

# Gas chromatographic retention behaviour of dibenzothiophene derivatives on a smectic liquid crystalline polysiloxane stationary phase

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(First received June 11th, 1991; revised manuscript received September 19th, 1991)

## ABSTRACT

The gas chromatographic retention behaviour, on a smectic liquid crystalline polysiloxane stationary phase, of methyl dibenzothiophenes and eighteen C<sub>2</sub>-dibenzothiophenes (sixteen dimethyldibenzothiophenes and two ethyldibenzothiophenes), out of twenty possible compounds, was investigated. The retention, in addition to vapour pressure and polarity, was greatly influenced by the molecular geometry of the solutes. The major factor affecting this behaviour was the length to breadth ratio ( $L/B$ ). The elution order of the dimethyldibenzothiophenes was fairly well correlated with  $L/B$  values: Roughly, the lower the  $L/B$  values, the earlier the dimethyldibenzothiophenes were eluted. However, the solute molecular shape, although of less importance, was also a significant retention-affecting factor, having the contrary effect to  $L/B$  values on the elution order. Arc-like molecules (dibenzothiophenes) with groups attached to the outer curved side (*i.e.*, in positions 3 and 4) were retained longer than predicted by  $L/B$  values. In contrast, isomers conforming to the arc-like arrangement (*i.e.*, with substituents in positions 1 and 2) were less retained than predicted. The application of these identifications to a crude oil sample is described.

## INTRODUCTION

Complex mixtures of alkylated polycyclic aromatic sulphur heterocyclic (PASHs) present in natural matrices contain numerous isomeric structures. Increasing attention has been paid to the separation and determination of individual alkylated PASHs both in organic geochemistry [1–3] (the distribution of methylated dibenzothiophenes may be typical of the origin or of the maturation of crude oils [4–6]) and in environmental chemistry [7] (isomeric forms often have different carcinogenic or mutagenic activities). Even if little is known about these properties of alkylated PASHs, the example of the ability of monomethyl derivatives of benzo[*a*]pyrene (BaP) to initiate mouse skin tumours may illustrate this well [8].

Much systematic work has been carried out on

the analysis of polycyclic aromatic hydrocarbons (PAHs), with systematic studies of the C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub> and C<sub>4</sub> derivatives of compounds such as naphthalene [9–11] and phenanthrene [12–14], often for geochemical reasons, but no corresponding systematic work is known for dibenzothiophenes. The detection of these compounds represents a real challenge because of the large number of isomers to be separated. Moreover, in most instances the dibenzothiophenes are minor constituents compared with the more abundant phenanthrenes. In order to obtain a good gas chromatographic separation, we chose a liquid crystalline stationary phase for the analysis of dibenzothiophene derivatives.

Liquid crystalline stationary phases have temperature-dependent ordered structures. The solute interacts with this ordered structure [15]. While compounds on non-polar stationary phases elute

according to their increasing boiling points, on liquid crystalline stationary phases retention is also governed by solute geometry. In liquid chromatography the length-to-breadth ratio ( $L/B$ ) had appeared as a reliable descriptor for polymeric  $C_{18}$  phases [16]. Nishioka *et al.* [17] also related gas chromatographic retention behaviour, on liquid crystalline phases, to this ratio and to the space molecular shape. In this work, retention data for  $C_1$ - and  $C_2$ -dibenzothiophenes (DBTs) on a liquid crystalline stationary phase were determined with a view to geochemical applications, and we also correlated these data with the  $L/B$  values for each molecule studied. Liquid crystals can demonstrate nematic, cholesteric or smectic properties within a certain temperature range. It has been generally accepted that nematic phases produced a better chromatographic resolution than did smectic phases [18]. However, Nishioka *et al.* [17] have developed smectic liquid crystalline stationary phases which demonstrated very good resolution for isomeric polycyclic aromatic compounds (PACs). These considerations led us to choose a smectic liquid crystalline phase for the separation of the  $C_1$ - and  $C_2$ -benzothiophenes.

