6-Methyl	219.29	212.54	212.86	298.65*	350.41 ·	375.38
7-Methyl	216.49	209.04	207.06	285.57	333.79	351.41
2-Ethyl	236.86	219.61	218.78	300.61*	332.76	351.24
3-Ethyl	238.71	224.01	223.91	320.21	361.80	397.50
5-Ethyl	238.23	224.72	223.75	317.20	362.49	387.79
6-Ethyl	238.25	224.32	223.75	315.04	357.26	381.78
7-Ethyl	233.50	219.48	216.05	298.68*	339.01	352.49
2,3-Dimethyl	242.19	226.24	225.49	312.80	347.81	373.94
2,4-Dimethyl	239.40	223.97	223.08	308.27	343.18	365.91
2,5-Dimethyl	238.73	222.20	220.16	306.79	341.94	360.58
2,6-Dimethyl	238.11	221.71	220.11	304.68	338.10	357.02
2,7-Dimethyl	235.74	218.79	215.17	293.37	321.48	334.29
3,4-Dimethyl	249.64	238.56	238.84	336.62	382.55	— **
3,5-Dimethyl	241.58	227.66	227.13	326.63	371.27	404.79***
3,6-Dimethyl	241.61	227.36	226.16	324.70	366.97	398.68
3,7-Dimethyl	238.60	223.50	220.64	312.02	350.89	374.53
4,5-Dimethyl	247.01	236.47	235.74	332.75	379.00	412.29***
4,6-Dimethyl	240.91	228.85	226.60	322.69	366.26	394.13
4,7-Dimethyl	238.53	224.25	220.96	311.09	350.69	370.27
5,6-Dimethyl	245.60	234.79	234.10	326.90	372.48	401.15§
5,7-Dimethyl	236.81	223.16	219.06	307.54	348.99	365.42
6,7-Dimethyl	242.88	230.18	227.99	313.33	354.42	373.92
2-Propyl	254.05	230.17	227.74	316.02	340.40	357.39

* Retention index determined with benzothiophene dioxide and 2,5-dimethylbenzothiophene dioxide as references (see text) and recalculated to the normal system.

** This compound does not elute from the column below the upper temperature limit.

*** Retention index based on dibenzothiophene and naphthobenzothiophene (see text).

§ Retention index determined with dibenzothiophene and 4,5-dimethylbenzothiophene dioxide as references (see text) and recalculated to the normal system.

imental) and their dioxides are given. The stationary phases are, in order of increasing polarity, DB-5, Carbowax 20M and CP Sil 88. DB-5 is a methylphenylsilicone phase containing 5% of phenyl groups and is considered to be non-polar. Carbowax 20M is a polyethylene glycol whose ether oxygen atoms make it a polar material. CP Sil 88 is 100% cyanopropylsiloxane and is highly polar. The sums of the five McReynolds constants for the three phases are *ca.* 337, 2300 and 3700, respectively.

Benzothiophenes on DB-5

From the data in Table II and Fig. 1A it is obvious that on DB-5 a mixture

of the six methylbenzothiophenes will give rise to four signals only; 3- and 4-methylbenzothiophene coelute, as do the 5- and 6-methyl isomers. This was also reported[17] for determinations on SE-54 (which is generally considered to be of the same polarity as DB-5) when the starting temperature was 40°C. Table III compares the RI of the eight compounds which were determined both in this work and on SE-54 by Vassilaros et al.[17]; the RI based on the thioaromatic scale (this work) have been converted into values based on the PAH scale, using the RI values 201.43 and 295.39 from ref. 17 for benzothiophene and dibenzothiophene, respectively. The following equation was used for the conversion:

$$RI_{PAH} = 0.9396(RI_{PASH} - 200) + 201.43$$

where 0.9396 is the scale factor for the RI units in going from the PASH RI scale to the PAH RI scale [0.9396 = (295.39 − 201.43)/100].

The difference between the two sets of determinations for the monomethyl compounds is ca. 2.4 RI units in all instances. This general agreement between the two sets of data, determined on different stationary phases under different conditions, is highly satisfactory.

