Abiotic oxidation of petroleum bitumens under natural conditions

A. Charrié-Duhaut\textsuperscript{a}, S. Lemoine\textsuperscript{a}, P. Adam\textsuperscript{a}, J. Connan\textsuperscript{b}, P. Albrecht\textsuperscript{a,*}

\textsuperscript{a}Laboratoire de Géochimie Organique, UMR 7509 du CNRS, Institut de Chimie, Université Louis Pasteur, 1 rue Blaise Pascal, 67000 Strasbourg, France
\textsuperscript{b}Centre de Recherches, Elf Exploration Production, 64000 Pau, France

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Abstract

Five series of crude oil samples exposed to atmospheric conditions have been analysed at the molecular level, each series comprising several samples originating from the same crude oil but altered to different extents. The aim of our investigation was to compare the specific impact of abiotic oxidation to other alteration processes such as biodegradation, evaporation and water washing. Bulk analyses revealed that increasing alteration is accompanied by an increase in oxygen content which parallels a relative increase of the proportions, as well as of the molecular weights of the macromolecular constituents of the bitumens. Gas chromatographic–mass spectrometric analyses of polar fractions showed the presence of oxygen-containing compounds (steroid ketones, benzothiophenic acids and sulfones) which result from oxidation of petroleum lipids. The hypothesis that part of these oxygenated compounds results from abiotic oxidation processes rather than from biodegradation is supported, notably, by the fact that oxygen incorporation generally occurred without diasteremic discrimination. This is also supported by simulation experiments performed on petroleum lipids, which showed that abiotic oxidation induces cleavage reactions affecting C–C and C–S bonds which may intervene in the transformation of geomacromolecules in the environment by degradation (“depolymerization”). Thus abiotic oxidation may play a major role in the fate of petroleum pollutants in the environment by transforming lipidic organic matter from petroleum into more water soluble and, therefore, more biodegradable constituents. However, these can be more toxic to the environment as the water-soluble fraction may be easily taken up by biota. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Abiotic oxidation; Biodegradation; Water washing; Geomacromolecules; Simulation experiments; Bitumen; Biomarkers

1. Introduction

Bituminous sands, asphalts, oil spillages and surface reservoirs represent enormous amounts of generally unexploitable crude oils which are exposed to the atmosphere and the fate of which is still the subject of many investigations. Indeed, when surface exposed, the organic matter undergoes various alteration processes, such as water washing, evaporation, biodegradation and abiotic oxidation. These physico-chemical processes can be induced by various external factors such as atmospheric oxygen, oxygen dissolved in rainwaters/percolating waters or by the action of aerobic and anaerobic micro-organisms. They can also be initiated by light, trace oxidants in the atmosphere (ozone, peroxides, e.g. Cooper and Zika, 1983; Cooper et al., 1989; Aneja et al., 1994) or by the chemical composition of the environment.

In recent years, much research has been dedicated to the study of such natural processes, particularly those following oil spillages (Gundlach et al., 1983; Wang et al., 1994; Bence et al., 1996) and releases of material in
relation to offshore petroleum activities (e.g. Fisher et al., 1998). For example, weathering processes such as evaporation and, to a smaller extent, water washing were shown to lead to a dramatic depletion of the low-molecular-weight constituents of bitumens ($\leq C_{15}$) (e.g. Gundlach et al., 1983; Volkman et al., 1984; Payne et al., 1987; Kagi et al., 1988; MacKay and McAuliffe, 1988; Kuo, 1994 and references therein). Biodegradation (Seifert and Moldowan, 1979; Goodwin et al., 1983; Wardroper et al., 1984; Volkman et al., 1984; Clayton and King, 1987; Chosson et al., 1992; Peters and Moldowan, 1993; Volkman et al., 1994; Fisher et al., 1998) has also received much interest, in particular within natural bioremediation, and enhanced bioremediation processes in terrestrial and stranded material (Volkman et al., 1984). Yet, little attention has been paid to abiotic oxidation as an independent phenomenon, although oxidation itself is a widespread process. Most studies in geochemistry have been restricted to the fate of low-molecular-weight constituents of petroleum using simulation experiments (e.g. Ehrhardt and Petrick; 1984, 1985; Giuliano et al., 1997). It is now common knowledge (e.g. Hostettler and Kvenvolden, 1994) that exposure of crude oils to atmospheric conditions results in dramatic changes in its chemical composition, e.g. modification of the colour and consistency of the bitumen and even incorporation of oxygen. However, little is known, at the molecular level, on the chemical impact of oxidation on fossil organic matter exposed to the atmosphere.

Several questions, therefore, still remain regarding which compounds are preferentially affected by oxidation and how oxygen is incorporated into petroleum lipids. Furthermore, is it possible to distinguish between biodegradation and abiotic oxidation processes? Are there parameters able to measure the extent of oxidation? What is the impact of abiotic oxidation on macromolecules which represent, by far, the major part of petroleum bitumens exposed to the atmosphere? From an environmental point of view, does abiotic oxidation contribute to the further degradation of lipidic organic matter of petroleum refractory to biodegradation and, hence, play an important role on the fate of petroleum pollutants in the environment?

We report here a detailed study of five series of samples, each series comprising several samples originating from the same crude oil, generally biodegraded, but altered to different extents. The aim of this investigation is to unravel the specific impact of abiotic oxidation but different from biodegradation. For this purpose, bulk measurements (elemental analysis, IR spectroscopy and size exclusion chromatography) in combination with GC–MS analyses, have been performed to observe the changes in organic matter composition as a result of oxidative alteration. Most of the work focusses on the GC–MS analysis of polar fractions that may contain oxidized compounds such as ketones, alcohols, acids and sulfones formed by abiotic oxidation. In parallel, simulation experiments of abiotic oxidation in the presence of light have been performed on certain reference compounds, in particular, aromatic biomarkers, in order to observe their behaviour under oxidative conditions and to identify the oxidation products formed.

2. Experimental

2.1. Description of samples

Five series of biodegraded samples of different origins (Western Europe and Middle East), exposed to natural atmospheric conditions for different lengths of time, were investigated. Their origin, description and apparent degree of alteration are summarized in Table 1. Several analyses (bulk and molecular) were used to assess the severity of the alteration undergone by the samples investigated. All samples were stored at $-20^\circ$ C prior to analysis. Liquid samples were stored in argon flushed bottles. Solid samples were wrapped in aluminium foil and stored in polyethylene bags. Samples were analysed immediately after extraction.

2.2. Instrumentation

2.2.1. Gas chromatography (GC)

GC was carried out either on a Carlo Erba 4160 or on a Fisons 8160 gas chromatograph equipped with an on-column injector, a FID (300 $^\circ$ C) and a J&W DB-5 fused silica column (30 m x 0.25 mm i.d., 0.1 $\mu$m film thickness). Hydrogen was used as carrier gas. Temperature programs: condition a: 40–$300^\circ$ C (4$^\circ$ C/min), isothermal 300 $^\circ$ C. Conditions b: 70–$300^\circ$ C (10$^\circ$ C/min), 100–$300^\circ$ C (4$^\circ$ C/min), isothermal 300 $^\circ$ C. Conditions c: 80$^\circ$ C (1 min), 80–100$^\circ$ C (10$^\circ$ C/min), 100–300$^\circ$ C (4$^\circ$ C/min), isothermal 300$^\circ$ C.

2.2.2. Gas chromatography–mass spectrometry (GC–MS)

GC–MS analyses were carried out either on a Finnigan INCOS 50 quadrupole mass spectrometer connected to a Varian 3400 gas chromatograph equipped, as above (oven temperature: conditions a or c) or on a triple quadrupole Finnigan TSQ 700 spectrometer connected to a Varian 3400 gas chromatograph (on-column injector, J&W DB-5 column, 60 m x 0.25 mm i.d., 0.1 $\mu$m film thickness or J&W DB-17, 60 m x 0.25 mm i.d., 0.1 $\mu$m film thickness. Temperature programs: Conditions d, 40$^\circ$ C (1 min), 40–$300^\circ$ C (10$^\circ$ C/min), 100–$300^\circ$ C (3$^\circ$ C/min), isothermal 300 $^\circ$ C. Mass spectra were produced at 70 eV, source 150 $^\circ$ C, in full detection mode over 40–800 amu (cycle time 1.1 s). In both cases, helium was used as carrier gas.
Coinjection experiments with reference compounds were performed on two different GC columns: J&W DB-5 (30 or 60 m × 0.25 mm i.d., 0.1 μm film thickness) and J&W DB-17 (60 m × 0.25 mm i.d., 0.1 μm film thickness).