## EXPERIMENTAL

### Materials

The methyl-dibenzothiophenes (MDBTs) and the  $C_2$ -dibenzothiophenes [dimethyl-dibenzothiophenes (DMDBTs) and ethyl-dibenzothiophenes (EtDBTs)] were synthesized at Brigham Young University (Provo, UT, USA) [19] and were kindly provided by M. L. Lee. The positions of substitution on the aromatic nucleus were numbered according to the

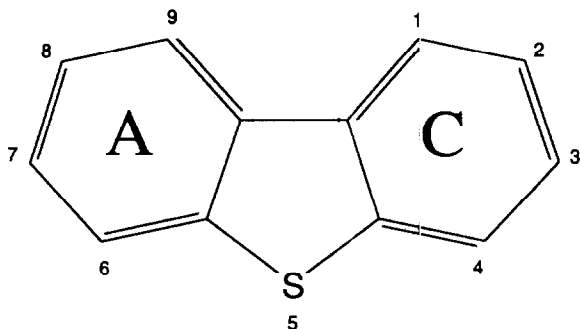


Fig. 1. Numbering of the dibenzothiophene molecule.

numbering system currently in use (Fig. 1) with the sulphur atom at position 5.

The crude oil sample came from a Tunisian oil field. Sample preparation and aromatic compound separation by high-performance liquid chromatography (HPLC) have been described elsewhere [20].

### Retention indices

Retention data are expressed in the form of retention indices ( $RI$ ). They are based on comparison of the retention times of the compounds of interest with those of calibration products. In the original Kováts retention index scale, they are the homologous  $n$ -alkanes. However, for the analysis of PAHs, Lee *et al.* [21] introduced a calibration scale specifically based on aromatic standards, benzene, naphthalene, phenanthrene, chrysene and picene, denoted in this work as  $RIN$  (retention indices for neutral aromatic compounds). According to this approach, Andersson [22] also defined a scale for the analysis of benzothiophenes based on the following PASHs: thiophene, benzothiophene, dibenzothiophene, benzonaphtho[2,1-*d*]thiophene and benzo-phenanthro[2,1-*d*]thiophene. These compounds were assigned  $RI$  values of 100, 200, 300, 400 and 500, respectively. The  $RI$  values based on this scale are denoted  $RIS$  (retention indices for sulphur aromatic compounds) and were calculated according to the equation [21]

$$RIS = 100 \cdot \frac{t_{r_x} - t_{r_z}}{t_{r_{z+1}} - t_{r_z}} \quad (1)$$

where  $t_r$  is retention time,  $x$  the compound of interest (in this study the DBT derivatives) and  $z$  and  $z + 1$  are the number of aromatic rings in the standard thiophenes eluting prior to and after the studied compounds (here  $z = 3$ ).

### Chromatographic and detection conditions

The DBT derivative standards and the collected HPLC fractions were analysed by gas chromatography-mass spectrometry, using an HP 5890 Series II gas chromatograph equipped with a splitless injector (purge delay 30 s, purge flow 60 ml/min) and a 50 m  $\times$  0.22 mm I.D. SB-Smectic phase column (Dionex, Lee Scientific Division), film thickness 0.1  $\mu$ m. Helium was used as the carrier gas with a column head pressure of 3.5 bar. The detector was an HP 5970 mass-selective detector [electron impact,

70 eV; selected ion monitoring (SIM) mode,  $m/z$  184 for DBT,  $m/z$  198 for the MDBTs,  $m/z$  212 for the  $C_2$ -DBTs; 2 scans/s; voltage, 2200 V]. The temperature programme consisted of an initial isothermal period of 2 min at 50°C, then programming at 10°C/min to 140°C, followed by an isothermal period of 2 min at 140°C, then programming at 2°C/min to 250°C with an isothermal period of 30 min at 250°C. The injector and detector were kept at 250°C.

The DBT derivatives were dissolved in cyclohexane at a concentration of 40 µg/ml. The retention time of each compound was determined three to five times; all the compounds showed a standard deviation below 0.20 *RIS*.

