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Gas chromatographic retention behaviour of alkylated phenanthrenes on a smectic liquid crystalline phase

Application to organic geochemistry

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ABSTRACT

The retention indices for the 30 C₂ phenanthrenes and 33 trimethylphenanthrenes were determined by gas chromatography on a smectic liquid crystalline phase. The retention behaviour of these alkylated phenanthrenes was related to molecular shape considerations: length-to-breadth ratio, dihedral angle and substitution pattern. In addition, the influence on the retention behaviour of the arc-like shape of the phenanthrene molecule was investigated. Based on these results, 12 C₂ phenanthrenes and 25 trimethylphenanthrenes were identified in natural samples, such as crude oils and rock extracts.

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INTRODUCTION

Aromatic compounds are major components of crude oils and rock extracts. They represent 20–45% by weight of the fraction boiling above 210°C of crude oils [1] and contain numerous isomeric structures. The relative distribution of the alkylated aromatic isomers provides information about the maturity levels of the samples [2–9] and about the origin of the organic matter [2,8,10,11]. In order to make valid assessments of maturity and origin, precise identifications of the isomers are required. Identification by gas chromatography (GC) on non-polar phases (DB-5 or similar) often suffers from coelution problems and, thus, must be complemented by measurements such as using phases of different polarity [12,13]. High-performance liquid chromatographic (HPLC) fractionation and high-resolution spectrofluorimetry (Shpol'skii effect; see refs. 14 and 15) were successfully applied in an alternative approach to individual isomer identification. Because those latter techniques were off-line-coupled, they were rather time-consuming and laborious. Development of a capillary GC technique allowing isomer identification in a single step was thus desirable.

Liquid crystalline phases were most promising since they have proved to be very selective in the GC analysis of aromatic compounds [16–18]. In contrast to the non-polar phases, elution is not only controlled by the boiling temperature but also by the geometry of the solutes [19]. In the present study, the retention indices, as determined for 30 C₂ phenanthrenes (C₂-Ps) on a smectic liquid crystalline phase, have been related on one hand to length-to-breadth (*L/B*) ratio and on the other hand to molecular shape. In subsequent tests, the identification of dimethylphenanthrenes (DMPs) in natural samples was examined.

With a view to exploring further the geochemical utility of alkylphenanthrenes, the smectic liquid crystalline phase was also used to investigate trimethylphenanthrenes (TMPs) in natural samples. They are ubiquitous in petroleum and in organic extracts of sedimentary rocks, but owing to the isomeric complexity of the mixtures and to the low concentrations of TMPs, only 1,2,8-TMP has been previously isolated and identified precisely in a Kuwait oil [20]. Some preliminary studies by liquid

and gas chromatography have been performed [21], but owing to numerous coelutions on capillary GC non-polar phases, precise identifications were difficult to obtain. In this study, the retention indices of 33 TMP isomers on the smectic phase are reported, and relationships between the retention behaviour and the molecular shape are discussed.

In addition, we report the identification of DMP and TMP isomers in crude oils and rock extracts using the liquid crystalline phase for capillary GC–mass spectrometry (MS) analyses.

EXPERIMENTAL

Materials

All the C₂-Ps [25 DMPs and five ethylphenanthrenes (EtPs)] and 33 TMPs (of 56 possible ones) isomers were available in this study (Tables I and II). Five TMPs were purchased from Chiron Labs. (Trondheim, Norway) and 28 TMPs were synthesized at the Institute of Petroleum and Organic Geochemistry (Germany), in the same way as the C₂-Ps [7,22] through oxidative photocyclization of alkylated stilbenes. Identification of DMP, EtP and TMP standards was based on ¹H NMR data. The numbering of the position of substitution on the aromatic rings is given in Fig. 1. In the following discussion, the term molecular shape will refer to the arc-like shape of the phenanthrene molecule, *i.e.*, the three aromatic rings are annulated in an arc arrangement (Fig. 1).

Three geological samples were investigated: a rock extract 20 from the Handil field, Kalimantan (Indonesia), a condensate 102 from the Aquitaine basin (France) and a crude oil 27 from the Handil field, Kalimantan (Indonesia). The diesel particulate sample was a methylene chloride extract of Standard Reference Material 1650, Diesel Particulate Matter (SRM 1650), which was obtained from the Standard Reference Material Program at the National Institute of Standards and Technology (NIST) [23]. Triaromatic fractions were collected after HPLC on an aminosilane stationary phase, as described elsewhere [14].

Chromatographic and detection conditions

The alkylated phenanthrene standards and the triaromatic HPLC fractions were analysed by GC–MS using an HP 5890 series II gas chromatograph

TABLE I

RETENTION INDICES, SHAPE PARAMETERS AND ENTHALPIES OF FORMATION FOR DMP AND EtP ISOMERS

I values were determined by experiment and *I_c* values were calculated using eqn. 2 (see text) (*D* = dihedral angle; n.d. = not determined).