For the dimethylbenzothiophenes some generalizations can be made. An isomer with a methyl group in the 2- or 7-position has a lower RI than the other isomers. If the two methyl groups are adjacent to each other, a considerably longer retention time is observed than would otherwise be expected. This is seen for all substitution patterns. This ortho-effect is known from methylbenzenes[20] and methylnaphthalenes[21] and also from chloroaromatics[22] and is said to be a function of the lower vapour pressure normally exhibited by ortho-isomers[20]; on non-polar columns there is a linear relationship between retention and the logarithm of the vapour pressure. This effect, which also operates for peri-substituted compounds (e.g., 3,4-dimethylbenzothiophene), is actually seen on all three columns.

The effect increases with increasing polarity of the stationary phase, which means that the elution range of compounds of type a,x-dimethylbenzothiophene, where a is fixed and x varies, is largest on CP Sil 88. The difference between the first and the last eluting x,6-dimethylbenzothiophene is 14 units on the most polar stationary phase but only 7.5 units on DB-5. This effect, which is displayed by both the non-polar benzothiophenes and the polar dioxides, is summarized in Table IV for the three columns.

Benzothiophenes on Carbowax 20M

Since some previous work[9] yielded indications that Carbowax or similar stationary phases might show increased selectivity toward PASHs, its inclusion in this study on alkyl derivatives of a simple PASH was justified. However, for the monosubstituted benzothiophenes there is no improvement in selectivity compared with DB-5; this time 3-, 5- and 6-methylbenzothiophene coelute (Fig. 1B). As on the other stationary phases, 2- and 7-methylbenzothiophene emerge from the column well separated ahead of the other isomers. Similar considerations apply to the ethyl derivatives; here the 3- and 6-ethyl peaks coalesce.

