Maria Gaviño[1]
Bernardo Hermosin[1]
Véronique Vergès-Belmin[2]
Witold Nowik[2]
Cesareo Saiz-Jimenez[1]

[1]Instituto de Recursos Naturales y Agrobiologia, CSIC, Apartado 1052, 41080 Sevilla, Spain
[2]Laboratoire de Recherche des Monuments Historiques, Champs-sur-Marne, France

Composition of the black crusts from the Saint Denis Basilica, France, as revealed by gas chromatography-mass spectrometry

The organic fraction of black crusts from Saint Denis Basilica, France, is composed of a complex mixture of aliphatic and aromatic compounds. These compounds were studied by two different analytical approaches: tetramethyl ammonium hydroxide (TMAH) thermochemolysis in combination with gas chromatography-mass spectrometry (GC-MS), and solvent extraction, fractionation by silica column, and identification of the fraction components by GC-MS. The first approach, feasible at the microscale level, is able to supply fairly general information on a wide range of compounds. Using the second approach, we were able to separate the complex mixture of compounds into four fractions, enabling a better identification of the extractable compounds. These compounds belong to different classes: aliphatic hydrocarbons (*n*-alkanes, *n*-alkenes), aliphatic and aromatic carboxylic acids (*n*-fatty acids, α,ω-dicarboxylic acids, and benzenecarboxylic acids), polycyclic aromatic hydrocarbons (PAH), and molecular biomarkers (isoprenoid hydrocarbons, diterpenoids, and triterpenoids). With each approach, similar classes of compounds were identified, although TMAH thermochemolysis failed to identify compounds present at low concentrations in black crusts. The two proposed methodological approaches are complementary, particularly in the study of polar fractions.

Key Words: Thermochemolysis; Black crusts; Polar compounds; Benzenecarboxylic acids

Received: June 3, 2003; revised: February 5, 2004; accepted: February 6, 2004

DOI 10.1002/jssc.200301626

1 Introduction

Air pollution causes the deposition of a variety of organic and inorganic compounds on the surface of historic monuments. Wet and dry deposition processes combined with gypsum crystal growth result in the formation of a hard, grey to black crust, in which airborne organic pollutants, aerosols, and a wide range of particulate matter, including dust, pollen, and spores, are entrapped in the mineral matrix. Black crusts cover marble, limestone, sandstone, granite, and terracotta statues, and have also been observed on bronze statues, tiles, bricks, and glass windows [1].

Previous studies have shown the complexity of the organic fraction of black crusts [2, 3], and it was indicated that a detailed study of their components would require an adequate analytical approach [2]. In fact, most modern analytical techniques currently in use require solubility and/or volatility of compounds for analysis. Thus, most of the work carried out with different materials has been focused on the analysis of organic molecules either with a relatively low molecular weight or readily volatilized by derivatization (e. g. methylation). Such samples can be extracted with organic solvents and fractionated by chromatography, prior to analysis, to separate different classes of compounds and facilitate their identification.

In organic geochemistry, there are many schemes for sample fractionation, depending on the nature of the compounds to be studied [4]. However, fractions eluted from chromatography columns with mixtures of increasingly polar solvents often contain too many compounds that are difficult to resolve by gas chromatography (GC). Furthermore, the final fractions, containing the most-polar compounds, are difficult to elute and could contaminate the analytical instruments, since polar species may be lost on cold or adsorbing sites. In addition, a great deal of information is lost when unsuitable methods are used to analyze the insoluble organic fractions of black crusts which are sometimes retained on the fractionation column.

In general, fractionation and separation of organic compounds result in tedious and time-consuming analyses, while contamination of samples with compounds intro-

Original Paper

Correspondence: Cesareo Saiz-Jimenez, Instituto de Recursos Naturales y Agrobiologia, CSIC, Apartado 1052, 41080 Sevilla, Spain. Fax: +34 954 624002, E-mail: saiz@irnase.csic.es.

Abbreviations: Benzene(poly)carboxylic acids , BPCA; Carbon preference index, CPI; Polycyclic aromatic hydrocarbons, PAH; Tetramethyl ammonium hydroxide, TMAH; trimethylsilyldiazomethane, $TMSCHN_2$.

duced during application of the protocol (e.g. dialkyl phthalates) is highly probable. However, when relatively large amounts of material are available, solvent extraction, fractionation, and analysis by gas chromatography in combination with mass spectrometry (GC-MS) are applied for the study of the organic components of black crusts [2, 3, 5].

The amount of sample is sometimes not enough for solvent extraction. In such cases, an analytical approach feasible at micro-scale level and able to supply fairly general information on a wide range of compounds is needed. A useful approach consists of analytical pyrolysis, a flash thermal desorption and degradation method, also in combination with GC-MS, which allows analyses from microgram quantities of samples. This method has been applied to a variety of samples obtained from cathedrals, churches, historic buildings, and sculptures in Belgium, Ireland, Italy, Spain, and The Netherlands, with satisfactory results [1, 2, 6].