Compound	<i>I</i>	<i>L/B</i>	<i>I_c</i>	Deviation (%)	<i>D</i> (°)	ΔH_f^0 (kcal/mol)
1,2-DMP	335.01	1.58	330.63	-1.3	0.10	37.1
1,3-DMP	316.51	1.28	318.51	+0.6	0.05	35.7
1,4-DMP	318.92	1.32	320.12	+0.4	6.95	43.7
1,5-DMP	320.81	1.31	319.72	-0.3	6.40	43.5
1,6-DMP	321.68	1.37	322.14	+0.1	0.05	35.6
1,7-DMP	333.69	1.59	331.03	-0.8	0.05	35.6
1,8-DMP	327.76	1.55	329.41	+0.5	0.00	37.5
1,9-DMP	317.01	1.28	318.51	+0.5	0.05	37.6
1,10-DMP	326.12	1.29	318.91	-2.2	0.45	42.8
2,3-DMP	322.51	1.38	322.55	0.0	0.00	34.8
2,4-DMP	321.24	1.38	322.55	+0.4	3.35	41.2
2,5-DMP	323.01	1.38	322.55	-0.1	3.10	41.1
2,6-DMP	322.84	1.40	323.35	+0.2	0.00	33.6
2,7-DMP	335.95	1.70	335.47	-0.1	0.00	33.6
2,9-DMP	323.67	1.37	322.14	-0.5	0.00	35.6
2,10-DMP	320.83	1.37	322.14	+0.4	0.00	35.6
3,4-DMP	321.94	1.31	319.72	-0.7	11.90	43.6
3,5-DMP	312.63	1.29	318.91	+2.0	3.90	41.3
3,6-DMP	308.63	1.33	320.53	+3.9	3.73	33.7
3,9-DMP	315.94	1.12	312.04	-1.2	0.00	35.7
3,10-DMP	313.36	1.12	312.04	-0.4	0.00	35.6
4,5-DMP	296.39	1.29	318.91	+7.4	27.20	52.3
4,9-DMP	315.21	1.29	318.91	+1.2	5.70	43.4
4,10-DMP	314.53	1.13	312.45	-0.7	6.50	43.7
9,10-DMP	323.11	1.28	318.51	-1.4	1.28	41.6
1-EtP	316.06	1.59	n.d.	n.d.	n.d.	n.d.
2-EtP	323.63	1.77	n.d.	n.d.	n.d.	n.d.
3-EtP	306.33	1.09	n.d.	n.d.	n.d.	n.d.
4-EtP	301.09	1.09	n.d.	n.d.	n.d.	n.d.
9-EtP	306.67	1.09	n.d.	n.d.	n.d.	n.d.

equipped with a splitless injector (purge delay, 30 s; purge flow-rate, 60 ml/min). The column was 50 m × 0.22 mm I.D. SB-Smectic phase (Dionex, Lee Scientific Division, Salt Lake City, UT, USA), film thickness 0.1 μm. Helium was used as the carrier gas with 2.5 bar as the inlet pressure. The detector was an HP 5970 mass selective detector (electron impact 70 eV; selected ion monitoring (SIM) mode: *m/z* 206 for C₂-Ps and *m/z* 220 for TMPs at 2 scans/s; voltage, 2200 V). The temperature programme included 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 the transfer line were kept at 250°C.

Retention indices

Retention data are expressed as retention indices (*I*). *I* values are based on a comparison of the retention times of the compounds of interest with those of reference standards included with the solutes. Kovats [24], in his original *I* scale, used isothermal conditions and homologous *n*-alkanes for calibration [24]. In contrast, for the analysis of polycyclic aromatic hydrocarbons (PAHs), which is

TABLE II

RETENTION INDICES, SHAPE PARAMETERS AND ENTHALPIES OF FORMATION FOR THE 33 TMP ISOMERS STUDIED

I values were determined by experiment and *I*_C values were calculated from eqn. 3 (see text) (*D* = dihedral angle; n.d. = not determined).

Compound	<i>I</i>	<i>L/B</i>	<i>I</i> _C	Deviation (%)	<i>D</i> (°)	ΔH_f^0 (kcal/mol)
1,2,5-TMP	353.23	1.40	336.99	-4.6	22.96	35.9
1,2,6-TMP	340.61	1.46	340.45	0.0	0.42	28.9
1,2,7-TMP	353.23	1.67	352.55	-0.2	0.23	28.9
1,2,8-TMP	348.70	1.40	336.99	-3.4	0.46	30.8
1,2,9-TMP	335.72	1.36	334.68	-0.3	0.43	30.9
1,3,5-TMP	322.43	1.16	323.16	+0.2	22.23	34.5
1,3,6-TMP	320.69	1.20	325.46	+1.5	0.25	27.4
1,3,7-TMP	334.96	1.38	335.84	+0.3	0.02	27.4
1,3,8-TMP	330.94	1.19	324.89	-1.8	0.18	29.4
1,3,9-TMP	321.46	1.01	314.51	-2.2	0.07	29.4
1,3,10-TMP	318.53	1.12	320.85	+0.7	2.78	34.5
1,4,6-TMP	327.13	1.31	331.80	+1.4	22.17	34.5
1,4,7-TMP	335.49	1.37	335.26	-0.1	22.38	34.5
1,4,8-TMP	329.89	1.27	329.50	-0.1	22.85	36.4
1,5,6-TMP	336.96	1.33	332.96	-1.2	24.79	36.3
1,5,9-TMP	322.75	1.20	325.46	+0.8	24.29	36.6
1,6,7-TMP	338.86	1.45	339.87	+0.3	0.19	28.0
1,6,9-TMP	324.69	1.22	326.62	+0.6	0.19	29.3
1,7,9-TMP	330.78	1.30	331.23	+0.1	0.00	29.3
2,3,5-TMP	329.25	1.39	336.41	+2.2	18.52	33.0
2,3,6-TMP	325.25	1.30	331.23	+1.8	0.16	26.0
2,3,7-TMP	340.08	1.46	340.45	+0.1	0.24	26.0
2,3,10-TMP	325.95	1.20	325.46	-0.1	0.15	28.0
2,5,6-TMP	338.24	1.48	341.60	+1.0	23.65	33.1
2,5,9-TMP	326.94	1.23	327.19	+0.1	21.76	34.5
2,5,10-TMP	327.86	1.24	327.77	0.0	21.22	34.4
2,6,9-TMP	327.62	1.29	330.65	+0.9	0.21	27.3
2,6,10-TMP	328.75	1.34	333.53	+1.5	0.26	27.3
2,7,9-TMP	337.82	1.45	339.87	+0.6	0.03	27.3
3,4,6-TMP	324.59	1.21	326.04	+0.4	23.44	34.0
3,4,10-TMP	319.72	1.22	326.62	0.0	25.10	32.3
3,5,9-TMP	322.46	1.11	320.28	-0.7	22.18	34.6
3,5,10-TMP	319.72	1.14	322.00	+0.7	21.74	34.4