#### Length to breadth ratio (*L/B*) calculations

The calculations were carried out on a desk-top microcomputer (TANDY 3000 NL) for the  $C_1$ - and  $C_2$ -DBT isomers using the MMX software (Serena Software, Bloomington, GA, USA), derived from the original MM2MP2 empirical force field method [23,24]. The calculations provided an estimate of the steric energy and the heat of formation ( $\Delta H_f^0$ ) at 298 K in the gas phase of the compounds; they also yielded the geometry of the molecule [bond lengths, angles and particularly the dihedral angle between rings A and C (see Fig. 1)]. In-house software also provided the length (*L*) and the breadth (*B*) of the molecule, which correspond to the dimensions of a minimized rectangle for each molecule studied.

## RESULTS AND DISCUSSION

#### Retention indices and molecular shape

A SIM gas chromatogram of DMDBT and MDBT standards used in the determination of retention indices is shown in Fig. 2.

The *RIS* and *RIN* values determined on the smectic liquid crystalline stationary phase for all  $C_1$ - and  $C_2$ -dibenzothiophenes (except for 1- and 3-EtDBT, which were not available) are given in Table I. One unit of *RIS* correspond to 30 s. Compounds differing by 0.60 *RIS* units were baseline resolved.

All the methyl-dibenzothiophenes are separated on the smectic phase and there are only three severe co-elutions, between 1,4- and 1,8-DMDBT, 2,7- and 3,6-DMDBT and 1,2-, 1,7- and 1,9-DMDBT, for

the eighteen studied  $C_2$ -DBT isomers. These results demonstrate once again the selectivity of liquid crystal phases, as shown previously for methylphenanthrenes [17].

The DMDBT isomers differ significantly in length, breadth and *L/B* values (see Table I). The elution order of DBT derivatives follows roughly increasing *L/B* values (see Fig. 3). For instance, 3,7- and 2,3-DMDBT, which exhibit high *L/B* values (1.81 and 1.48, respectively) are eluted latter whereas 4,6- and 2,4-DMDBT with low values of *L/B* (1.11 and 1.04, respectively) are eluted first (see also Fig. 3).

The correlation equation obtained for the fifteen DMDBTs is

$$RIS = 281.75 + 32.23L/B \quad (2)$$

with a correlation coefficient  $r = 0.74$ . Predicted *RIS* values ( $RIS_c$ ) were calculated from eqn. 2 and compared with measured values. The average deviation was *ca.* 1%. Higher deviations of *ca.* 4% were obtained for 3,4- and 2,8-DMDBT. The correlation coefficient ( $r$ ) increases to 0.93 when 2,8- and 3,4-DMDBT are omitted and reaches 0.98 when, in addition, 1,8- and 1,9-DMDBT (deviation higher than 1%) are not considered. This means that the retention behaviour of the compounds is not exactly and entirely dependent only on the *L/B* values. The spatial molecular shape (as discussed previously by Nishioka *et al.* [17]) and dihedral angles or, more precisely, the distortion of the molecules (as mentioned and shown more precisely for dimethylphenanthrene isomers in the case of liquid chromatography [16]) are also important factors affecting retention.

A correlation coefficient of 0.57 is obtained when the retention indices (*RIS*) of all sixteen DMDBTs are plotted as a function of the molecular length (*L*) and 0.72 for the molecular breadth (*B*) (Table I). This indicates that the retention behaviour of DMDBT isomers is mainly controlled by the breadth of the molecules. When 3,4-, 2,8-, 1,8- and 1,9-DMDBT are not considered in the DMDBT set, the latter correlation coefficient reaches 0.96.

The relatively anomalous behaviour of 3,4-, 2,8- and 1,8-DMDBT is dependent on the molecular shape of the solutes. As can be seen in Table I, by comparison of the *RIS* and  $RIS_c$  values, 3,4-DMDBT is more retained and 2,8- and 1,8-DMDBT are eluted earlier than predicted from their *L/B* values.