2.2.3. Nuclear magnetic resonance spectroscopy

NMR experiments were performed on either a BRUKER WP-200 SY (200 MHz operating frequency) spectrometer or on a high resolution BRUKER ARX 500 (500 MHz) spectrometer. Chemical shifts δ are reported in ppm vs tetramethylsilane using the solvent CDCl₃ (δ¹H 7.26 ppm) as internal reference.

2.2.4. Infrared Spectroscopy

Infrared spectra were obtained on a BRUKER IFS 25 spectrometer.

2.2.5. Size exclusion chromatography

Size exclusion chromatographic analyses were performed at room temperature using tetrahydrofuran (THF) as eluent on a Hewlett Packard Series 1050 chromatograph equipped with an UV spectrophotometer, an ICS 8110 differential refractometer and two μStyragel columns (Waters Associates) containing beads of styrene-divinylbenzene copolymer (10³ and 10⁴ Å respectively). Calibration was obtained with a series of linear polystyrenes with M_w values ranging from 480 to 675 000 and polydispersion indexes between 1.05 and 1.07. Calibration curves giving retention times as a function of molecular weight of polystyrene were made using 0.05% solutions. The UV-detector was set at 254 nm. Analyses were performed on 1 wt.% solutions of the samples dissolved in THF. All samples were filtered on an acrodisc 13CR PTFE 0.45 μm and injected 15 min.
after the solutions were prepared. The eluent (THF) flow was set at 2 ml/min. Absorptions were measured at 350 nm.

2.3. Extraction and fractionation

Samples were extracted and fractionated as shown in Fig. 1. Acids were separated following the method described by McCarthie and Duthie (1962). Asphaltenes were precipitated as described by Speight (1984). Samples containing water (from a spring) were dried by azeotropic distillation with toluene prior to separation.

2.3.1. Extraction and precipitation of “humic” substances (adapted from Parsons, 1988)

Typically, the crushed sample (∼20 g) was ultrasonically extracted (3×) with an aqueous sodium hydroxide solution (0.1 M, 100 ml, 30 min) and the aqueous extracts recovered by centrifugation. The three extracts were combined and concentrated to 100 ml. This solution was then acidified to pH 1 with HCl (6 M). The precipitate (“humic” substances) formed after 18 h was centrifuged and the supernatant removed. The recovered “humic” substances were dried in a desiccator and weighed prior to IR spectroscopy.

2.4. Reduction of carbonyls to methylene groups

2.4.1. Wolff-Kishner reduction of ketone fractions (Huang-Minlon, 1946)

The ketone fraction, dissolved in dichloromethane (250–500 ml), was added to a mixture of diethylene glycol (6 ml), n-butanol (4 ml) and hydrazine hydrate.

Fig. 1. Extraction and fractionation scheme.
(1.5 ml) and was refluxed under argon for 5 h. Excess hydrazine, n-butanol and water was removed by distillation. After cooling to room temperature, potassium hydroxide (850 mg) was added and the mixture again heated (200°C, 5 h). After dilution with distilled water and extraction with diethyl ether, saturated and aromatic hydrocarbons were separated from the crude mixture by TLC as shown in Fig. 1.

2.4.2. Reduction of ketone fractions with Pd/C (after Novak et al., 1954)

Typically, the ketone fraction dissolved in ethylacetate (1 ml) and acetic acid (5 ml) was hydrogenated in a hydrogen atmosphere using Pd/C 10% (40 mg). The crude mixture, obtained after filtration over celite, was fractionated by TLC (SiO2/DCM) to yield the hydrocarbons (Rf: 0.85-1), unreacted ketones (Rf: 0.10-0.85), and polars (Rf: 0–0.10).

2.4.3. Deuterated Clemmensen reduction of ketone fractions (after Enzell, 1966)

Typically, D2O (1 ml) and trimethylsilyl chloride (1 ml) were added to a solution of a ketone fraction in anhydrous toluene (1 ml). Zinc powder (20 mg) was added and the mixture was stirred under argon for 2 h (0°C). After extraction, saturated and aromatic hydrocarbons were fractionated by TLC (SiO2/hexane) as shown in Fig. 1.

2.5. Simulation experiments

All simulation experiments were carried out in quartz glassware.

2.5.1. Oxidation of triaromatic steroid 1a.

Water (<1 ml) was added to triaromatic steroid 1a (8 mg) in hexane (10 ml) and exposed to sunlight (summer season) under oxygen atmosphere for 14 days. Combined DCM and diethyl ether extracts had solvent removed under reduced pressure before analysis by GC and GC–MS.

2.5.2. Oxidation of alkylbenzothiophenes

Water (500 µl) was added to 2-methylbenzothiophene 2 (8 mg) (also 4-methylbenzothiophene 3, 9 mg; 2-butyl-4-hexylbenzothiophene 4, 2 mg; synthesized by Perakis, 1986) in hexane (5 ml): the mixture was exposed to air and to lamp light (OSRAM HQI TS 250/D), the spectrum of which is very close to that of sunlight. After 48 h (respectively, 72 h, 64 h) exposure, solvents were removed under reduced pressure and the mixture was analyzed by GC and GC–MS. Identification of oxidized species, in particular the acids, was made by GC and GC–MS analysis after esterification of the crude reaction mixture by diazomethane.

2.6. Synthesis of reference compounds

2.6.1. Deuterated C27 triaromatic steroid 5 and C27 triaromatic steroid ketone 6

Deuterated reference compounds were synthesized following the procedure described by Lemoine (1996).

2.6.2. 2-carboxybenzothiophenes 7

Butyllithium in hexane (0.6 M, 1.9 ml, 1.11 mmol) was added, under argon at ~78°C, to a solution of benzothiophene (124 mg, 0.93 mmol) in anhydrous tetrahydrofuran (5 ml). After 15 min, carbon dioxide was bubbled through the solution (30 min). The reaction mixture was then poured in water, acidified to pH 1 with aqueous HCl and extracted with diethyl ether (3×). After removal of the solvent under reduced pressure, the crude mixture was chromatographed on a silica gel column with CH2Cl2/ACOEt as eluent. 2-Carboxybenzothiophene 7 (53 mg) was obtained in 32% yield. The corresponding methylester, necessary for the coinjection experiments, was obtained by treating 7 with etheral diazomethane. 2-carbomethoxybenzothiophene: TLC: Rf=0.5 (DCM); MS (IE, 70 eV), m/z (rel. int.): 192 [M+](67%), 162 (10), 161 (100), 133 (18), 89 (18); 1H NMR (CD2Cl2, 200 Mhz): 3.92 (3H, s, H-2), 7.43 (1H, s, H-3), 7.89 (2H, m, H-4 or H-7), 8.05 (2H, m, H-5 and H-6), 8.79 (2H, m, H-4 or H-7), 8.05 (1H, s, H-3).

2-methylbenzothiophene 2 and 4-methylbenzothiophene 3 were synthesized following the procedure described by Perakis (1986).

Triaromatic steroid ketones 8 (a and b) and 9 (a and b) were synthesized following the procedure described by Schaeffer et al. (1993).