commonly performed by linear programmed temperature GC, Lee *et al.* [25] have introduced a calibration scale based on the following aromatic standards: benzene, naphthalene, phenanthrene, chrysene and picene. These compounds were assigned *I* values of 100, 200, 300, 400 and 500, respectively, and *I* was calculated according to eqn. 1 [25]:

$$I = 100 \cdot \frac{t_{r(X)} - t_{r(z)}}{t_{r(z+1)} - t_{r(z)}} + 100z \quad (1)$$

where *t_r* is retention time, X is the compound of interest (in this study the phenanthrene derivatives) and *z* and *z* + 1 are the number of aromatic rings in the standard aromatics eluting prior to (phenanthrene) and after (chrysene) the compounds studied (here *z* = 3).

Molecular mechanics and *L/B* ratio calculations

The calculations for the *L/B* values were carried out for the C₂-Ps [26] with a VAX computer using

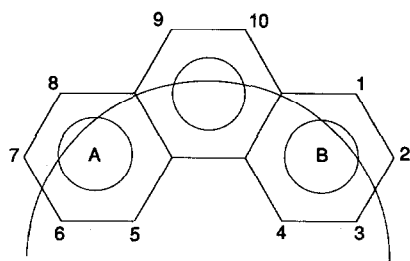


Fig. 1. Numbering of the phenanthrene molecule.

the MM2MMP2 empirical force field method (version 1982, Molecular Design, San Leandro, CA, USA [27,28]). For the TMP isomers, they were performed on a desk-top microcomputer (Tandy 3000 NL), using the MMIIPC software (QCPE, Indiana University, Bloomington, IN, USA), derived from the original MM2MMP2 empirical force field method [27,28]. The calculations provided an estimate of the steric energy and the heat of formation of the compounds (ΔH_f°) at 298 K in the gas phase. They also yielded the geometry of the molecule [bond lengths, angles and particularly the dihedral angle between ring A and ring B (see

Fig. 1)]. In-house software was used to provide the length (L) and the breadth (B) of the molecule: these values correspond to the dimensions of a minimized rectangle for each molecule studied, calculated according to the approach of Radecki *et al.* [29] and Wise *et al.* [30]. Both calculation softwares give comparable results for geometry and enthalpy values.

RESULTS AND DISCUSSION

C_2 -P analysis

An SIM chromatogram of some C_2 -P standards used in the determination of retention indices is shown in Fig. 2. These particular isomers were chosen because they are those generally present in crude oils and rock extracts [7] and are also representative of the retention behaviour of C_2 -Ps. The I values determined on the smectic liquid crystalline stationary phase for all the C_2 -P isomers are given in Table I. One unit of I corresponds to a difference in retention times of 40 s. Compounds differing by 0.50 I unit are baseline-resolved, those differing by 0.25 I unit are 50% resolved, and those differing by 0.15 I unit are 30% resolved. From the data presented in Table I, it follows that severe coelution

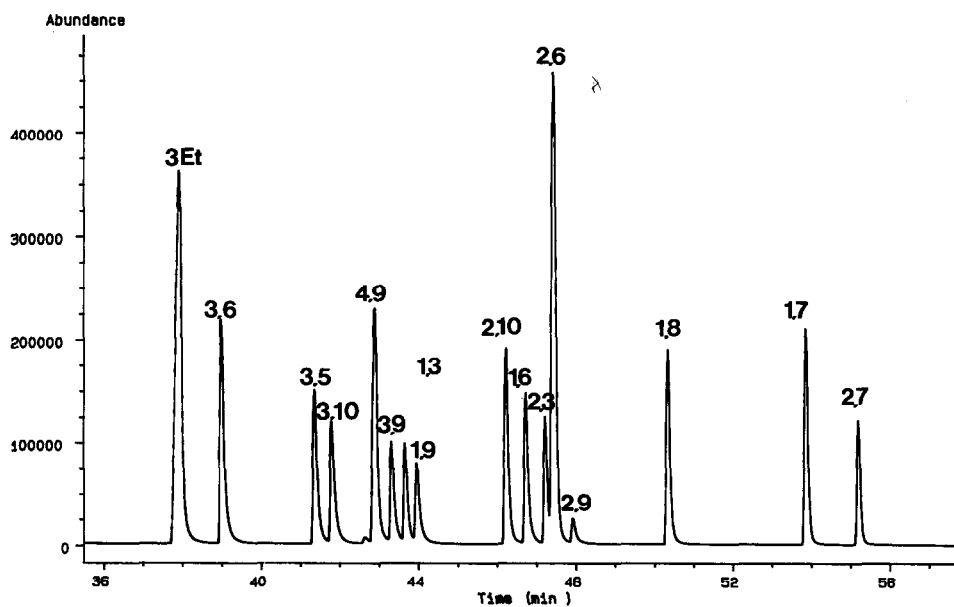


Fig. 2. Partial gas chromatogram of reference C_2 -P standards used in the determination of GC retention indices (see text for experimental conditions). Peaks: 3Et = 3-ethylphenanthrene; 1,3 = 1,3-dimethylphenanthrene.