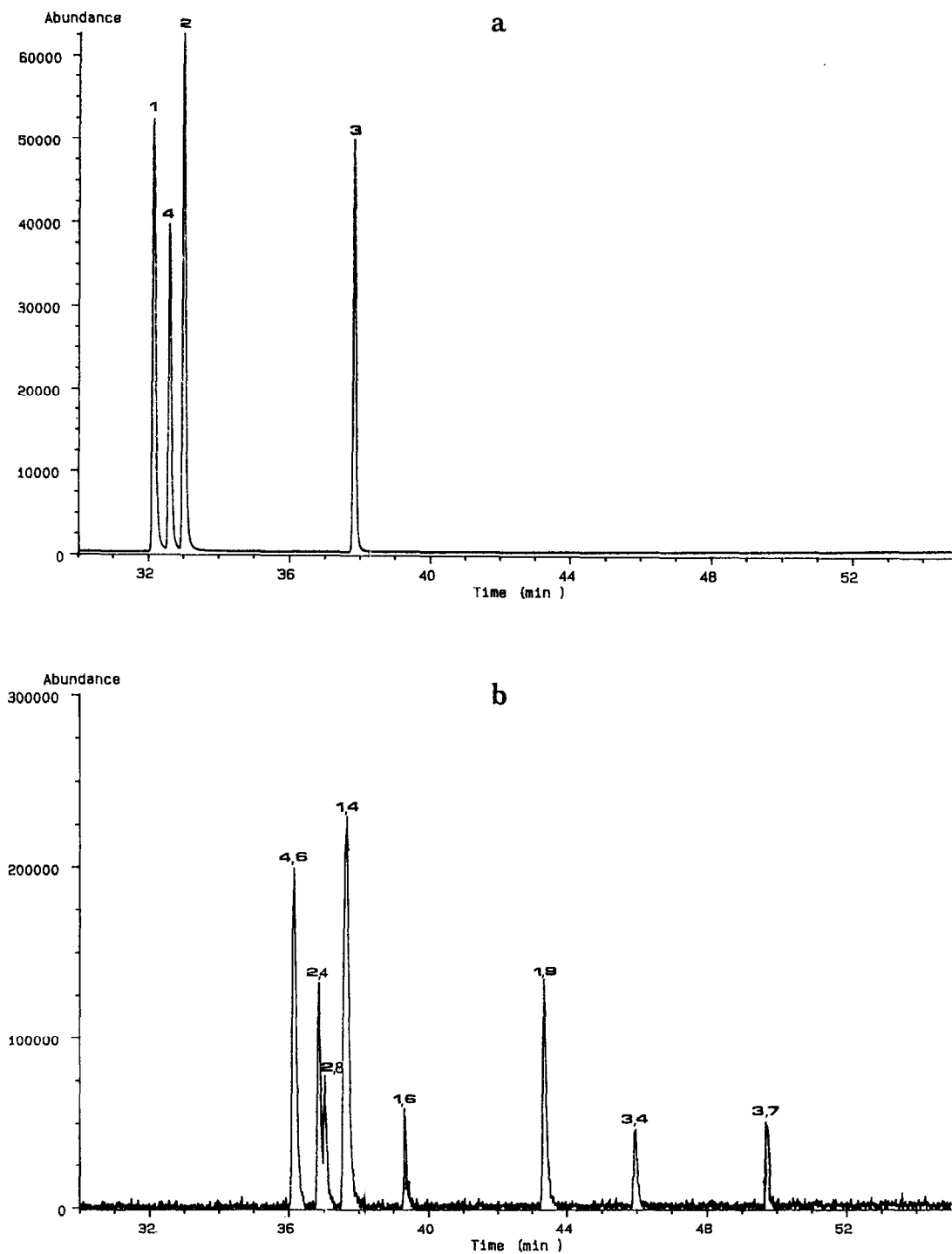


Fig. 2. Partial SIM gas chromatograms of DBT derivative standards used in the determination of gas chromatographic retention indices (see text for experimental conditions). (a) MDBT,  $m/z$  198; (b)  $C_2$ -DBT,  $m/z$  212.