3. Results and discussion

3.1. Bulk analyses

3.1.1. Elemental analysis

An indication of the strong structural modifications undergone by fossil organic matter exposed to the atmosphere is given by its elemental composition. As illustrated in Table 2 oxidative degradation is characterized by a significant incorporation of oxygen increasing, for instance, from 1.5% in the less degraded samples to 8.7% in the most degraded samples of the Gokhurt series. Hydrogen, sulfur and nitrogen values showed few changes. Interestingly, the O/H ratio increases in the more severely degraded samples indicating that hydrogen has been replaced by oxygen. A slight decrease in the H/C ratio is observed and may be explained either by oxidation of carbon (leading to the removal of H atoms) or by a higher aromaticity. The S/C ratio generally decreases with increasing alteration.
3.1.2. Composition of the samples

Among criteria measuring the severity of oxidative degradation, the relative yields of the extractable fractions (saturates, aromatic hydrocarbons, macromolecular asphaltenes and resins) reveal interesting features (Table 3). Indeed, in all samples investigated, aromatic and saturated hydrocarbon fractions have shown low yields in the most altered samples. For example, 22% of the organic matter in the less degraded sample is made up of aromatic hydrocarbons whereas only 1% of the organic matter in the most degraded sample is composed of aromatics in the Gokhurt series. Similarly, the proportion of saturated hydrocarbons is lower (1–3%) in the most degraded samples. The preferential removal of aromatic hydrocarbons compared to saturated hydrocarbons has been observed previously in weathered shales (Leythaeuser, 1973; Clayton and Swetland, 1978; Forsberg and Bjorøy, 1983) and severely altered samples from oil spills (e.g. Wang et al., 1994). Contrastingly, yields of asphaltenes in weathered samples increase relative to low-molecular-weight fractions with values reaching 90% (Bichri 430; Table 3) (cf. Connan et al., 1980; Wang et al., 1994). The formation of organic material, insoluble in organic solvents, is also observed in the most oxidized samples.

The physical appearance of the samples is further proof that they have been affected by atmospheric exposure. At relatively early stages of degradation (although already biodegraded and water washed) the bitumens still appear black and viscous while at more advanced stages of alteration, they tend to appear brown and friable (Table 1) as observed by Hostettler and Kvenvolden (1994) on some aged oil spills. Samples that have been moderately altered (such as Hit 142) are very hard and black. This might be related to an increase in the relative amount of macromolecular material.

An increase in the oxygen content of macromolecular fractions is revealed by IR spectroscopy (see below and Fig. 2). Thus, fossil organic matter becomes progressively more hydrophilic which may be one reason why part of it becomes insoluble in organic solvents. On the other hand, a substantial part of the more altered samples is more soluble in alkaline (0.1 M NaOH) aqueous solutions. This soluble material can be reprecipitated (and isolated) by lowering the pH. In the case of the Bichri samples, the proportion of organic material extractable by alkaline aqueous solutions is higher in the most altered samples. As much as 90% can be extracted by aqueous alkali. In the less altered samples, the amount of substances extractable with aqueous alkali is about 2% relative to the organic extracts. For the samples from

### Table 2
Elemental composition of the total organic extracts (at.%) and H/C, O/C O/H and S/C ratios

<table>
<thead>
<tr>
<th>Samples</th>
<th>C (%)</th>
<th>H (%)</th>
<th>S (%)</th>
<th>N (%)</th>
<th>O (%)</th>
<th>H/C</th>
<th>O/C</th>
<th>O/H</th>
<th>S/C</th>
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<tr>
<td>Gaujacq 3</td>
<td>37.8</td>
<td>61.2</td>
<td>0.58</td>
<td>0.12</td>
<td>0.23</td>
<td>1.62</td>
<td>6.1E-3</td>
<td>4.7E-3</td>
<td>1.5E-2</td>
</tr>
<tr>
<td>Bastennes 732</td>
<td>41.4</td>
<td>54.8</td>
<td>1.72</td>
<td>0.13</td>
<td>1.97</td>
<td>1.35</td>
<td>5.0E-2</td>
<td>9.5E-3</td>
<td>4.2E-2</td>
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<td>56.7</td>
<td>0.86</td>
<td>0.14</td>
<td>0.60</td>
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<td>1.4E-2</td>
<td>1.1E-2</td>
<td>2.1E-2</td>
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<tr>
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<td>54.3</td>
<td>1.20</td>
<td>0.16</td>
<td>2.14</td>
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<td>5.1E-2</td>
<td>3.9E-2</td>
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<td>Maestu 829</td>
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<td>52.8</td>
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<td>0.18</td>
<td>2.12</td>
<td>1.23</td>
<td>4.9E-2</td>
<td>4.0E-2</td>
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<td>46.5</td>
<td>1.50</td>
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<td>11.9</td>
<td>1.17</td>
<td>29.9E-3</td>
<td>25.4E-2</td>
<td>3.8E-2</td>
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### Table 3
Relative yields of the extractable fractions and of the asphaltenes isolated from the total organic extracts

<table>
<thead>
<tr>
<th>Samples</th>
<th>Saturates (wt.%)</th>
<th>Aromatics (wt.%)</th>
<th>Polars (res. + asph., wt.%)</th>
<th>Asphaltenes (wt.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaujacq 3</td>
<td>37</td>
<td>33</td>
<td>30</td>
<td>nd(^a)</td>
</tr>
<tr>
<td>Bastennes 732</td>
<td>7</td>
<td>15</td>
<td>78</td>
<td>33</td>
</tr>
<tr>
<td>Maestu 830</td>
<td>10</td>
<td>10</td>
<td>80</td>
<td>29</td>
</tr>
<tr>
<td>Maestu 794</td>
<td>3</td>
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<td>96</td>
<td>41</td>
</tr>
<tr>
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<td>1</td>
<td>96</td>
<td>68</td>
</tr>
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<td>41</td>
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<tr>
<td>Gokhurt 326</td>
<td>5</td>
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<td>91</td>
<td>63</td>
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<td>Mehrgahr 334</td>
<td>3</td>
<td>&lt; 1</td>
<td>97</td>
<td>84</td>
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<tr>
<td>Bichri 425</td>
<td>8</td>
<td>19</td>
<td>73</td>
<td>37</td>
</tr>
<tr>
<td>Bichri 430</td>
<td>1</td>
<td>&lt; 1</td>
<td>99</td>
<td>95</td>
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\(^a\) nd = Not determined.
the Maestu series, the yields are even lower. Thus, incorporation of oxygen enables transformation of the organic matter from petroleum into humic-like substances (e.g. Parsons, 1988) favouring their progressive solubilization and transformation.

3.1.3. IR Spectroscopy

IR spectroscopic analyses of the asphaltenes reveal the type of oxygen functionalities arising from degradation (Speight, 1984). Similar trends were observed for all our samples as illustrated for the Maestu series (Fig. 2). In the less degraded bitumens (Fig. 2a), the aliphatic (2920–2855 and 1455–1375 cm$^{-1}$) and aromatic (1600–1650 and 700–930 cm$^{-1}$) signals prevail whereas absorptions corresponding to oxygenated functions are low. At a higher level of alteration (Fig. 2b and c), new IR bands appear such as hydroxyls and carboxyls (large O–H bands at 3650–3100 cm$^{-1}$) suggesting the presence of constituents such as phenols, primary/secondary/tertiary alcohols, aliphatic or aromatic mono- or polycarboxylic acids (although the samples have been carefully dried water may interfere at 3400–3450 cm$^{-1}$). Ketones (C=O, 1650–1730 cm$^{-1}$), aldehydes, acids and esters (1600–1650 cm$^{-1}$), sulfones (1110 cm$^{-1}$), sulfones (1030 cm$^{-1}$) and possibly ethers or sulfonates (1000–1300 cm$^{-1}$) could also be detected. These bands become more intense, even predominant, for samples more severely affected by degradation. Similar qualitative spectra, and evolutions, were recorded for the total extracts.

The “humic” material isolated from Bichri 430 was also investigated by IR spectroscopy (not shown). A predominant band in the 3650–3100 cm$^{-1}$ range was observed suggesting OH groups of phenols, alcohols and aliphatic or aromatic mono- or polycarboxylic acids. A band in the 1600–1730 cm$^{-1}$ range also suggests the presence of aldehydes, ketones and mono- or polycarboxylic acids.

3.1.4. XANES spectroscopy

The XANES spectroscopy experiments carried out on the bitumen from Hit, Gokhurt, Bastennes, Maestu and Bichri series confirmed the trend observed for the organic sulfur compounds. The relative sulfur content of the samples (thiophenic, alkyl or aryl sulfur, Brown et al., 1992) estimated by the intensity of the S L-edge signals, decreased with increasing alteration. On the other hand, the most degraded samples contained oxidized forms of sulfur: sulfides, sulfones and probably sulfonates and sulfates were detected (S K-edge signals; Sarret et al., 1999).