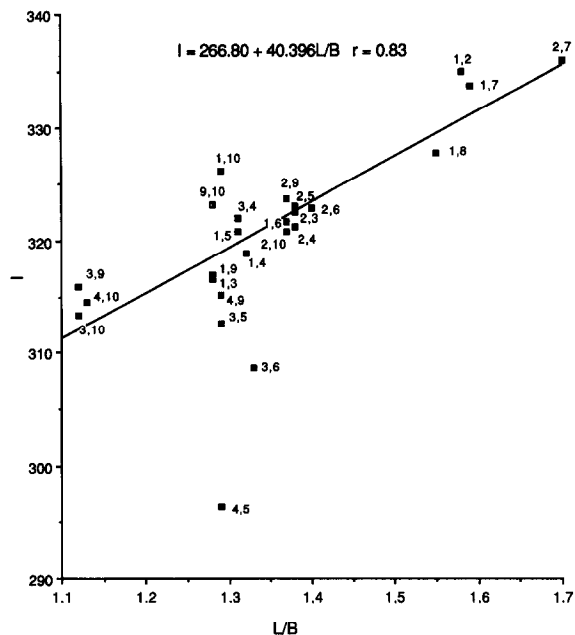


Fig. 3. Plot of GC retention indices versus L/B values for all 25 DMP isomers.

occurs only in one case, *i.e.*, between 2,10- and 1,5-DMP. Otherwise, the partial resolution (I difference in parentheses) between 2,10- and 2,4-DMP (0.41 I), between 2,4- and 1,6-DMP (0.44 I), between 1,6- and 3,4-DMP (0.27 I), between 2,3- and 2,6-DMP (0.33 I), between 2,6- and 2,5-DMP (0.17 I), and between 2,5- and 9,10-DMP (0.1 I) is sufficient for the identification of these isomers in natural samples. Furthermore, some of these partially resolved isomers (2,4-, 3,4-, 2,5- and 9,10-DMP), which exhibit strained molecular structures with rather large dihedral angles (see Table I), are quite unstable (they have the largest ΔH_f° values, see Table I) and seem to be not present in natural samples [7]. Only one of the partially resolved pairs (2,3-DMP and 2,6-DMP) has been reported to be present in natural samples [7]. The 50% resolution obtained in this case is sufficient for identification and even for quantification.

The DMP isomers differ significantly in L/B values and also in dihedral angle values (see Table I). The elution order of DMP isomers roughly follows increasing L/B values (see Fig. 3). Unstrained long or narrow DMP molecules are generally more

retained than those having a square-like shape. For instance, 2,7- and 1,7-DMP, which exhibit high L/B values of 1.7 and 1.6, respectively, are eluted later, whereas 3,9- and 3,10-DMP with low values of L/B (1.1) are eluted more rapidly. On the other hand, compounds with large dihedral angles generally elute before the unstrained DMP with a low L/B . For example, 4,5-DMP exhibits a dihedral angle of 27.20° and, despite a rather high L/B value of 1.3, elutes much earlier ($I = 296.39$) than the other isomers.

Fig. 3 shows the correlation between I and L/B for the 25 DMP isomers. The correlation equation obtained for DMP, is

$$I = 266.80 + 40.396 L/B \quad (2)$$

with a correlation coefficient $r = 0.83$ (4,5-DMP has been omitted from the calculation because it obviously has very different retention behaviour from the other DMP isomers). Predicted retention indices (I_C) are calculated from eqn. 2 and compared with measured I values. The average deviation between I_C and I is *ca.* 0.83 when 4,5-DMP is not considered. The highest deviation is 7.4% for 4,5-DMP. Two other DMP, 3,6- and 1,10-DMP, exhibit rather significant deviations when compared with the average deviation, *i.e.*, 3.9% and -2.2% , respectively. The other DMP isomers show deviations of less than 2%, with the majority less than 1%. Although the linear model represented by eqn. 2 generally has a reasonable predictive ability, parameters other than L/B values must be considered in order to understand the strong deviations observed in some cases. Thus, the molecular shapes, which have already been shown to have an influence on the retention of PAHs on liquid crystalline phases [31,32], and the dihedral angles, which have already been mentioned as an important factor affecting retention in HPLC on polymeric C_{18} phases [26,33], were investigated.

4,5-DMP, which deviates in retention behaviour the most significantly from the global set of DMP (deviation of 7.4%), is eluted much earlier than predicted. This isomer has a very strained molecular structure (dihedral angle greater than 27°), and its retention behaviour is compatible with the previous "slot" model proposed by Wise and Sander [34] in reversed-phase liquid chromatography. Strained molecules such as 4,5-DMP, which interact weakly with the stationary phase, will be retained less. The

distortion of the molecules hinders penetration of the solutes into the slots. The effect is less pronounced with 4,9-DMP, despite the large dihedral angle (5.7°). However, steric exclusion is indicated also in this case by the earlier than expected elution (deviation 1.2%).

The other DMP isomers showing “anomalous” behaviour are 3,6- and 3,5-DMP, which elute earlier than predicted with deviations of 3.9% and 2.0%, respectively, and 9,10-, 3,9-, 1,10- and 1,2-DMP, which elute later than predicted with deviations of -1.4% , -1.2% , -2.2% and -1.3% , respectively. For these isomers the dihedral angle does not account for these deviations and the molecular shape has to be considered.

Other authors have introduced the concept of the arc-like arrangement to explain certain retention behaviour on liquid crystalline phases [32]. Phenanthrene is a molecule with aromatic rings annelated in a curved arrangement (see Fig. 1). Methyl groups in positions 3, 4, 5 and 6 are “inside the arc”. Methyl groups in positions 3 and 6 retain the arc arrangement and even continue the arc, whereas methyl groups in positions 1, 8, 9 and 10 are attached to the outer curved side of the arc. The elution of 3,6-DMP earlier than expected from its L/B value (I deviation 3.9%) is attributed to the fact that the two methyl groups are in line with the arched arrangement of the aromatic rings, hence continuing and even emphasizing the arc-like topography of the phenanthrene molecule. Likewise, the relative early elution of 3,5-DMP (I deviation 2.0%) is believed to result from an extension of the molecular arc by the methyl group in position 3. Because the methyl group inside the arc (position 5) does not contribute to the effect, the deviation is less pronounced in this case.