The first application of analytical pyrolysis investigating the organic compounds present in black crusts was reported by Saiz-Jimenez [2]. The temperatures used in these studies (500–700°C) were shown to be adequate for evaporation/pyrolysis of organic materials within mineral matrices. However, no fatty acids were detected in the pyrolysate, although they were by far the most abundant compounds in solvent extracts. Several factors contributed to this absence, such as the relatively high pyrolysis temperature and the presence of a considerable amount of salts producing decarboxylation. Inorganic additives resulted in the production of unwanted thermal reactions, namely cyclization and aromatization reactions of unsaturated fatty acids [7, 8]. A decarboxylation process in fatty acids was noted in the pyrolysis of black crusts, resulting in alkenyl compounds with one C atom fewer than the original fatty acid, together with the formation of some other artifacts [2, 8].

Some of the advantages and disadvantages of solvent extraction vs analytical pyrolysis were discussed a few years ago [6], although at that time direct methylation of the samples with tetramethyl ammonium hydroxide (TMAH) prior to pyrolysis was not considered.

In an effort to solve the bias of data in pyrolysis, mainly from the loss of polar compounds, TMAH thermochemolysis has been proposed [8, 9]. This consists of the derivatization of samples containing carboxyl and/or hydroxyl groups with the alkylating reagent. Tetramethyl ammonium salts of organic acids can be converted into methyl esters and the corresponding by-products in the pyrolysis unit; thereby, the functional groups are directly protected [8].

In this paper, we report our studies on the composition of the organic compounds present in black crusts, after extraction and fractionation on a silica column. We were able to separate the complex mixture of compounds into four fractions, enabling a better identification of almost all extractable compounds. We also compared the data obtained by direct analysis of the black crusts using TMAH thermochemolysis.

2 Materials and methods

Black crusts were collected from the Basilica of Saint Denis, located in the suburbs of Paris, France, in an area heavily affected by industrial pollution until the sixties. Saint Denis Basilica is considered a good representative of early Gothic architecture. Many original forms of the Gothic style are found in this basilica, such as the cross-rib vault and flying buttresses, which represented structural innovations.

The black crusts were collected from the walls and vaults of a limestone arch, located on a terrace at the top of the west front of the basilica.

The analytical protocol is described in **Figure 1**. For solvent extraction, 20 g of black crusts were ground in an agate mortar and extracted in a Soxhlet apparatus with dichloromethane-methanol (2 : 1) for 72 h. The extracts were evaporated under vacuum at low temperature (below 40°C) and redissolved in dichloromethane-methanol (2 : 1). The resulting extract was chromatographed using a silica column, eluted with hexane (Fraction I), hexane-dichloromethane (1 : 1) (Fraction II), dichloromethane (Fraction III), and finally with methanol (Fraction IV). Compounds present in fractions III and IV were methylated with trimethylsilyldiazomethane ($TMSCHN_2$) [10]. The residues from the black crust extraction were also analyzed using TMAH thermochemolysis.

For analysis, 2 μL of extract was injected in a Fisons instrument GC 8000/MD 800, using a 30 m × 0.25 mm TRB-5HT column (film thickness 0.1 μm). The GC oven was programmed from 80°C to 120°C at 30 K min^{-1} and then to 320°C. The final temperature was held for 20 min.

TMAH thermochemolysis was accomplished as described by Saiz-Jimenez et al. [6]. Briefly, a few mg of sample was deposited in a Curie-point small hollow ferromagnetic cylinder (temperatures 300 or 590°C) and wetted with 5 μL of a 25% *w/w* methanolic solution of TMAH. The cylinder was gently dried with an N_2 flow and immediately inserted in the pyrolyser. The analysis was performed in the same instrument as described above, to which a Fischer 0316 Curie-point pyrolyser was coupled. The GC oven was programmed from 50°C to 280°C, at a rate of 5 K min^{-1}. This temperature was held for 100 min and then raised to 310°C at 20 K min^{-1}, where the final temperature was held for 2 min. Additional analytical protocols can be found elsewhere [6, 9].

In the analytical procedure followed in this work, the carboxylic acids were recovered as the corresponding methyl