3.1.5. Carbon and hydrogen isotopic compositions

All the samples from the same series, independent of the severity of alteration, have almost identical carbon isotopic compositions: deviations of less than 1% are observed except in the case of Mehrghahr (Table 4). Therefore, $\delta^{13}$C can be used as a tool for establishing correlations between severely degraded bitumens (Connan et al., 1990 and 1992a,b; Connan, 1999). However, a slight enrichment in $\delta^{13}$C is observed in one severely altered sample (e.g. Mehrghahr), a feature which was observed previously by Leythaeuser (1973) in the case of weathered shales. In contrast to the $\delta^{13}$C values, the $\delta^D$ values are strongly affected by the alteration processes. Thus, for the Hit series, for instance (Table 4), the $\delta^D$ values of the asphaltenes increase from $-92\%$ in the less altered sample to $-68\%$ in the more altered ones. The increase in deuterium content with increasing alteration can be explained by exchange reactions with rainwater or percolating waters (less negative $\delta^D$ values; DeNiro and Epstein, 1981; da Silveira Lobo Sternberg, 1988). Thus, incorporation of oxygen (Table 2) induced by atmospheric alteration, in particular as hydroxyls or carboxyls or as ketones as shown by IR spectroscopy favours such a process. Indeed, H/D atoms from hydroxyl groups or H/D atoms located at an enol
position can undergo rapid exchange with water contrary to those located on aliphatic or aromatic skeletons. Variations in $\delta D$ values which parallel an increase in oxygen content can therefore be used to measure the degree of alteration of the bitumens exposed to atmospheric conditions.

3.1.6. Size exclusion chromatography

Molecular weight distributions of organic extracts, and asphaltenes, at various degrees of alteration have been compared by size exclusion chromatography (SEC) for all samples. A calibration of the molecular weight scale was compiled (see Experimental, and Bynum et al., 1970; Rosset et al., 1994). Since retention times on SEC columns depend not only on molecular weight but also on molecular size and shape, quantitative data, in terms of molecular weight, cannot be obtained by this method (Speight et al., 1985). However, within a series of samples, the relative molecular weights can be compared (Calemma et al., 1995).

An increase in the apparent molecular weights with degree of alteration appears in the case of the organic extracts (illustrated for the Maestu series in Fig. 3) and for the asphaltenes in all samples investigated except the Gokhurt series. The increase in the apparent molecular weight of the most oxidized samples can be interpreted as being, partly at least, the result of the formation of aggregates. In fact, this can be deduced from the molecular weight distributions which becomes polymodal (Fig. 3b and c) in the more altered samples (Snyder, 1969). Formation of aggregates was further confirmed by an increase of high molecular weight populations accompanied by the appearance of a trimodal distribution when time between solubilisation of the asphaltenes and their injection on the SEC column was increased from 15 min to 105 min (Speight et al. 1985). In accordance with this observation and interpretation, dilution of the samples, which renders the formation of aggregates less likely, led to a disappearance of the polymodal distributions which became monomodal and shifted towards a lower molecular weight range.

This phenomenon of intermolecular association, leading to polymodal distributions and to apparent higher molecular weights, is particularly clear for the most altered samples and can be explained by a change in chemical composition. In the altered samples, intermolecular association may be favoured by the higher abundance of oxygen-containing functionalities (e.g. formation of hydrogen bonds). A similar trend was observed for asphaltenes.

In conclusion, the substantial increase in molecular weight of the most altered samples may be due, partly, to a concentration of the high molecular weight fractions, since water washing was able to remove the lower molecular weight constituents of the organic extract. Moreover, the formation of aggregates may also partly explain the apparent molecular weight increase. However, it may be that part of the increase is due to crosslinking induced by oxidation. These results may partly explain the alteration of the physical aspect of the most altered samples (e.g. hardening) and the formation of solvent-insoluble material.

3.2. Molecular composition

Saturated and aromatic hydrocarbons were investigated by GC–MS in order to identify the structures more particularly affected by exposure to the atmosphere and to evaluate and distinguish, if possible, the specific impact of the different processes intervening in the alteration (water washing, biodegradation, abiotic oxidation). Emphasis was put on the analysis of ketones, alcohols and acids which may have been formed by biodegradation or abiotic oxidation.

3.2.1. Saturated hydrocarbons

For samples from the Hit series (Fig. 4), the gas chromatograms of the saturated hydrocarbons display unresolved complex mixtures (UCM). Linear and isoprenoid hydrocarbons, are absent, typical of severely biodegraded oils or of oils exposed to natural surface alteration (Gundlach et al., 1983; Volkman et al., 1984; Peters and Moldowan, 1993; Hostettler and Kvenvolden, 1994; Fisher et al., 1998). The $m/z$ 217 fragmentograms (not shown) of steranes display $5a(H)$, $14b(H)$, $17b(H)$ R and S isomers (Mackenzie, 1984) indicating that these samples are derived from relatively mature oils. The distribution of the hopanes ($C_{27}$–$C_{35}$; $m/z$ 191; not shown) dominated by the $17b(H)$, $21b(H)$ isomers (Ensminger et al., 1974, Van Dorsellaer et al. 1977;
Seifert and Moldowan, 1980) is typical for relatively mature oils. Detailed analysis of the distribution of steranes also revealed changes which can be ascribed to biodegradation; for example, a relative enrichment of the diasteranes and an unusual depletion of the 5α(H), 14α(H), 17α(H) S isomers is observed in the intermediate sample. In the most altered samples of this series (Hit 141; Fig. 4c, and Mari 90, not shown), the almost complete removal of steranes may also be explained by biodegradation (Seifert and Moldowan, 1979, Seifert et al., 1984; Goodwin et al., 1983; Chosson et al., 1992). Modification of the distribution of the hopanes may also be due to biodegradation. Thus, an increase in the relative proportion of Tm and Ts as well as of the higher homologues (C35) in Hit 141 are typical effects of biodegradation (Seifert et al., 1984; Requejo and Halpern, 1989; Moldowan and McCaffrey, 1995).

Water washing, and evaporation, to which bitumens were exposed, led to the depletion of the low molecular weight components (up to C15). Similarly, a skewing of the UCM towards higher molecular weight components (typically C25–C35) probably relates to the same effects observed at the highest degree of alteration (Fig. 4b and c). Similar features are observed for the Bichri, Gokhurst and Bastennes samples. Hexahydrobenzohopanes 10 (C31–C35; Connan and Dessort, 1987), 8,14-secohopanes 11 (Schmitter et al., 1982; Rullkötter and Wendisch, 1982; Wang et al., 1990), 8,14 secohexahydrobenzohopanes 12 (Dessort and Connan, 1993) and tricyclic terpanes 13 (Seifert and Moldowan, 1979; Connan et al., 1980; Aquino Neto et al., 1982, 1983) are known to be particularly resistant to alteration and can be detected, at least in traces, in almost all the samples investigated. The relative proportion of the 8,14-secohopanoids increases relative to hopanes when the degree of alteration increases (compare the m/z 123 and m/z 191 fragmentograms).

For samples from the Maestu series, biodegradation is even more pronounced. Linear and isoprenoid hydrocarbons and steranes have completely disappeared in all the samples. Gas chromatograms of the saturates (Fig. 5) revealed beside the predominant UCM a small contribution of residual hopanes, hexahydrobenzo hopanes 10, 8,14-secohopanes 11 and 8,14 secohexahydrobenzohopanes 12 a high abundance of the newly identified regular polycyclic polymeric hydrocarbons 14 and 15 (up to 7 rings; Fig. 5) (Schaeffer et al., 1994; Poinset et al., 1995; Li et al., 1996; Poinset, 1997) which seem to be especially resistant towards biodegradation.

In the most altered sample, regular hopanes have almost
completely disappeared and even the distribution of the resistant polycyclic polyprenic hydrocarbons seems to be modified by biodegradation as the relative proportion of the higher homologues increases. In this series of samples, the degree of alteration, as deduced from IR spectroscopy and elemental analysis (oxygen content) for instance, does not correlate with the intensity of the water washing/evaporation undergone by the samples. Indeed, the skewing of the UCM towards higher molecular weight in the gas chromatograms of the saturates is more pronounced in the case of Maestu 794 (Fig. 5b) which has been rated as an intermediate sample (Table 1).