1,2-, 9,10- and 1,10-DMP, which have their methyl substituents attached to the outer curved side of the arc, are eluted later than predicted from their L/B values. 3,9-DMP has one methyl group outside the arc (position 9) and is also retained longer than predicted. In the cases of 1,2- and 9,10-DMP, the two adjacent methyl groups may also explain retention times that are longer than predicted. This *ortho*-effect is already known for alkylated benzenes [35], alkylated naphthalenes [36] and alkylated benzothiophenes [37], and it is explained as a function of the lower vapour pressure exhibited by *ortho*-isomers [35]. Furthermore, this *ortho*-effect

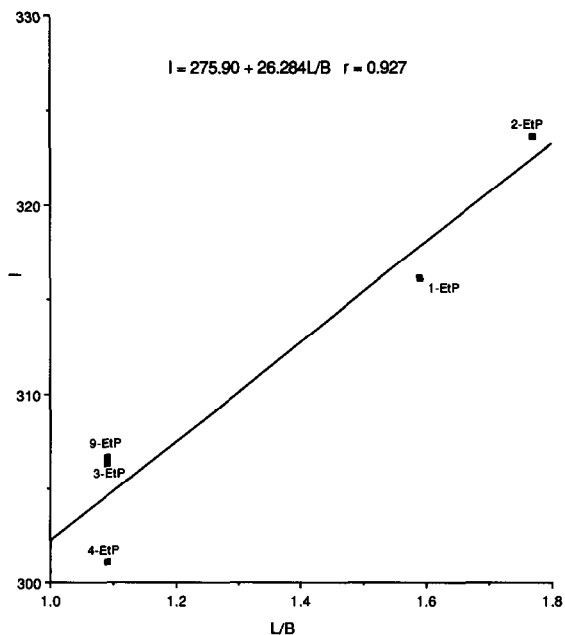


Fig. 4. Plot of GC retention indices versus L/B values for all EtP isomers.

has been already mentioned for liquid crystalline phases [38].

The same considerations apply to EtPs, which are eluted according to their L/B values (see Fig. 4), *i.e.*, 2-EtP, which has the largest L/B value, is eluted after 1-, 3- and 9-EtP. 4-EtP, as a very strained molecule, should have a significant dihedral angle. This would explain its elution before 3- and 9-EtP despite coincident L/B values.

Among the different factors governing the retention behaviour of EtP and DMP isomers, the L/B ratio is still the major factor. However, other parameters, such as the dihedral angle, the molecular shape and the *ortho*-effect, also influence the elution order. For the majority of the C_2 -P isomers, the second and third parameters seem to compensate one another and the retention behaviour is determined mainly by the L/B ratio. In those cases when one of these parameters is stronger than the others, deviations are observed: a large dihedral angle is responsible for early elution; the *ortho*-effect results in late elution of the isomers; finally arc-like molecules with substituents that maintain the arc arrangement (3,6- and 3,5-DMP) seem to elute earlier than expected, whereas molecules with substituents

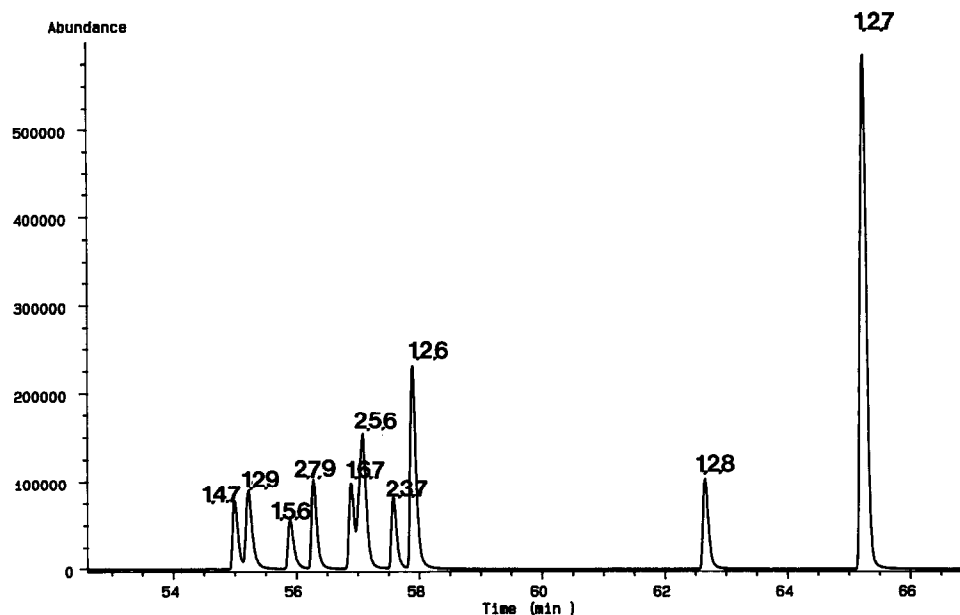


Fig. 5. Partial gas chromatogram of reference TMP standards used in the determination of GC retention indices (see text for experimental conditions). 1,2,8 = 1,2,8-Trimethylphenanthrene.

attached to the outer curved side of the arc are retained longer. This behaviour is compatible with previous studies [29,30] and perhaps could be attributed to specific interaction of arc-like molecules with the liquid crystalline stationary phase.