TABLE I

RETENTION INDICES AND SHAPE PARAMETERS FOR METHYL-, ETHYL- AND DIMETHYLDIBENZOTHIOPHENE ISOMERS

*RIS* and *RIN* were determined by experiments whereas *RIS<sub>c</sub>* were calculated through the correlation eqns. 2 and 3 (see text).

Compound	<i>RIN</i>	<i>RIS</i>	<i>RIS<sub>c</sub></i>	Deviation (%)	Length ( <i>L</i> ) (Å)	Breadth ( <i>B</i> ) (Å)	<i>L/B</i>	Dihedral angle (°) <sup>a</sup>
1-MDBT	302.41	308.53	310.33	+0.6	11.40	8.30	1.37	n.d.
2-MDBT	303.85	310.30	314.24	+1.2	11.95	7.95	1.50	n.d.
3-MDBT	312.08	320.35	316.85	-1.1	12.55	7.90	1.59	n.d.
4-MDBT	303.08	309.34	307.08	-0.7	11.40	8.90	1.28	n.d.
1,2-DMDBT	319.75	329.73	327.19	-0.8	12.10	8.55	1.41	8.4
1,3-DMDBT	316.28	325.31	328.81	+1.1	12.60	8.60	1.46	5.6
1,4-DMDBT	309.81	317.40	319.78	+0.7	11.65	9.90	1.18	5.8
1,6-DMDBT	312.63	320.85	321.07	+0.1	11.70	9.55	1.22	6.5
1,7-DMDBT	319.53	329.28	329.77	+0.1	12.50	8.40	1.49	5.9
1,8-DMDBT	309.81	317.40	323.33	+1.9	12.30	9.50	1.29	5.4
1,9-DMDBT	319.53	329.28	323.33	-1.8	11.40	8.85	1.29	20.0
2,3-DMDBT	322.40	332.97	329.77	-1.0	12.50	8.40	1.49	0.1
2,4-DMDBT	308.51	315.80	316.88	+0.3	11.50	10.55	1.09	0.1
2,6-DMDBT	314.87	323.59	323.33	+0.1	12.60	9.75	1.29	0.4
2,7-DMDBT	321.50	331.70	331.38	-0.1	13.00	8.45	1.54	0.1
2,8-DMDBT	308.81	316.17	328.16	+3.8	12.05	8.35	1.44	0.1
3,4-DMDBT	323.87	334.60	321.07	-4.0	11.65	9.55	1.22	0.1
3,6-DMDBT	321.58	331.79	331.06	-0.2	12.80	8.35	1.22	0.6
3,7-DMDBT	330.21	342.34	340.09	+0.7	13.50	7.45	1.81	0.1
4,6-DMDBT	307.30	314.33	317.52	+1.0	11.65	10.50	1.11	0.1
2-EtDBT <sup>b</sup>	305.55	312.19	n.d. <sup>c</sup>	n.d.	n.d.	n.d.	1.32	n.d.
4-EtDBT	306.54	313.39	n.d.	n.d.	n.d.	n.d.	1.37	n.d.

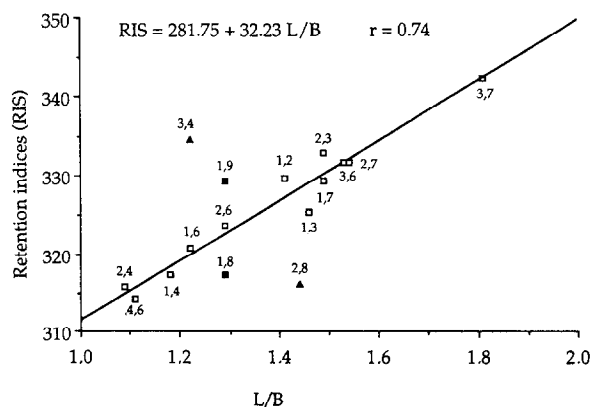
<sup>a</sup> Angles between rings A and C (see Fig. 1).<sup>b</sup> Et = Ethyl.<sup>c</sup> n.d. = Not determined.