3.2.2. Aromatic hydrocarbons

As indicated above, aromatic hydrocarbons are highly sensitive to atmospheric alteration processes as their relative proportion to the total organic extract decreases, in the samples from the Hit series, from 22% in the less altered bitumen to 2% in the most altered sample (Table 3). This high sensitivity towards alteration is also revealed by the GC and GC–MS analyses of the aromatic fractions in the samples from the Hit series.
In the less altered sample (Fig. 6a) alkyl-naphthalenes, benzothiophenes and dibenzothiophenes, as well as ring C monoaromatic 16 and 17 and triaromatic 1 and 18 steroids, can still be detected. They tend to disappear in the more altered samples (Fig. 6b and c) partly due to water washing/evaporation, as revealed by the more pronounced depletion in the low-molecular-weight constituents. In these severely altered samples, the aromatic fraction appears almost exclusively as an UCM. Some series of aromatic biomarkers, such as the aromatic steroids which have been reported to be particularly resistant to biodegradation (Connan, 1984), have completely disappeared in the most altered samples. Other series seem to be particularly resistant to atmospheric alteration as they could be found, at least in traces, in almost all the investigated samples, including those most altered. This is so for benzo hopanes 19, the ring D monoaromatic 8,14 secobenzohopanes 20 (Hussler et al., 1984a,b; Dessort and Con nan, 1993) which could be used to evaluate the maturity of biodegraded oils (He Wei and Lu Songnian, 1990) and 8,14-secobenzohopanes 21 (Dessort and Con nan, 1993). Their resistance parallels that observed for the structurally-related saturated hydrocarbons i.e. hexahydrobenzohopanes 10, 8,14-secobenzo hopanes 11, 8,14-secobenzohopanes 12. As for the saturated hydrocarbons, the low-molecular-weight constituents are removed with increasing severity of alteration. This is probably due to water washing/evaporation processes. Similar features are observed for the aromatic fractions of the Gokurt, Bichri, Bastennes and Maestu samples. In the case of the Maestu series, however, the aromatic fractions, even in the less altered sample, almost exclusively appear as an UCM constituted by a mixture of isomeric alkyl(poly)aromatic hydrocarbons. Furthermore, the amount of aromatic compounds in this series decreases more rapidly than the saturates (Table 3) suggesting that an alteration process affecting the aromatic constituents of UCM is operative.

3.2.3. Ketones

Gas chromatograms of the ketone fractions of all samples contain a large UCM. However, for the Gokurt series, some individual peaks appear in the GC trace (not shown) and structures, based on the interpretation of mass spectral data, was proposed for these components. Thus, a series of hopanoid ketones, ranging from C_{27} to C_{34} was detected in the two less oxidized samples with the predominant configurations 17β(H), 21β(H) 22R and 17α(H), 21α(H) 22R, characteristic of immature samples and soils (Dastillung, 1976). This indicates that these compounds are not transformation products of fossil hopanoids from the bitumen which only contains hopanoids having a 17α(H), 21β(H) configuration typical for mature oils (Ensminger et al., 1974, Van Dorsselaer et al. 1977; Seifert and Moldowan, 1980). These hopanoid ketones must, therefore, originate from recent organic contributions: this is not surprising since one sample is an impregnated soil and the other was collected in a water spring.

Beside the hopanoids, a series of components characterized by the presence of small molecular ions (m/z 274 + nx14) and an intense fragment at m/z 245 or 259 were detected in the Gokurt series, displaying mass
spectra very similar to those of the triaromatic steroids (8 and 9) bearing a keto group at the benzylic position (Schaeffer et al., 1993). This hypothesis was confirmed in the case of the C20 (8a) and C26R (9a) homologues and for their 4-methylated counterparts C21 (8b) and C27R (9b) by comparison of their chromatographic behaviour (coelution on two different GC columns, see experimental) and mass spectral data with those of standards synthesized according to the reaction pathway described by Schaeffer et al. (1993).

Detailed analysis of the ketones from the Gokhurt samples also allowed us to detect compounds derived from ring D monoaromatic 8,14-secohopanoids 22 (m/z 173; m/z 188 + nx14; M⁺: 380 + nx14, Fig. 7), ketones derived from ring C monoaromatic steroids 23 and 24 (m/z 267 or 271 and M⁺: 296 + nx14; Schaeffer et al., 1993) as well as ketones 25 derived from the newly identified 14-methyldiaromatic steroids 26 (Lemoine et al., 1996) (M⁺: 292 + nx14; m/z 195; 25a Fig. 8). Interpretation of the mass spectra of the ketones was confirmed by the analysis of the hydrocarbon fractions obtained by Wolff-Kishner reduction of the ketone fractions (Huang-Minlon, 1946) which is able to quantitatively reduce carbonyl groups to the corresponding saturated or aromatic hydrocarbons. Indeed, this fraction of hydrocarbons contains series of ring C monoaromatic 16 and 17 and triaromatic 1 and 18 steroids, ring D monoaromatic 8,14-secohopanoids 20 and 14-methyldiaromatic steroids 26.

Location of the carbonyl group at the benzylic position on the hydrocarbon skeletons of the compounds which could not be identified by comparison with standards, could be demonstrated by means of selective catalytic hydrogenation of the benzylic ketones carried out with Pd/C and acetic acid (Novak et al., 1954). When two benzylic positions were available, as for ring C monoaromatic steroids 16 and 17 and monoaromatic 8,14 secohopanoids 20, it was not possible, at this stage, to determine which one was preferentially oxidized. In fact, subsequent simulation of oxidation carried out on a ring C monoaromatic steroid (Lemoine, 1996) confirmed that both benzylic positions could be oxidized, either sequentially or simultaneously. Aromatic ketones...
have been detected previously by Ehrhardt et al. (1989) in a Baltic sea concentrate where they were supposed to result from the oxidation of aromatics originating from fossil fuels.

The distributions of each series of aromatic hydrocarbons (aromatic steroids 16 and 17, 1 and 18, ring D monoaromatic 8,14-secohopanoids 20...) obtained by Wolff-Kishner reduction of the ketone fractions from the Gokhurt samples are very similar to those of the aromatic hydrocarbons present in the less altered samples of the same series. This is exemplified for the ring C monoaromatic and triaromatic steroid hydrocarbons by comparison of m/z 253 (Fig. 9) and m/z 231 fragmentograms (Fig. 10). Distributions of the isomers and homologues suggests that neither diastereomeric nor significant side-chain size discrimination occurred during the oxidation which led to the formation of the ketones. This also suggests that the analysis of the ketone fraction might be used as a geochemical tool for the study of maturity, or for correlation purposes, in the case of severely oxidized bitumens where the aromatic hydrocarbons have completely disappeared.

The triaromatic steroids, abundant in the less altered sample from the Gokhurt series, have completely disappeared in the most oxidized sample. The corresponding ketones detected in trace amounts only in the less altered sample are present in the most oxidized one (Fig. 11). Similar trends have been observed for other aromatic biomarkers such as the ring C monoaromatic steroids 16 and 17 or the ring D monoaromatic 8,14-secohopanoids 20 (Fig. 12). To check if the formation of triaromatic steroid ketones can account for the removal of the triaromatic steroid hydrocarbons when the degree of alteration increases, a quantitative study of their depletion and the parallel appearance of the corresponding ketones was undertaken using two deuterated standards to achieve better selectivity (Dahl et al., 1985).

Thus, solutions of deuterated 4-methyl triaromatic steroid 5 and deuterated 4-methyl triaromatic steroid ketone 6 were used as internal standards and added respectively to the aromatic and ketone fractions of each sample. The amount of the predominant C28S 18b homologue in the samples (m/z 231; Fig. 11) was calculated from peak areas in typical GC-MS fragmentograms (m/z 231 and m/z 247 for the standard). Similarly, the amount of the C28S ketone 9c (m/z 245; Fig. 11) could be deduced by comparison with the standard ketone (m/z 261). Three separate injections were performed. To make sure that the results were not biased

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**Fig. 9.** Mass fragmentograms (m/z 253; conditions c) showing the distribution of the ring C monoaromatic steroids (a) present in Gokhurt 330 and (b) formed upon Wolff-Kishner reduction of the ketones from Gokhurt 326.