TMP analysis

An SIM chromatogram of TMP standards used in the determination of retention indices is shown in Fig. 5. The I values determined on the smectic liquid crystalline phase for the 33 available TMP isomers are given in Table II. As in the case of C_2 -P isomers, one unit of I corresponds to a difference in retention times of 40 s. Compounds differing by 0.50 I unit are baseline-resolved, those differing by 0.25 I unit are 50% resolved, and those differing by 0.15 I unit are 30% resolved. From the data presented in Table II, it follows that severe coelution occurs in five cases, *i.e.*, 1,6,9-/3,4,6-TMP, 1,7,9-/1,3,8-TMP, 3,5,9-/1,3,5-TMP, 1,2,7-/1,2,5-TMP and 3,4,10-/3,5,10-TMP. Otherwise the partial resolution (I difference in parentheses) between 3,5,9-/1,3,5- and 1,5,9-TMP (0.32 I), between 2,5,9- and 1,4,6-TMP (0.19 I), between 2,6,9- and 2,5,10-TMP (0.24 I), between 1,4,7- and 1,2,9-TMP (0.23 I) and between 2,7,9-

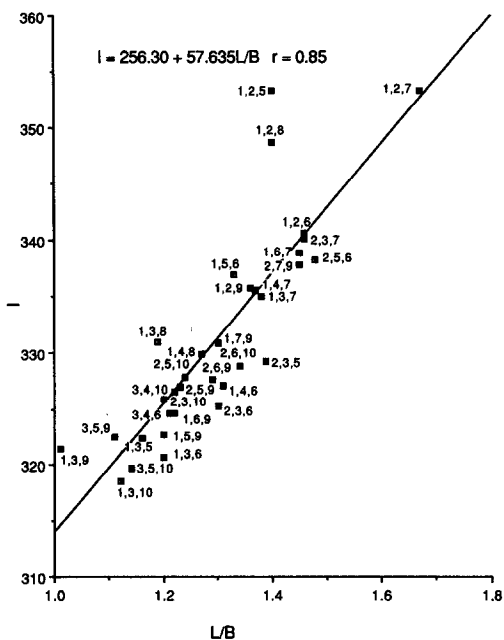


Fig. 6. Plot of GC retention indices versus L/B values for 33 TMP isomers.

and 2,5,6-TMP (0.42 *I*) is sufficient for the identification of these isomers in natural samples.

As in the case of DMPs, TMP isomers differ significantly in *L/B* values (see Table II). The elution order of TMPs generally follows increasing *L/B* values. For instance, 1,2,7- and 1,2,6-TMP, which exhibit the highest *L/B* values (1.7 and 1.5, respectively) elute later, and 3,5,9- and 1,3,9-TMP, with low *L/B* values (1.1 and 1.0, respectively) elute very early.

Fig. 6 shows the correlation obtained for the 33 TMPs when *I* is plotted versus *L/B*, where linear regression gives

$$I = 256.30 + 57.635 L/B \quad (3)$$

and a correlation coefficient $r = 0.85$. Predicted I_C values were calculated from eqn. 3 and compared with the measured *I* values. The average deviation is *ca.* 1%. The highest deviations are -4.6% and -3.4% for 1,2,5- and 1,2,8-TMP. The remaining TMP isomers have deviations of *ca.* 2% or less, the majority being less than 1%. TMP isomers showing deviations greater than 1% are the following: 1,3,9-TMP (-2.2%), 1,5,6-TMP (-1.2%), 1,3,8-TMP (-1.8%), 2,6,10-TMP (1.5%), 1,4,6-TMP (1.4%), 2,3,5-TMP (2.2%), 2,3,6-TMP (1.8%) and 1,3,6-TMP (1.5%). The retention behaviour is predicted quite well by eqn. 3; however, as was the case with the DMP isomers, the molecular shape, dihedral angle and *ortho*-substitution pattern must also be taken into account to explain the deviations.

Like 1,2-DMP, the two TMP isomers (1,2,8- and 1,2,5-TMP) with the most significant deviations elute much later than predicted, as was also observed for 1,2-DMP, for these isomers also possess the 1/2 substitution sequence, *i.e.*, the *ortho*-effect could explain the deviation. Moreover, they have two (1,2,5-TMP) or three (1,2,8-TMP) methyl substituents attached to the outer curved side of the arc. Two other TMPs that are retained longer than predicted (1,3,9- and 1,3,8-TMP) are also in this category, with two substituents outside the arc. Their retention behaviour should be compared with that of 3,9-DMP. 1,5,6-TMP has two substituents inside the arc and, one outside, and shows an *ortho*-effect. These last two characteristics seem to control the retention behaviour of this compound, *i.e.*, elution is delayed.

In the case of 1,3,6- and 2,3,6-TMP, the methyl

substituents maintain the arc shape. These isomers are retained less than predicted from the *L/B* values, and can be compared with 3,6-DMP, which is also retained less than expected. 2,6,10- and 1,4,6-TMP have two substituents maintaining the arc shape and are eluted earlier than predicted. 2,3,5-TMP has its substituents inside the arc and also shows the *ortho*-effect. Since it is eluted earlier than predicted, the *ortho*-effect must be of minor importance compared with the molecular shape. Its retention behaviour should be compared with that of 3,5-DMP, which is also eluted earlier than expected.

It seems that, as in the case of DMP isomers, the TMP isomers with substituents outside the arc elute later than expected and those with substituents that maintain the arc are retained less than predicted from *L/B* values. The contribution of the dihedral angle to the retention behaviour of TMP isomers is difficult to estimate because of the lack of very strained reference compounds (*i.e.*, with 4/5 substitution pattern).

In conclusion it can be stated that, as was the case for the DMP, the *L/B* ratio is the major TMP retention behaviour parameter. It appears that secondary factors are the molecular shape (arc arrangement) and the *ortho*-effect. The molecular shape appears to have a stronger influence on retention behaviour than the *ortho*-effect (see the case of 2,3,5-TMP). Finally, the dihedral angle appears to have an influence only for very strained isomers, but this last point needs to be confirmed with additional reference compounds.