Fig. 3. Plot of gas chromatographic retention indices (*RIS*) versus *L/B* for the sixteen studied DMDBT isomers. ▲ = Deviation > 3%; ■ = deviation > 1%; □ = deviation < 1%.

The same features can be observed for the MDBTs. The correlation equation obtained for the four MDBTs (see Fig. 4) is

$$RIS = 265.86 + 32.24L/B \quad (3)$$

with a correlation coefficient  $r = 0.80$ . *RIS<sub>c</sub>* values were calculated from eqn. 3 and compared with the measured values (*RIS*). Despite a good agreement between the *RIS* and *RIS<sub>c</sub>* values (see Table I; the highest deviations were near 1%), the 1- and 2-MDBTs are eluted earlier than predicted by their *L/B* values, with the largest difference for 2-MDBT (see Table I). In comparison, the 3- and 4-MDBTs are more strongly retained, with the greatest deviation for 3-MDBT (see Table I).

As previously reported by Nishioka *et al.* [17], arc-like molecules (such as DBT or DBT with methyl groups in position 1 or 2; see Fig. 1) seem to

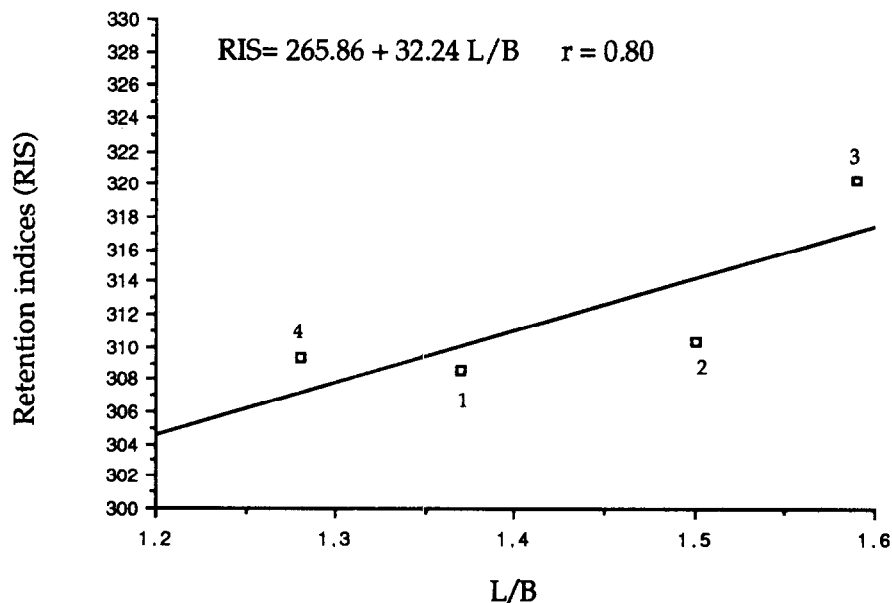


Fig. 4. Plot of gas chromatographic retention indices (*RIS*) versus *L/B* for the 4 MDBT isomers.

be eluted earlier than predicted by the *L/B* ratios. In contrast, molecules with substituents attached to the outer curved side (*i.e.*, DBT with methyl groups in positions 3 and 4; see Fig. 1) seem to be retained longer than expected. The apparently anomalous behaviour of 3,4-, 2,8- and 1,8-DBT fits these

observations, but no retention mechanism has been proposed.