The ortho-effect is even more pronounced than on DB-5, as is the lowering of the RI values for 2- and 7-substituted compounds. 2-Propylbenzothiophene elutes
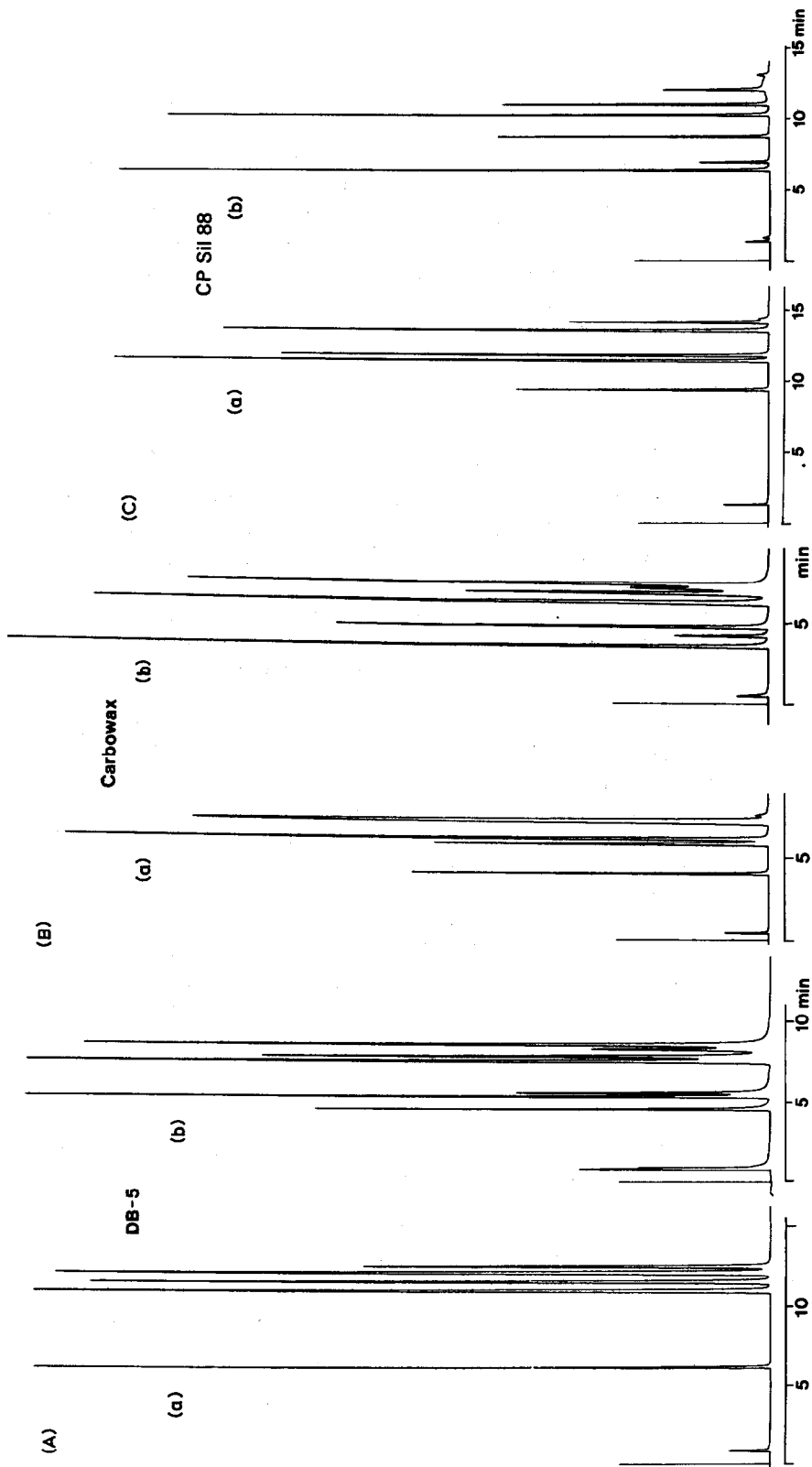
Fig. 1. Gas chromatograms of (a) benzothiophene and the six monomethylbenzothiophenes and (b) the corresponding dioxides. Except for the oven temperature, the conditions are as reported under Experimental. The peaks are identified here by the numbers in parentheses, which refer to the position of the methyl group. 0 = benzothiophene. A + sign indicates coelution. (A) DB-5: (a) 75°C (0, 7, 2, 5 + 6, 3 + 4); (b) 135°C (0, 2, 7, 6, 5, 4, 3). (B) Carbowax 20M: (a) 75°C (0, 2, 7, 3 + 5 + 6, 4); (b) 175°C (0, 2, 7, 6, 5, 4, 3). (C) CP Sil 88: (a) 105°C (0, 7, 2, 5 + 6, 3, 4); (b) 230°C (2, 7, 0, 6, 5, 4, 3).

ahead of four of the C_2 derivatives, as opposed to DB-5, where the elution order strictly follows the number of carbon atoms in the side-chain. Obviously vapour pressure is no longer the sole determining factor for the retention but polar interactions become significant.

Benzothiophenes on CP Sil 88

Also on the very polar stationary phase CP Sil 88 the six monomethyl derivatives appear as four peaks only. The 3- and 4-methyl isomers coelute, as do the 5- and 6-methyl isomers (Fig. 1).

The *ortho*-effect is very prominent, as is the low RI of 2- and 7-substituted compounds. 2-Propylbenzothiophene elutes ahead of four C_2-benzothiophenes. Although the C_2 compounds are spread out over a larger interval on the more polar columns (*cf.*, Table IV) (16.14 units between the first and the last eluting compound on DB-5, 19.81 units on Carbowax 20M and 26.06 units on CP Sil 88), the number of overlapping peaks is only slightly less than on DB-5. Twelve to fifteen of the twenty C_2-isomers coelute with one or more other isomers on the different columns. Just as none of the stationary phases will separate all the monomethylbenzothiophenes, less surprisingly, none will separate all the C_2 compounds either.