Identification of C_2 -Ps and TMPs in natural samples

Figs. 7 and 8 show identifications performed for C_2 -Ps and TMPs in a sample of a crude oil. True identifications were achieved by capillary GC-MS and co-injection with pure standards.

Tables III and IV give the relative abundances of C_2 -Ps and TMPs in natural samples (crude oil, rock extract, condensate and diesel exhaust). It is evident that the relative amounts of the isomers vary from sample to sample. The origin of these individual variations would be of great interest in geochemical studies, as discussed elsewhere [39].

Nevertheless, it is interesting to note that 1,2-DMP, which has not been identified previously in natural samples, was observed in all the investigated samples. We can also note the presence of 2-EtP,

TABLE III
RELATIVE DISTRIBUTION OF C₂-Ps IN NATURAL SAMPLES
Values are percentages of the total C₂-P content.

Sample	Relative abundance of C ₂ -phenanthrenes (%)																
	9Et	3Et	3,6	3,10	3,9	1,3	1,9	2,10	1,6	2,3	2,6	2,9	1,8	1,7	2,7	1,2	2Et
Crude oil 27	0.0	2.9	5.3	8.1	7.3	4.8	4.7	7.8	6.0	3.8	13.9	7.3	2.3	12.8	7.0	2.1	3.7
Rock 20	0.0	3.3	2.9	5.9	6.2	4.6	4.5	5.5	6.4	3.7	11.8	7.9	5.0	17.7	4.7	4.2	5.5
Condensate 102	0.0	0.0	12.5	8.6	7.3	6.1	2.2	6.1	5.7	6.9	21.9	6.3	1.4	5.3	8.4	1.3	0.0
Diesel	0.6	1.6	6.5	7.0	6.1	6.7	3.0	8.1	6.3	7.5	15.2	7.4	2.0	8.1	9.0	2.4	2.5

TABLE IV
RELATIVE DISTRIBUTION OF TMPs IN NATURAL SAMPLES
Values were calculated using the chromatographic peaks corresponding to the TMP isomers identified in natural samples.

Sample	Relative abundance of trimethylphenanthrenes (%)																							
	1,3,10	3,5,10	1,3,6	1,3,9	1,3,5	1,5,9	1,6,9	2,3,6	2,3,10	2,6,9	2,6,10	1,7,9	1,3,7	1,2,9	2,7,9	1,6,7	2,3,7	1,2,6	1,2,8	1,2,7	+	1,2,5		
Crude oil 27	7.6	1.0	5.3	3.6	2.5	1.4	4.7	5.1	6.8	10.6	7.2	10.9	6.3	1.5	7.3	3.4	5.9	3.4	4.3	3.2				
Rock 20	4.7	0.0	2.9	3.9	2.9	2.8	5.6	3.8	2.9	11.8	4.3	10.2	5.3	n.d.	6.1	3.0	8.7	4.9	9.1	4.1				
Condensate 102	11.1	0.0	8.7	3.1	0.2	0.6	2.9	9.3	4.8	10.9	9.1	7.9	8.0	0.4	7.5	2.8	9.0	1.3	1.4	1.2				
Diesel	6.2	0.0	6.2	3.0	1.1	2.3	3.7	7.1	4.0	8.5	6.6	10.1	8.1	1.1	8.8	4.3	11.0	2.3	3.0	2.7				

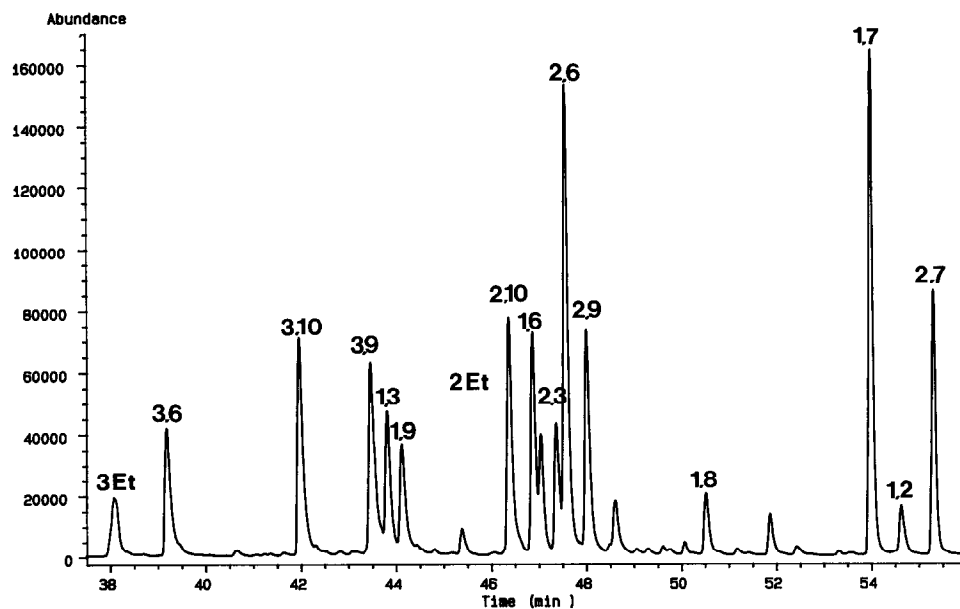


Fig. 7. Partial SIM (m/z 206) chromatogram of a triaromatic fraction of crude oil #27; identification of C_2 -P isomers by co-injection with standard compounds. Peaks: 2Et = 2-ethylphenanthrene; 2,6 = 2,6-dimethylphenanthrene.