**Fig. 10.** Mass fragmentograms (m/z 231; conditions c) showing the distribution of the triaromatic steroids (a) present in Gokhurt 330 and (b) formed upon Wolff-Kishner reduction of the ketones from Mehrgahr.
by the presence of contaminating organic matter ("dilution") or by the effect of evaporation or water washing ("concentration"), the amount of steroid, either as hydrocarbon or ketone, was expressed relative to the amount of C₃₀ hopane in each sample which is known to be highly resistant to degradation (Prince et al., 1994) and was quantified using a deuterated homologue. It appears that the triaromatic steroid hydrocarbons progressively disappear as the degree of alteration increases (Table 5): at the same time, the amounts of triaromatic steroid ketone increase. Only 10% of the triaromatic ketone, relative to the related hydrocarbon initially present in the less altered sample, could be recovered in the more altered sample. Therefore, we infer that the triaromatic ketones are further transformed into more polar compounds which were not recognized, or which have been removed by water washing, or biodegradation, because of their enhanced solubility in water.

In summary, the results point to an abiotic origin for the benzylic ketones formed during exposure to the atmosphere. Notably, the formation of steroid triaromatic ketones which occurred without diastereomeric or side-chain discrimination (Fig. 10), not expected from a biological process, clearly supports this hypothesis.

Indeed, biological oxidation would favour loss of long chain aromatic steroids and primarily affect the aromatic steroids with a 20R configuration (Wardroper et al., 1984). Although biological oxidation at benzylic positions has been reported (e.g. Van der Linden and Thijssse, 1965), benzylic positions are particularly sensitive to autoxidation. Indeed, benzylic ketones are formed as major transformation products when alkylbenzenes are exposed to light in the presence of a photosensitizer (Ehrhardt and Petrick, 1984). Also, we have exposed the C₂₆ triaromatic steroid hydrocarbon 18a to air and sunlight (without sensitizer) which led to the predominant formation of the corresponding benzylic ketone 9a (60% yield) detected in the altered sample from the Gokhurt series. Consequently, as benzylic ketones most probably have an abiotic origin, we propose to use the ratio of triaromatic steroid/triaromatic steroid ketone (Table 5) as a parameter for the extent of abiotic oxidation undergone by bitumens. It varies from about 1 (unoxidized) down to 0 (most oxidized) (Table 5).

The ketone fractions of the Bichri samples were shown to contain the same type of oxidation products: triaromatic steroid ketones 8 and 9 were detected in the most oxidized sample, whereas they are only present

Fig. 11. Mass fragmentograms (m/z 231; conditions c) showing the distribution of the triaromatic steroids (a) present in Gokhurt 330 and (b) in Mehrgahr. Mass fragmentograms m/z 245 showing the distribution of the triaromatic steroid benzylic ketones (c) present in Gokhurt 330 and (d) in Mehrgahr.
in trace amounts in the non-altered sample. Again, their appearance is accompanied by their depletion as aromatic hydrocarbons in the aromatic fractions of the degraded sample. The same applies to ring D monoaromatic 8,14-secohopanoids 20 and to ring C monoaromatic steroids 16 and 17. For both series again the corresponding benzylic ketones were monitored. The ketones derived from ring C monoaromatic steroids 23 and 24, were only detected in the less altered sample, suggesting that they are formed at an early stage of degradation and are further converted into more complex or more polar components which could not be detected.

Although aromatic hydrocarbons progressively disappear with increasing alteration for the Hit samples, especially those in the low molecular weight range, the corresponding benzylic ketones could not be detected except in the Mari 90 sample.

In the samples from Bastennes and Maestu, aromatic hydrocarbons are present only as a UCM. As shown by GC analysis, the ketones as well as the fraction of the aromatic hydrocarbons obtained by Wolff-Kishner reduction also occur as a UCM. This suggests that the aromatic hydrocarbons forming the UCM from the Maestu samples are transformed during atmospheric

Fig. 12. Mass fragmentograms (m/z 365; conditions c) showing the distribution of the ring D monoaromatic 8,14-secohopanoids (a) present in Gokhurt 330 and (b) in Mehrgahr. Mass fragmentograms (m/z 173; conditions c) showing the distribution of the ring D monoaromatic 8,14-secohopanoids benzylic ketones (c) present in Gokhurt 330 and (d) in Mehrgahr.

Table 5
Amounts of C28 S 18b steroid triaromatic hydrocarbon and of the corresponding benzylic ketone 9c expressed versus the amount of C30 17a(H), 21β(H) hopane in the samples from the Gohkurt series

<table>
<thead>
<tr>
<th>Samples</th>
<th>Amount of C28S</th>
<th>Amount of C28S ket</th>
<th>Amount of C30 2ββ hopane</th>
<th>C28S / C30 2ββ hopane</th>
<th>C28S ket/C30 2β hopane</th>
<th>C28S/(C28S + C28S ket)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gokhurt 330</td>
<td>109 ppm</td>
<td>0.8 ppm</td>
<td>136 ppm</td>
<td>0.80</td>
<td>0.006</td>
<td>0.99</td>
</tr>
<tr>
<td>Gokhurt 326</td>
<td>8 ppm</td>
<td>7 ppm</td>
<td>105 ppm</td>
<td>0.08</td>
<td>0.067</td>
<td>0.53</td>
</tr>
<tr>
<td>Mehrgahr 334</td>
<td>0 ppm</td>
<td>6 ppm</td>
<td>79 ppm</td>
<td>0.00</td>
<td>0.076</td>
<td>0.00</td>
</tr>
</tbody>
</table>
alteration into the corresponding benzylic ketones. This may play an important role in the fate of hydrocarbons occurring in the UCM and which are known to be resistant to biodegradation (Gough et al., 1992). However, some non-aromatic ketones were detected as resolved peaks in the GC trace of the ketone fraction from Bastennes 732. Based on mass spectral data, these components were interpreted as being ketones derived from hopanes (C_{27–C_{33}}) and 8,14-secohopanes (C_{27–C_{29}}) (C_{27} homologue: Fig. 13) and bearing the carbonyl group in the side chain or on ring E (C_{27}). Indeed, hopanes with 17α(H), 21β(H) configuration as well as 8,14-secohopanes which are generally present in mature oils and, in particular, in the Gaujacq oil were obtained by Wolff-Kishner reduction of these ketones. A series of steranes (C_{21–C_{22}}, C_{27–C_{29}}) with 5α(H), 14β(H), 17β(H) 20R and 20S configurations typical for mature oils were also obtained (in trace amounts) although the corresponding ketones could not be detected by direct GC–MS analysis of the ketone fractions.

Clemmensen reduction using D₂O (Enzell, 1966) allowed us to locate the carbonyl group on the saturated skeletons. Mass spectra indicated that, in the case of hopanes and 8,14-secohopanes, the deuterium atoms were mainly located on the side chain. Thus, fragments m/z 191 and 369 in the case of the hopanes (except C_{27}) and m/z 123 in the case of the 8,14-secohopanes which correspond to the A/B rings remained unaffected whereas those corresponding to the D/E rings were shifted by two mass units. However, detailed analysis of the mass spectrum of the coeluting dideuterated C_{21} steranes, obtained by Clemmensen reduction, seems to indicate a random location of the dideuterated methylene group. For example, fragment 218 (deuteration on the D ring or side-chain) and fragment 220 (deuteration on the A, B or C rings) are clearly present in the spectrum (Fig. 14). Thus, the random location of the deuterated methylene group on the steroid hydrocarbons suggests that these coeluting dideuterated steroidal products result from the reduction of a mixture of isomers of steroid ketones bearing the carbonyl group at various locations. The multiplicity of ketone isomers present before reduction may also explain why the steroid ketones could not be detected as such by GC–MS analysis of the ketone fractions. The non-specific location of the carbonyl group on the steroid skeleton suggests that it results from non-specific abiogenic oxidation. The possibility that non-aromatic ketones may be formed by abiogenic oxidation is further supported by simulated photooxidation of n-alkanes in the presence of a photosensitizer (Ehrhardt and Petrick, 1985; Rontani and Giusti, 1987; Giuliano et al., 1997) which led, notably, to the formation of the corresponding ketones. Also, benzylic ketones bearing an additional carbonyl group in the aliphatic side-chain were identified in experiments using the C_{26} triaromatic steroid (Lemoine, 1996).