Benzothiophene dioxides

The three types of stationary phases investigated here cover the whole range of polarities available. The very similar results obtained make it unlikely that other stationary phases would provide much enhanced selectivities unless they exhibit very special properties. For the analysis of complex samples, the selectivity of the common stationary phases is simply insufficient.

However, at least with the lower PASHs, there might be a simple way to improve this situation, namely to use a rapid and quantitative derivatization reaction. Oxidation to the dioxides with *m*-chloroperbenzoic acid in, *e.g.*, chloroform is complete in a few minutes and side-reactions appear not to play a role. This oxidant is superior to hydrogen peroxide, which requires higher reaction temperatures and fre-

TABLE III

COMPARISON OF RETENTION INDICES* OF EIGHT ALKYLATED BENZOTHIOPHENES DETERMINED ON DB-5 (STARTING TEMPERATURE 60°C, THIS WORK) WITH THOSE DETERMINED ON SE-54 (STARTING TEMPERATURE 40°C, REF. 17)

Compound	SE-54**	DB-5	Difference
2-Methyl	220.76	218.31	2.45
3-Methyl	223.08	220.78	2.30
4-Methyl	223.15	220.72	2.43
5-Methyl	222.09	219.74	2.35
6-Methyl	222.11	219.55	2.56
7-Methyl	219.16	216.92	2.24
5-Ethyl	236.14	237.35	−1.21
3,5-Dimethyl	243.56	240.50	3.06

* Based on naphthalene/phenanthrene as standards (see text).
** From ref. 17.

TABLE IV

SIZE OF THE ELUTION RANGE FOR a,x-DIMETHYLBENZOTHIOPHENES AND THEIR DIOXIDES (IN RETENTION INDEX UNITS)

Compounds	a	DB-5	Carbowax 20M	CP Sil 88
a,x-Dimethylbenzothiophenes	2	6.45	7.45	10.32
	3	11.04	15.06	18.20
	4	11.11	14.59	17.88
	5	10.20	14.27	16.68
	6	7.49	13.08	13.99
	7	7.14	11.39	12.82
a,x-Dimethylbenzothiophene	2	19.43	26.33	39.65
dioxides	3	24.60	34.74	> 30.85*
	4	28.35	39.37	> 46.38*
	5	25.96	37.06	51.71
	6	22.22	34.38	44.13
	7	19.96	32.94	40.24

* 3,4-Dimethylbenzothiophene dioxide could not be determined on CP Sil 88 (see text).

quently gives rise to side-products. The reaction is exemplified by the oxidation of 3,7-dimethylbenzothiophene (eqn. 3).

$$ (3) $$
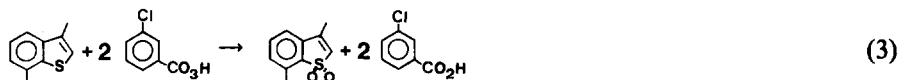
The dioxides have a vapour pressure high enough to allow their analysis by GC. They have been shown to be stable under normal GC conditions[23]. In order to explore their behaviour on the three columns used here, all 28 dioxides of the C_0-, C_1-, C_2- and 2-propylbenzothiophenes were synthesized and analysed in the same way as the parent compounds.

Benzothiophene dioxides on DB-5

Owing to their polar nature, the dioxides have a lower vapour pressure than the parent compounds and consequently elute considerably later. On DB-5 the monomethyl dioxides have retention times comparable to that of dibenzothiophene. The most striking difference from the methylbenzothiophenes, however, is the complete resolution of the six isomers. In Fig. 1 the chromatograms obtained for benzothiophene and its six monomethyl derivatives and also for the corresponding dioxides are shown for all three stationary phases. The excellent improvement on going from the benzothiophenes to the dioxides is obvious, regardless of the stationary phase.

The influence of the substituent position is very striking. While the 3-, 4-, 5- and 6-methyl dioxides elute fairly close together, the 2- and 7-methyl dioxides display a much shorter retention time. While this trend is also true for the unoxidized parents, the effect is considerably more pronounced for the dioxides (and is further enhanced on the more polar stationary phases, see below). This strong dependence on the substitution pattern has the undesirable consequence of moving some C_2-oxides into the region of the C_1-oxides. There are three of the former (2-ethyl, 7-ethyl and 2,7-

dimethyl) eluting before the last eluting C_1-oxide. It is thus clear that isomers with substituents flanking the functional group elute ahead of the others.

