

## Organic Geochemical Evidence for a Series of C<sub>25</sub>–C<sub>28</sub> Sulphur-containing Lipids comprising Regular and Irregular Isoprenoid and Unusual Linearly Extended Phytane Skeletons

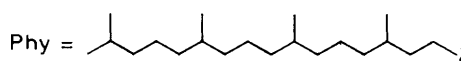
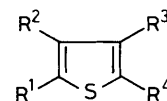
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A series of alkylthiophenes (C<sub>25</sub>–C<sub>28</sub>), consisting of regular and irregular isoprenoid and unusual linearly extended phytane carbon skeletons, some of which are presently unknown in living organisms, have been identified in a bituminous marl.

The 'aromatic hydrocarbon' fraction of the extractable organic matter from a bituminous marl layer from the northern Apennines (Italy; Miocene, 6 × 10<sup>6</sup> years) which was deposited under hypersaline conditions,<sup>1</sup> contains series of alkylthiophenes and thiolanes.<sup>2</sup> The origin of these sulphur-containing compounds is now believed to involve reaction of

functionalised lipids with inorganic sulphur species such as hydrogen sulphide and polysulphides during early diagenesis.<sup>3–5</sup>



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**Table 1.** Alkyl thiophene standards prepared, the nature of their carbon skeletons, and their abundance in the bitumen ( $\mu\text{g/g}$ ). Compounds (1) and (2) were obtained as a mixture (ca. 63:37).

		R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	Nature of carbon skeleton	Abundance ( $\mu\text{g/g}$ )
(1)	C <sub>25</sub>	Phy	Me	H	H	Regular isoprenoid	n.d. <sup>a</sup>
(2)	C <sub>25</sub>	Phy	H	Me	H	Irregular isoprenoid	n.d. <sup>a</sup>
(3)	C <sub>25</sub>	Phy	H	H	Me	Linearly extended phytane	78 <sup>b</sup>
(4)	C <sub>26</sub>	Phy	H	Me	Me	Irregular isoprenoid	59
(5)	C <sub>26</sub>	Phy	Me	H	Me	Regular isoprenoid	}139 <sup>c</sup>
(6)	C <sub>26</sub>	Phy	H	H	Et	Linearly extended phytane	
(7)	C <sub>27</sub>	Phy	H	H	Pr	Linearly extended phytane	27
(8)	C <sub>28</sub>	Phy	H	H	Bu	Linearly extended phytane	Trace <sup>d</sup>

<sup>a</sup> n.d. not detected. <sup>b</sup> Includes an additional minor, as yet uncharacterised, C<sub>25</sub> isoprenoid thiophene. <sup>c</sup> Includes an additional minor, as yet uncharacterised, C<sub>26</sub> isoprenoid thiophene. <sup>d</sup> Abundance difficult to determine owing to co-elution with another, as yet uncharacterised, C<sub>28</sub> isoprenoid thiophene.

**Table 2.** Mass spectral characteristics and retention indices of the thiophene standards and retention indices of the corresponding alkanes formed by desulphurisation.<sup>a</sup>

Mass spectrum, $m/z$	<i>I</i> (thiophenes)	<i>I</i> (alkanes)
(1)	2544	2239
(2)	2544	2233
(3)	2536	2270
(4)	2631	2339
(5)	2610	2321
(6)	2613	2367
(7)	2698	2466
(8)	2793	2562

<sup>a</sup> The mass spectra were obtained by g.c.–m.s. at 70 eV. Retention indices were measured against co-injected n-alkanes on a CP Sil 5 fused silica capillary column (25 m  $\times$  0.32 mm, film thickness 0.12  $\mu\text{m}$ , hydrogen carrier 40 kPa, 130–300 °C at 4 °C min<sup>-1</sup>). <sup>b</sup> Mass spectrum for a mixture of thiophenes (1) and (2) (ca. 63:37).