### 3.2.4. Acids

Studies were mainly focussed on mono-acids which were analysed as methylesters. In all the samples studied, except in the more altered sample from the Hit...
series, linear acids dominated. Their distribution (C_{12}–C_{32}) presented a slight even carbon number pre-dominance. The major products were C_{16} and C_{18} compounds. Several other series of polycyclic acids appeared in the oxidized samples. From their mass spectra, these acids possess structures, and configurations at the asymmetric carbon atoms, typical for lipids from mature petroleum or bitumens, excluding the possibility that they result from recent contamination. Acids identified include tricyclic acids 30 (C_{20}–C_{26}, Cyr and Strausz, 1983), steroid acids 31 and 32 (C_{21}–C_{24}, C_{27}–C_{32}) bearing a carboxyl group in the side-chain (Paulus, 1993) and 3-carboxysteroids 33 and 34 (C_{23}, C_{25}, C_{29}–C_{30}) having 5\(\alpha\)(H), 14\(\beta\)(H), 17\(\beta\)(H) 20R and 20S configurations (as deduced from mass spectra and those of standards; Schaeffer et al., 1993), 17\(\alpha\)(H), 21\(\beta\)(H)-hopanoic acids (C_{28}–C_{34}) and acids derived from 8,14-secohopanes 35 and 36 (C_{28}–C_{32}). According to their mass spectra, the hopanoids were mainly functionalized on their side-chains. Although these acids probably derive from mature bituminous organic matter, no direct correlation could be established between the saturated hopanoids and the hopanoic acids since the respective distributions were quite different.

In the mono-ester fraction of the two most altered samples from the Hit series, novel sedimentary organic sulfur compounds were detected which correspond to a series of alkylated benzenothiophene carboxylic acids extending from C_{9} to C_{16} (several isomers) as deduced from their mass spectra. The latter are characterized by an intense molecular ion (M\(^{+}\): 192 + n\times 14) and an intense fragment at M\(^{+}\)–31 (Fig. 15) suggesting that the carboxyl group is located at the benzylic position. Comparison of the mass spectra and chromatographic behaviour with those of standards (see Experimental) established that the major isomer of the lower homologues is the methylester corresponding to 2-carboxybenzothiophenes 7. The second major isomer may possibly be the methylester deriving from 4-carboxybenzothiophene 37. Its mass spectrum is very similar to that of 7 and nearly identical to that of a carboxybenzothiophene (as methylether) obtained by reaction of 4-methylbenzothiophene with oxygen in the presence of light (see below) and to which the structure of 4-carboxybenzothiophene could be ascribed. Furthermore, the compound obtained in the simulation experiment, and that occurring in the natural sample, coelute on two GC columns coated with different phases.

The carboxylic acids detected may have been formed by biodegradation of “free” hydrocarbons, or alkylated benzenothiophenes, originally present in the bitumen. Thus, the steroid acids bearing the acid function in the side-chain [present in the most altered samples of the Hit series (Mari 90)] are formed during in vitro biodegradation of steranes (Paulus, 1993). Similarly, linear carboxylic acids have been shown to be possible biodegradation products of linear alkanes (Jones and Howe, 1968; Klug and Markovetz, 1971; Fonken and Johnson, 1972). Formation of carboxylic acids by biodegradation of hopanones, tricyclic terpanes or alkylated benzothiophenes is also possible. Indeed, carboxylic acids related to hopanes (configuration at the asymmetric carbon atoms typical of mature petroleum) and tricyclic terpanes have been detected in biodegraded oils (e.g. Cyr and Strausz, 1983; Mackenzie et al., 1983; Behar and Albrecht, 1984). However, formation of these acids by biological oxidation of the corresponding saturated hydrocarbons has, to our knowledge, never been demonstrated.

Alternative explanations can be proposed for at least a part of the carboxylic acids present in the altered samples. The aliphatic acids (linear, hopanoid, tricyclic) may have been released from the macromolecular constituents of the bitumen by hydrolysis of esters. Alternatively, these acids may have been formed by an abiotic oxidation process affecting either free lipids or lipids linked to the macromolecular matrix, in particular those linked by C–C bonds to alkylaromatic sub-units (a type of linkage which has been demonstrated to occur in geomacromolecules; Trifilieff et al., 1992). Indeed, the latter possibility is supported by simulation experiments in which 9-heptadecylphenanthrene 39 was exposed to oxygen and light (Lemoine, 1996) resulting in the formation of the related benzyl ketone 40 but also in the partial cleavage of the side-chain: a mixture of linear...
acids (C_{13}–C_{18}, C_{16} and C_{17} being predominant) was recovered, and degradation of the aromatic skeleton gave the lactone 41 (Fig. 16). For the C_{15} acid, 17 of the carbon atoms must come from the alkyl side-chain of 9-heptadecylphenanthrene and one from the aromatic part of the molecule. This can only be explained by oxidation of the aromatic rings by singlet oxygen which can be formed photochemically, the benzylic ketone related to 9-heptadecylphenanthrene 40 acting as a photosensitizer.

Thus, alkyl skeletons linked to aromatic subunits in geomacromolecules, or in low-molecular weight aromatics, will be oxidized at the benzylic position to the corresponding ketones. The latter can then be further oxidized, resulting in the cleavage of C–C bonds and, subsequently, in the release of carboxylic acids (Fig. 17). Alternatively, release of carboxylic acids may result from oxidation at the aromatic rings (Fig. 17) induced, for example, by singlet oxygen. If the benzylic position is a secondary position, such an oxidative degradation reaction could also result in the release of ketones. Therefore, the C_{27} and C_{29} hopanoid or 8,14-secohopanoid ketones present in the 732 may have been formed by a process similar to that affecting hopanoan thiophenes or hopanes and 8,14-secohopanes linked to aromatic units.

The benzothiophenic acids identified in the most altered samples may also have been formed by abiotic oxidation. Indeed, 2-carboxybenzothiophene 7 present in the Hit samples has been obtained when 2-methylbenzothiophene 2 was exposed to air and light (Andersson and Bobinger, 1996). Similarly, 4-carboxybenzothiophene 37 (mass spectrum almost identical to that of 7) also present in the Hit samples was formed by the reaction of 4-methylbenzothiophene 3 with oxygen in the presence of light. Furthermore, 2-butyl-4-hexylbenzothiophenes 4 ([M^+] = 388) synthesized by Perakis (1986) led, in addition to the predominant formation of the corresponding benzylic ketones 42 and 43, ([M^+] = 402) to the cleavage of one alkyl substituent and to the formation of a benzothiophenic monoacid 44 (as methylester: [M^+] = 290, fragment at m/z 259 ([M^+] – 31)). These results suggest that the benzothiophenic acids in the altered samples could have been formed by abiotic oxidation of methyl- or alkylbenzothiophenes initially present in the aromatic fraction (Fig. 18). Alternatively, they may have been formed by release of alkylbenzothiophenic subunits linked to macromolecular structures by oxidation of the alkyl chain at the benzylic position (Fig. 18a).

3.2.5. Alcohols

Alcohols have not been detected in our samples, except for the Gokhurt series where hopanoid alcohols, ranging from C_{30} to C_{32}, have been identified. Mass spectral data indicate that 17α(H) 21β(H), 17β(H) 21α(H) and 17β(H) 21β(H) isomers are present. Therefore, these hopanoids probably derive from recent organic matter.

3.2.6. Organic sulfur compounds

The Hit series was particularly rich in organic sulfur compounds. In the less degraded oil, numerous polycyclic sulfides, isolated by silver ion TLC, were recognized (Fig. 19a). Three series (45, 46, 47) of hopanoid sulfides (C_{30}–C_{35}, C_{31} predominant) (Philippe, 1993; Schaeffer, 1993) were present as well as steroid sulfides (C_{23}–C_{29}, 48 and 49) (Schmid, 1986; Behrens et al., 1997), bicyclic sulfides 50 (C_{17}–C_{24}, C_{18} and C_{20} predominant) and tetracyclic sulfides 51 (C_{23}–C_{29}) (Payzant et al., 1986). In the intermediate sample, all low molecular weight components had disappeared but the hopanoid sulfides were still detectable (Fig. 19b). Sulfides were totally absent in the most degraded samples (Hit 141 and Mari 90).