This effect is numerically expressed by the difference in RI between a dioxide and its parent compound. This difference is in a sense the RI increment of the dioxide group. As can be seen in Table V, in which the increment is listed for the monosubstituted compounds, this increment can be sensitive to changes in substitution pattern, generally being lower for ethyl than for methyl derivatives and much lower for 2- and 7-substituted dioxides, causing them to elute ahead of the isomeric compounds. Again, the extremes are displayed by the most polar stationary phase where this increment can vary between 178 units (for 3,5-dimethyl-) and 119 units (for 2,7-dimethylbenzothiophene dioxide).

The chain length of the substituents still plays a role, of course. The difference in RI units between the dioxides of 2-propyl-, 2-ethyl- and 2-methylbenzothiophene dioxide is *ca.* 16 units per methylene group (less than the value for the unoxidized parent compounds), but this is overshadowed by the effect of the substituent position. For the dimethyl dioxides, the difference in RI units between the last and the first eluting isomer is no less than 43 units (compared with 11.6 units for the parent compounds), meaning that the exact structure of the dioxides plays a much larger role for the retention than with the parent compounds. That the effect of the chain length on the retention is subordinate to the effect of the substitution pattern is further demonstrated by 2-propylbenzothiophene dioxide, which elutes ahead of eight (of the twenty) C_2-dioxides.

Several dioxides nearly coalesced with dibenzothiophene. Their RI values were determined (under the usual chromatographic conditions) with 2,5-dimethyl- and benzothiophene dioxides as reference compounds and the values thus obtained were recalculated to the normal scale used.

Dioxides on Carbowax 20M

The general trend for the monomethyl derivatives is the same as on DB-5, but

TABLE V

DIFFERENCE IN RETENTION INDEX BETWEEN MONOSUBSTITUTED BENZOTHIOPHENE DIOXIDES AND THE CORRESPONDING BENZOTHIOPHENES (INCREMENTS FOR THE DIOXIDE FUNCTIONAL GROUP)

Substituent	DB-5	Carbowax 20M	CP Sil 88
None	76.77	139.06	164.64
2-Methyl	66.47	118.09	137.80
3-Methyl	82.63	142.83	174.80
4-Methyl	81.70	140.57	169.44
5-Methyl	80.33	140.36	166.40
6-Methyl	79.36	137.87	162.52
7-Methyl	69.08	124.75	144.35
2-Ethyl	63.75	113.15	132.46
3-Ethyl	81.50	137.79	173.59
5-Ethyl	78.97	137.77	164.04
6-Ethyl	76.79	132.94	158.03
7-Ethyl	65.18	119.53	136.44
2-Propyl	61.97	110.23	129.65

the more polar stationary phase will retard the (polar) dioxides more than the (non-polar) reference compounds and thus the RI values will tend to be higher on this stationary phase. On Carbowax 20M benzothiophene dioxide itself elutes later than all those monosubstituted dioxides which carry a methyl or ethyl group in the 2- or 7-position. Possibly the flanking alkyl group exerts some steric hindrance to efficient interactions with the stationary phase. This is evident for 2-propylbenzothiophene dioxide also, which elutes ahead of all but four of the C_2- and all but two of the C_1-dioxides.

Dioxides on CP Sil 88

The results on this very polar stationary phase are similar to those on Carbowax 20M, but more pronounced. Several C_1- and C_2-dioxides, substituted in the 2- and/or 7-positions, including the 2-propyl derivative, elute before benzothiophene dioxide itself, underlining the hindering effect of the flanking alkyl groups on the interactions of the SO_2 group with the stationary phase. Also in this instance, four of the six monomethyl dioxides are more strongly retained than the 2-propyl derivative.