The structure of the major thiophene, 2,3-dimethyl-5-(2,6,10-trimethylundecyl)thiophene, whose carbon skeleton is identical to that of phytane, has recently been established by comparison of the mass spectrum and relative g.c. retention time data with those of a synthesised standard.<sup>3</sup> Other major isoprenoid thiophenes present in the 'aromatic hydrocarbon' fraction from the marl extract occur in the C<sub>25</sub>–C<sub>28</sub> range.<sup>2</sup> Some structural information on these compounds was obtained from their mass spectra after g.c.–m.s. analysis. The major fragment ions (corresponding to cleavage  $\beta$  to the thiophene ring) indicated thiophene units coupled to a long alkyl chain and smaller alkyl fragments (methyl, dimethyl, ethyl, propyl, and butyl). These cleavages were sometimes accompanied by a significant McLafferty rearrangement ion at 1  $m/z$  unit higher. The relative g.c. retention times of these thiophenes indicated that the long alkyl chain was isoprenoidal.

Desulphurisation of the 'aromatic hydrocarbon' fraction with Raney nickel afforded several C<sub>25</sub>–C<sub>28</sub> isoprenoid alkanes.<sup>5</sup> On the basis of the mass spectra (g.c.–m.s.), relative g.c. retention time data, and molecular connectivity rules,<sup>6</sup> these alkanes were tentatively assigned as regular and irregular isoprenoids and components consisting of the phytane skeleton with a linear carbon chain extension.

To aid the identification of these isoprenoid thiophenes, several compounds (Table 1) were prepared by acylation of various alkylated thiophenes (2-methyl-, 3-methyl-, 2,3-dimethyl-, 2,4-dimethyl-, 2-ethyl-, 2-propyl-, and 2-butylthio-

phene) with 3,7,11,15-tetramethylhexadecanoic acid [i, (COCl)<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, dimethylformamide (DMF); ii, SnCl<sub>4</sub>], followed by reduction of the resulting ketone (AlCl<sub>3</sub>/LiAlH<sub>4</sub>, Et<sub>2</sub>O). In the case of the acylation of 3-methylthiophene, a mixture of the 2,3- and 2,4-disubstituted isomers was obtained. The thiophenes and intermediate ketones were fully characterised by mass spectrometry, and high field <sup>1</sup>H n.m.r. and i.r. spectroscopy. Desulphurisation of the synthesised thiophenes (W-2 Raney Ni, EtOH, heat) followed by hydrogenation to remove trace amounts of alkenes (PtO<sub>2</sub>, HOAc, H<sub>2</sub>) afforded the corresponding alkanes, which were characterised by g.c. and g.c.–m.s. Mass spectral and g.c. retention index data for the alkylthiophenes and g.c. retention index data for the alkanes are given in Table 2.

The alkylthiophenes present in the northern Apennines marl were identified as having structures (3)–(8), by comparison of their mass spectra and by coelution with the synthesised standards on three stationary phases (CP Sil 5, CP Sil 88, and DB 1701). Similar comparisons were also made with the respective desulphurisation products.

The carbon skeleton of thiophene (5) is comparable with that of known lipids<sup>7</sup> which have been identified in bacteria. Thiophene (4) has a carbon skeleton which is related to these bacterial lipids, although such a C<sub>26</sub> compound has yet to be identified. To our knowledge there are no known natural product counterparts with a carbon skeleton similar to those of thiophenes (3), (6), (7), and (8) which consist of a linearly extended phytane structure. It is unlikely that these compounds arise from regular or irregular isoprenoids since this would require the breaking of two carbon–carbon bonds during early diagenesis. The origin of such compounds is therefore unknown at present but may reflect an input of functionalised lipids having the same carbon skeleton at the time of sediment deposition. Since the thiophene ring is located at the same relative position, this would suggest that the natural products contain functionalities at the same position, which can then react with inorganic sulphur species during early diagenesis. The biosynthetic origin of such compounds would appear to be unusual since they contain isoprenoidal and n-alkyl units. Examples of known compounds consisting of isoprenoidal and n-alkyl portions are the extended hopanes<sup>8</sup> although in this case the n-alkyl portion derives from defunctionalisation of the polyhydroxy side chain during diagenesis.

An alternative, but less likely, explanation for the formation of (3), (6), (7), and (8) may be a reaction in sediments between phytol and 2-n-alkylthiophenes (cf. Freidel–Crafts alkylation). Such a process has recently been suggested to account for the presence of steroids bonded by a carbon–carbon bond to aromatic units in asphaltenes and coals.<sup>9</sup>

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