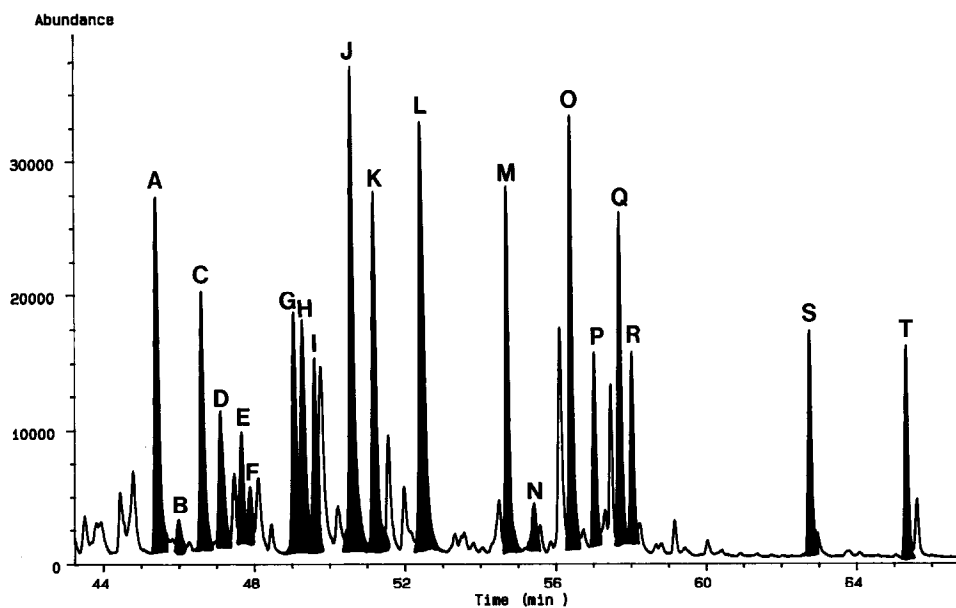


Fig. 8. Partial SIM (m/z 220) chromatogram of a triaromatic fraction of crude oil 27; identification of TMP isomers by co-injection with standard compounds. Peaks: A = 1,3,10-TMP; B = 3,5,10-TMP; C = 1,3,6-TMP; D = 1,3,9-TMP; E = 1,3,5-TMP + 3,5,9-TMP; F = 1,5,9-TMP; G = 1,6,9-TMP + 3,4,6-TMP; H = 2,3,6-TMP; I = 2,3,10-TMP; J = 2,6,9-TMP; K = 2,6,10-TMP; L = 1,7,9-TMP + 1,3,8-TMP; M = 1,3,7-TMP; N = 1,2,9-TMP; O = 2,7,9-TMP; P = 1,6,7-TMP; Q = 2,3,7-TMP; R = 1,2,6-TMP; S = 1,2,8-TMP; T = 1,2,7-TMP + 1,2,5-TMP.

which was difficult to confirm otherwise. The strained C₂-P isomers (with methyl or ethyl groups in positions 4 and 5), which are unstable, were absent in these samples, as reported previously in other studies [7].

Among the 33 available TMP standards, the most stable ones (*i.e.*, the 18 showing the lowest ΔH_f^0 values, see Table II) were all found in the natural samples investigated. In highly thermally stressed samples, such as diesel exhaust and oil condensate, an increase in the abundance of the most stable TMP isomers (*i.e.*, 2,3,6-, 2,3,7-, 1,3,6- and 1,3,7-TMP), compared with the TMP distribution in the crude oil and in the rock extract, can be noted. Conversely, the abundance of a less stable TMP, such as 1,2,8-TMP, decreases with increasing thermal maturity (from the rock extract to the oil condensate). The least stable isomers (*i.e.*, with $\Delta H_f^0 > 32$ kcal/mol) were not present.

The case of 1,3,10-TMP is ambiguous because, based on its retention time, it is present in natural samples; however, owing to its rather high ΔH_f^0 (34.5 kcal/mol) it should not be present in the amount found in the investigated samples. Possible coelution with another TMP not yet available but more stable could explain these results. Theoretical calculations suggest 2,3,9-TMP as a possible candidate. Indeed, it has a *L/B* value of 1.20 and a ΔH_f^0 value of 27.96 kcal/mol. From this value of the enthalpy of formation compared with the others (see Table II, *e.g.*, it is as stable as 2,3,10-TMP and more stable than 1,6,9- or 1,3,10-TMP, which are present in natural samples), it is reasonable to suspect its presence in natural samples. Its calculated *I* value from eqn. 3 (with *L/B* = 1.20) is 325.46. This is consistent with possible coelution between 1,3,10- and 2,3,9-TMP. In such a case, the deviation, for 2,3,9-TMP, of 1.4% between $I_C = 325.46$ and $I = 318.53$ (the value obtained for 1,3,10-TMP) could be explained by the influence of the molecular shape as in the case of 1,3,5-TMP (see above). These assumptions need to be proved, and the synthesis of the 2,3,9-TMP is now under progress.

The quantitative results (percentage relative abundance) for the TMP isomers corroborate quite well the thermodynamic calculations performed on them.

CONCLUSIONS

The smectic liquid crystalline phase used in this study has provided an alternative tool for individual identification of C₂-Ps, and it has allowed the determination of C₂-Ps not yet identified in natural samples. In the case of TMP analysis, the smectic liquid crystalline phase provides useful GC patterns that allow identification of some of the most abundant isomers.

Furthermore, this study illustrates the potential of theoretical methods to generate molecular parameters, such as *L/B* ratios and dihedral angles, which can then be used to predict or at the least to explain the elution order of C₂-P and TMP isomers on liquid crystalline phases, even if, up to now, the contribution to the final elution order of parameters such as the dihedral angle or the molecular shape cannot be quantitated. The natural occurrence of TMP isomers could also be predicted according to their calculated ΔH_f^0 values.

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