On this column some dioxides emerge after benzo[2,1-*d*]naphthothiophene (II), the standard used to define the RI value 400. As the next standard, benzophenanthro[2,1-*d*]thiophene, did not elute below the upper temperature limit of the stationary phase, the RI values for those compounds have been calculated on the basis of dibenzothiophene/benzonaphthothiophene. 5,6-Dimethylbenzothiophene dioxide nearly coincided with the latter standard and its RI value had to be determined indirectly using dibenzothiophene and 4,5-dimethylbenzothiophene dioxide as secondary reference compounds. 3,4-Dimethylbenzothiophene dioxide did not elute at all at temperatures below 260°C, above the upper temperature limit for the stationary phase; consequently, no RI could be determined for this compound on CP Sil 88.

Some of the effects discussed above are further demonstrated by the data in Table VI, which shows the RI increments for a methylene unit in the side-chain of the monosubstituted benzothiophenes and their dioxides. For the parent compounds, the RI increment is larger for the first methylene unit (going from a methyl to an ethyl derivative) than for the second one (from an ethyl to a propyl derivative); this is true for all the stationary phases. The increment varies slightly with the position of the substituent, mainly in that the 7-substituted compounds generally show a smaller increment than other isomers.

TABLE VI

RETENTION INDEX INCREMENTS FOR A METHYLENE UNIT IN SOME ALKYLATED BENZOTHIOPHENES (BT) AND THEIR DIOXIDES (BTO$_2$)

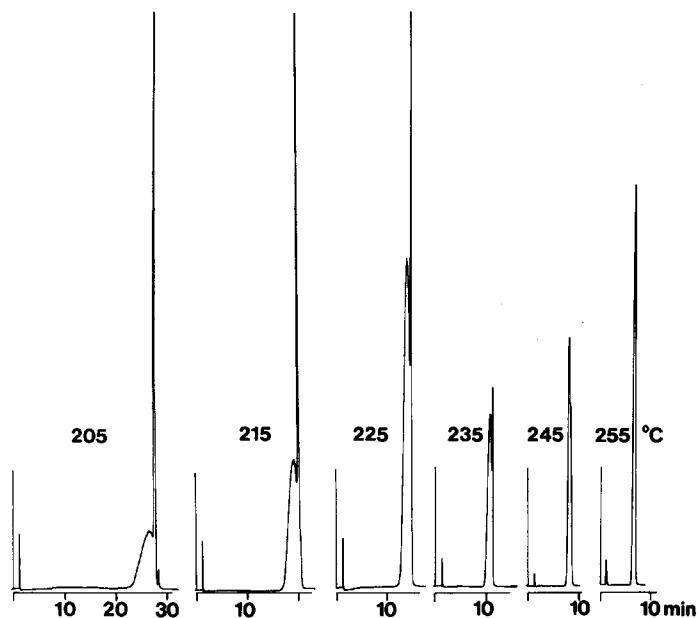| Substituents compared	DB-5		Carbowax 20M		CP Sil 88	
	BT	BTO$_2$	BT	BTO$_2$	BT	BTO$_2$
2-Pr–2-Et	17.19	15.41	10.56	7.64	8.96	6.15
2-Et–2-Me	18.89	16.17	11.79	6.85	10.48	5.14
5-Et–5-Me	18.74	17.38	11.84	9.25	10.90	8.54
7-Et–7-Me	17.01	13.11	10.04	5.22	8.99	1.08

Fig. 2. 3-Methylbenzothiophene dioxide chromatographed at different temperatures on CP Sil 88. Injector and detector temperature, 250°C.

Elongation of the alkyl chain by a methylene unit has a smaller effect on the RI the more polar the stationary phase and the more polar the molecule is, reflecting how polar interactions become more important for the retention. The extreme case is the 7-alkylated dioxides on CP Sil 88, where the RI difference between the ethyl and the methyl derivatives is only 1 unit.