The sulfones corresponding to the cyclic sulfides described above appeared in polar fractions (“alcohol” fraction) from the two most degraded samples (Hit 141 and Mari 90). They were identified from their mass spectra, comparison with reference compounds (Philippe, 1993) and by means of their distribution. Thus, the hopanoid sulfones (C_{30}–C_{32}, C_{31} predominant), related to sulfides 45, 46, 47, display a distribution similar to that of the sulfides. Tetracyclic sulfones were recognized as steroid sulfones (C_{22}). The lack of long chain steroid sulfones may be explained by the preferential biodegradation of steroids with a long side-chain. Another type of tetracyclic sulfone, based on a tricyclic C_{23} hydrocarbon skeleton, was identified as the C_{23} sulfone found in Athabasca bitumens by Strausz et al. (1990). Sulfones corresponding to the bicyclic sulfides 50 were absent. Because of their low molecular weight, and their enhanced solubility in water, they may have been removed by water washing.

Fig. 16. Predominant oxygenated compounds formed upon reaction of 9-heptadecylphenanthrene with oxygen under sunlight irradiation.
Several studies have shown that bacteria are able to oxidize sulfides to sulfones and sulfoxides (e.g. Fedorak, 1990, Jenisch et al., 1995). Also, the high sensitivity of aliphatic sulfides to several oxidants (hydrogen peroxide, ozone, singlet oxygen; Oae, 1991) is well known. The fact that all the sulfides in the Hit series were converted to the related sulfone, regardless of structure (tetracyclic terpanes, steroids, hopanoids etc) suggests...
that those in the most weathered samples result predominantly from abiotic processes.

Aromatic sulfur compounds are present in significant amounts in the less altered samples from the Hit and the Bichri series: among them, alkylated dibenzothiophenes prevail. Based on their mass spectra, they bear one to eight aliphatic carbon atoms. A complex alkylbenzothiophene series was detected by mass spectrometry. Alkyldibenzothiophenes and alkylbenzothiophenes are absent from the most altered bitumen, partly due to water washing/evaporation but probably also to biological or abiotic oxidation processes (Fedorak, 1990).

Interestingly, as described above, some carboxylic acids, derived from the alkylbenzothiophenes, were found in the oxidized samples from the Hit series. However, no sulfones or sulfoxides related to these aromatic sulfur compounds (alkylated benzo-thiophenes and dibenzothiophenes) could be detected in the altered samples suggesting that, under the given conditions, sulfur in aromatic rings is more difficult to oxidize than sulfur in thianes or thiologes.

3.3. Oxidants and abiotic oxidation mechanisms in natural environments

Among the oxidants occurring in nature, and liable for the formation of the oxygenated compounds detected in our samples, triplet oxygen is the most abundant and is known to participate in radical-type autoxidation reactions (Ingold, 1969; Voronenkov et al., 1970; Korcek et al., 1972; Sheldon and Kochi, 1981). Alkyl radicals are able to react with triplet oxygen to form hydroperoxide radicals, which can then abstract hydrogen on another hydrocarbon skeleton, leading to a new alkyl radical, and a radical chain reaction can proceed. The intermediate peroxides and peroxide radicals are further transformed into several oxygenated compounds such as acids, alcohols and ketones or can undergo cleavage reactions (Perkel et al., 1994) leading to smaller oxygenated molecules. The most likely location for this type of oxidation are benzylic or allylic positions, as well as tertiary alkyl positions, which, by abstraction of hydrogen, lead to stable radicals. Thus, this type of reaction can account for the formation of aromatic ketones, or the benzo-thiophenic acids bearing an oxygen functionality at the benzylic position, as identified in the most weathered samples from the Hit series. Such processes may also induce cleavage and may contribute to the degradation of high molecular weight constituents of the bitumens, leading to the release of low-molecular weight oxygenated components and explain (see below), some of the carboxylic acids which have appeared in the most altered samples.

![Gas chromatograms (Conditions c) of the sulfide fractions isolated from Hit 143 (a) and from Hit 142 (b).](image-url)
However, triplet oxygen alone is not very reactive and free radicals are generally necessary to initiate the oxidation reaction by abstraction of hydrogen. Free radicals are present in nature, for example in humic substances (Senesi and Steelink, 1989). Free radicals can also be formed photochemically in the presence of radical initiators. Aliphatic as well as benzylic ketones, which are the suspected products of such radical oxidation affecting bitumens are also good photochemical radical initiators (Kopecky, 1992). In consequence, the presence of light may favour the oxidation of the organic matter from petroleum, and the reaction products may even accelerate the process.

Beside triplet oxygen, other oxidants may be responsible for the formation of the oxygen-containing compounds, such as singlet oxygen, ozone, hydroxyl or peroxy radicals, superoxides, nitrogen oxides and hydrogen peroxide (Cooper et al., 1989; Aneja et al. 1994). Some, although present in low concentrations, are ubiquitous (hydrogen peroxide, nitrogen oxides), others are transient, highly reactive species (e.g. hydroxyl radicals, singlet oxygen). All may occur, in nature, from photochemical reactions involving photosensitizers (Cooper et al., 1989) such as humic substances, aromatic hydrocarbons, benzylic ketones and porphyrins. Relatively stable oxidants such as hydrogen peroxide, formed by photochemical reactions in nature can be transported to the bitumens by rainwater. Alternatively, oxidants may be formed by photochemical reactions between sensitizers already present in bitumens and react directly with the bitumens' organic matter. In such a situation, unstable, highly reactive oxidants such as hydroxyl radicals or singlet oxygen may also participate in the oxidation processes affecting bitumens.

4. Conclusions

Analyses of five series of crude oil samples exposed to the atmosphere, but altered to different extents, revealed that dramatic changes have affected the more degraded samples. Elemental analysis and IR spectroscopy showed that atmospheric alteration results in an increase in the oxygen content which parallels an increase in the proportions of the macromolecular constituents. A substantial depletion of the low-molecular-weight constituents, in particular of the saturated and aromatic compounds, is also observed. $\delta D$ has been shown to be particularly sensitive to alteration processes as it increases from values of about $-100\%$ in the less altered samples up to about $-50\%$ in the more altered ones. This may be the result of H/D exchange reaction with water on oxygenated functionalities formed during atmospheric alteration by biological and abiotic oxidation processes.

Although some of the saturated and aromatic hydrocarbons have probably been removed by water washing and evaporation, we have shown that some of these fractions have been transformed into oxygenated compounds. Indeed, analysis of the polar fractions (ketones, sulfones, carboxylic acids) allowed us to identify oxygen-containing compounds clearly resulting from the oxidation of typical petroleum components. For example, benzylic ketones are related to aromatic biomarkers (mono- and triaromatic steroids, ring $D$ monoaromatic 8,14-secohopanoids), and carboxylic acids are related to steroids, tricyclic terpenoids, hopanoids and benzothiophenes. Sulfones, which are the result of the oxidation of steroid, terpenoid or hopanoid sulfides were also detected.

The hypothesis that these oxygenated compounds result at least partially from an abiotic oxidation process, rather than from biodegradation, is supported by quantitative data obtained for steroid triaromatic ketones; these compounds appear with increasing alteration and are absent in the less altered, but already biodegraded samples. Furthermore, oxygen incorporation generally occurred without diastereomeric discrimination, a feature unexpected for biological processes. Simulation experiments, in which typical constituents of mature petroleum (triaromatic steroids, alkylated benzothiophenes, alkylphenanthrenes) were exposed to oxygen and light, further support this hypothesis, since they were transformed into benzylic ketones or acids similar to those detected in our samples.

The results also suggest that abiotic oxidation can severely alter macromolecular constituents of petroleum, as well as aromatic constituents of the UCM. Simulation experiments suggest that abiotic oxidation may favour the release, by oxidative cleavage, of subunits from the macromolecules as oxygenated low molecular weight components (e.g. carboxylic acids or ketones). Finally, it has been shown that incorporation of oxygen functionalities transforms part of the highly lipidic macromolecular fractions of petroleum into more hydrophilic humic-like substances.

In summary, our results suggest that alteration by abiotic oxidation may play a key role in the fate of petroleum pollutants in the environment. Indeed, abiotic incorporation of oxygen may lead to the formation of oxygenated components by the oxidation of low molecular weight lipids but also by inducing cleavage reactions in macromolecular substances. The increased water solubility of these oxygenated constituents probably allows a better bio-availability and, therefore, an increased rate of biodegradation. However, it may be that some of these oxygenated compounds are toxic to biota especially since they are more bioavailable. Furthermore, the important incorporation of hydroxyl and carboxylic functions into petroleum macromolecules (transforming them into humic-like substances) combined with oxidative cleavage reactions may also favour their progressive solubilization in nature.
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