#### Analytical applications

Fig. 5 shows the identification of the  $C_1$ - $C_2$ -DBTs in a crude oil. True identifications

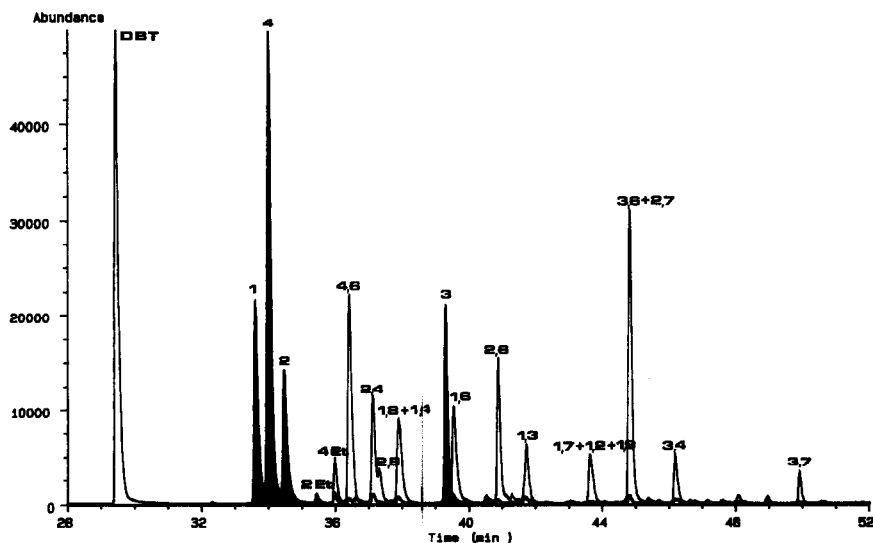


Fig. 5. SIM gas chromatogram ( $m/z$  184 for DBTs;  $m/z$  198 for the MDBTs;  $m/z$  212 for  $C_2$ -DBTs) of a dibenzothiophenic fraction from crude oil (see text for experimental conditions). DBT = Dibenzothiophene; 1, 2, 3 and 4 = 1-, 2-, 3- and 4-methyldibenzothiophene respectively; 2-Et and 4-Et = 2- and 4-ethyldibenzothiophene, respectively; 4,6, etc. = 4,6-dimethyldibenzothiophene, etc.

achieved by capillary gas chromatography–mass spectrometry and co-injections with pure standards. It can be seen that even though the resolution is not complete between 2,4- and 2,8-DMDBT (they differ by 0.37 *RIS* unit), it is more than 50%, which is sufficient to identify and determine them correctly in natural samples.

Although all the MDBTs and C<sub>2</sub>-DBTs studied were present in natural samples, the more abundant isomers were those with a methyl group in position 4 or 6, as predicted by molecular mechanics calculations. Such trends illustrate well the opportunity to obtain theoretical information in order to predict retention behaviour and abundance of alkylated PASHs in natural samples. This will be of a great interest for future geochemical applications.

#### CONCLUSIONS

This study illustrates well the great selectivity of smectic liquid crystalline phases for analyses of PAHs (in this instance DBT derivatives). Twelve out of eighteen C<sub>2</sub>-DBTs available in this study could be baseline resolved. There were only two severe co-elutions, between the 3,6- and 2,7-DMDBT and between 1,2-, 1,9- and 1,7-DMDBTs. The 2,8- and 2,4-DMDBTs were not baseline resolved but the separation was sufficient to determine each independently. The very good separation obtained for the MDBTs and C<sub>2</sub>-DBTs will be very useful for further geochemical studies based on DBT isomers. Further, this study provides a good illustration of the applicability of theoretical calculations to predict elution order or at least to obtain an idea of the general retention behaviour of DBT derivatives on smectic liquid crystalline stationary phases. Parameters such as the arc-like arrangement and the distortion angles of the molecules have not been taken into account, and further investigations are needed to develop descriptors which include these two molecular parameters.

#### ACKNOWLEDGEMENTS

M. L. Lee (Brigham Young University, Provo, UT, USA) is acknowledged for the gift of dimethyl-

dibenzothiophene isomers. Thanks are also due to J. Connan and J. L. Oudin for discussions. SNEA(P) and TOTAL-CFP are acknowledged for financial support in these studies.

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