Finally, the curious behavior displayed by 3-methylbenzothiophene dioxide on CP Sil 88 should be mentioned. On this stationary phase only, this dioxide, but no other isomer, appeared as a broad peak followed by the normal narrow peak [compare the peaks in Fig. 1C (b)]. The retention time of the latter peak was used and fits well into the general picture. Probably a decomposition occurs on the column. The temperature dependance is illustrated in Fig. 2. At lower temperatures (205°C) relatively little of the broad peak is visible and the normal sharp peak dominates. However, at 255°C only the "broad" peak appears, which at this temperature is narrow and more "normal" looking.

The initial assumption that it is a temperature effect and for that reason only seen on CP Sil 88, where the elution temperature under normal conditions is considerably higher than for the other phases (ca. 152, 183 and 245°C for DB-5, Carbowax 20M and CP Sil 88, respectively) was tested in a separate experiment. The compound was sealed in a melting point capillary and heated at 250°C for 5 min. However, the product of this treatment gave a gas chromatogram which was indistinguishable from that of the starting material. When another column with a cyanopropylsiloxane stationary phase (SP 2340) was used, no sign of decomposition in the temperature range 185–225°C was observed and a chromatogram of all the monomethylbenzothiophene dioxides contained a peak of the correct size for the 3-methyl

derivative. Hence it is probably a specific effect of the CP Sil 88 column, the origin of which is unknown.

Many of the features discussed above can be brought out more clearly graphically, and for that reason some of the data in Table II are plotted in Fig. 3. In Fig. 3a the good correlation between the RI on CP Sil 88 and those on DB-5 is displayed. Different straight lines are obtained depending on the number of carbon atoms in the substituents. Compounds with a substituent in the 2-position (filled circles in Fig. 3a) and, to a lesser extent, those with a 7-substituted pattern generally lie below the other points because they are less retained than other isomers on CP Sil 88.

A similar good correlation is obtained for the dioxides on CP Sil 88 and DB-5 (Fig. 3b), with a correlation coefficient for the C_2 compounds of 0.991. The slope of
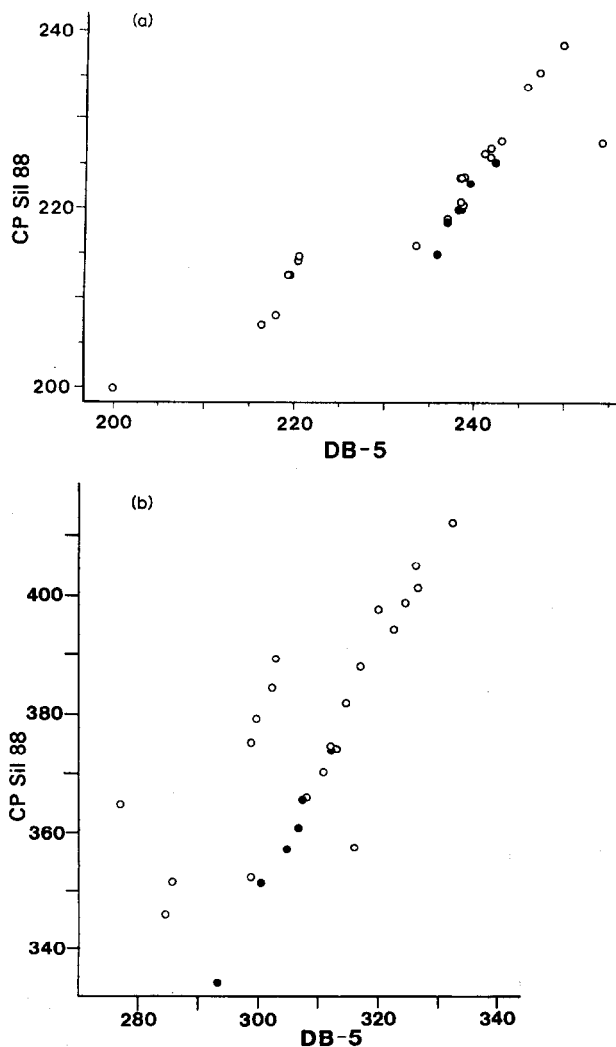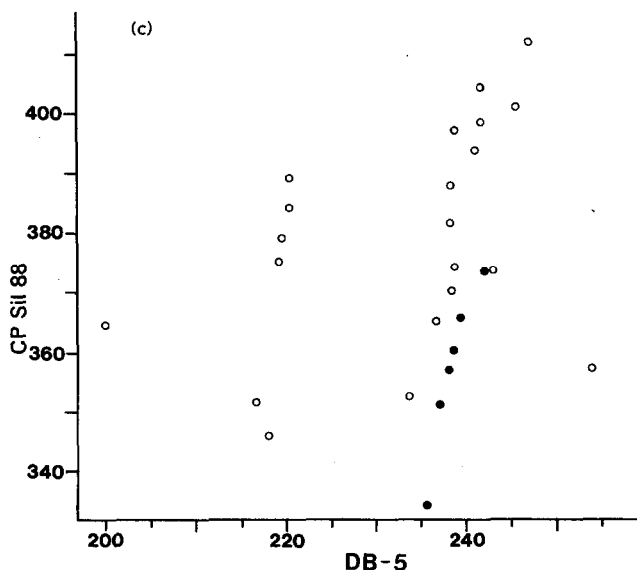

Fig. 3.

Fig. 3. Plots of retention indices for (a) alkylated benzothiophenes on DB-5 and CP Sil 88, (b) alkylated benzothiophene dioxides on DB-5 and CP Sil 88 and (c) alkylated benzothiophene dioxides on CP Sil 88 vs. alkylated benzothiophenes on DB-5. Filled circles represent 2-substituted compounds.

1.99 shows how CP Sil 88 spreads the compounds over a larger elution interval. There are fewer overlapping peaks with the polar stationary phase, but this may simply be due to the fact that the compounds elute over a larger interval (slope > 1) and less to some unique selectivity of this phase which should have manifested itself in a low correlation coefficient. In this plot the 2-substituted C_2 isomers lie on the same straight line as the other isomers. Fig. 3b also makes it visually clear how the dioxides of benzothiophene and the C_1-benzothiophenes on this polar stationary phase fall in the same interval as those of the C_2-benzothiophenes. Similar correlations are obtained if the data from Carbowax 20M are plotted against those from the other stationary phases.

The high degree of correlation makes it unlikely that any combination of these three stationary phases would lead to much additional information compared with the use of only one column. The high correlation also shows that the choice of stationary phase appears not to be very critical, unless specific separation problems must be solved.

The last case, a plot of the RI values of dioxides against those of the parent compounds, is presented in Fig. 3c. The unoxidized benzothiophenes were measured on DB-5 and their dioxides on CP Sil 88. In this instance the points are much less correlated with each other; this is true also if the same stationary phase is used for both classes of compounds, but the effect is not as pronounced as in Fig. 3c.

The procedure of oxidizing a sample and chromatographing both the unoxidized and oxidized samples for the identification of individual alkylated benzothiophenes is currently under investigation.

CONCLUSIONS

Of the three stationary phases studied in this work, the most polar, consisting of 100% cyanopropylsiloxane, proved more capable of resolving a large number of alkylated benzothiophenes than the less polar Carbowax and DB-5. On oxidation with *m*-chloroperbenzoic acid, benzothiophenes yield the corresponding dioxides, which can be chromatographed with ease.

The substitution pattern of both parent and oxidized benzothiophene has a great influence on their retention:

(a) 2- and 7-substituted compounds always elute faster than other isomers; this effect is particularly striking for the dioxides;

(b) *ortho*-substitution contributes to a longer retention time;

(c) on polar stationary phases, the contribution of the dioxide group to the retention often alters the normal elution order of C_1-substituted derivatives eluting before C_2-substituted derivatives and so on;

(d) the substitution pattern influences the retention of the dioxides more than that of the unoxidized benzothiophenes, causing them to elute over a larger interval;

(e) to obtain the largest number of resolved peaks, benzothiophenes should be chromatographed as dioxides.

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