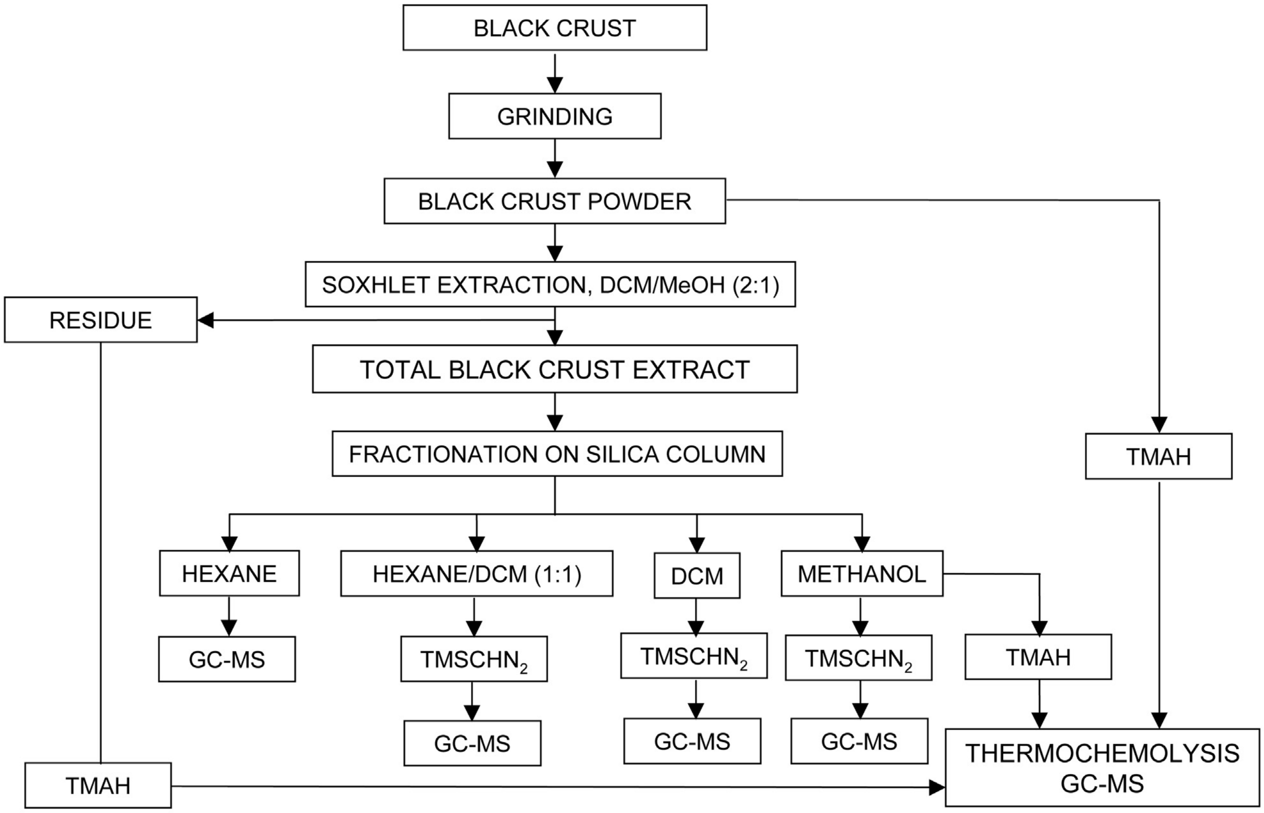

Figure 1. Analytical protocol followed for black crust analysis.

esters, and the hydroxyls as methoxyls. Throughout this paper they are referred to as acids and hydroxyls – their original forms – rather than as derivatized methyl esters and methoxyls. No attempt was made to identify all compounds, only the most-abundant or most-representative (e.g. molecular markers); therefore, the lists of compounds are undoubtedly incomplete.

The compounds were identified by comparison of their mass spectra with a self-compiled data bank of compounds from a variety of samples [1, 2, 5–9, 11–13]. In some cases, identification was achieved by computer analysis from a National Bureau of Standards library of about 54000 spectra, with the computer matching being checked against standards whenever possible.

3 Results

The results from TMAH thermochemolysis followed by GC-MS analysis of Saint Denis black crusts are shown in **Figure 2**. Some of the most abundant thermochemolysis products are listed in **Table 1**. The chromatogram from Saint Denis black crusts compares well with previously published data from other European monuments [1, 2, 5, 6]. Previous studies [6] have shown that the most abundant thermochemolysates in the black crusts from an Irish building were *n*-fatty acids, benzenecarboxylic acids, aliphatic dicarboxylic acids, and polycyclic aromatic hydrocarbons (PAH) having from two to five rings. Homologous series of *n*-alkanes and *n*-alkenes were also present, although their peaks were of lower intensity. This trend was also observed in Saint Denis black crusts. In fact, the major peaks were those of *n*-hexadecanoic acid (26), 1,2-benzenedicarboxylic acid (15), *n*-octadecanoic acid (32), 1,9-nonanedioic acid (19), benzoic acid (3), 1,3-benzenedicarboxylic acid (17), methylbenzenecarboxylic acid (6), 2-butenedioic acid (1), fluoranthene (29), and 1,5-pentanedioic acid (4).

The chemical structures of the identified compounds indicated that a representative picture of all major classes of compounds present in black crusts is provided by TMAH thermochemolysis.

In addition, black crusts were extracted with organic solvents, and the extracts were fractionated on a silica column. Four fractions were obtained. Fraction I, eluted with hexane, was mainly composed of a homologous series of *n*-alkanes in the range $C_{14}–C_{32}$ with a carbon preference index (CPI) of ca. 1.6. A maximum at C_{17} and a secondary maximum at C_{27} were observed (**Figure 3**, **Table 2**). This distribution suggests a petroleum origin with a superimposed plant contribution. Series of *n*-alkenes ($C_{15}–C_{27}$)

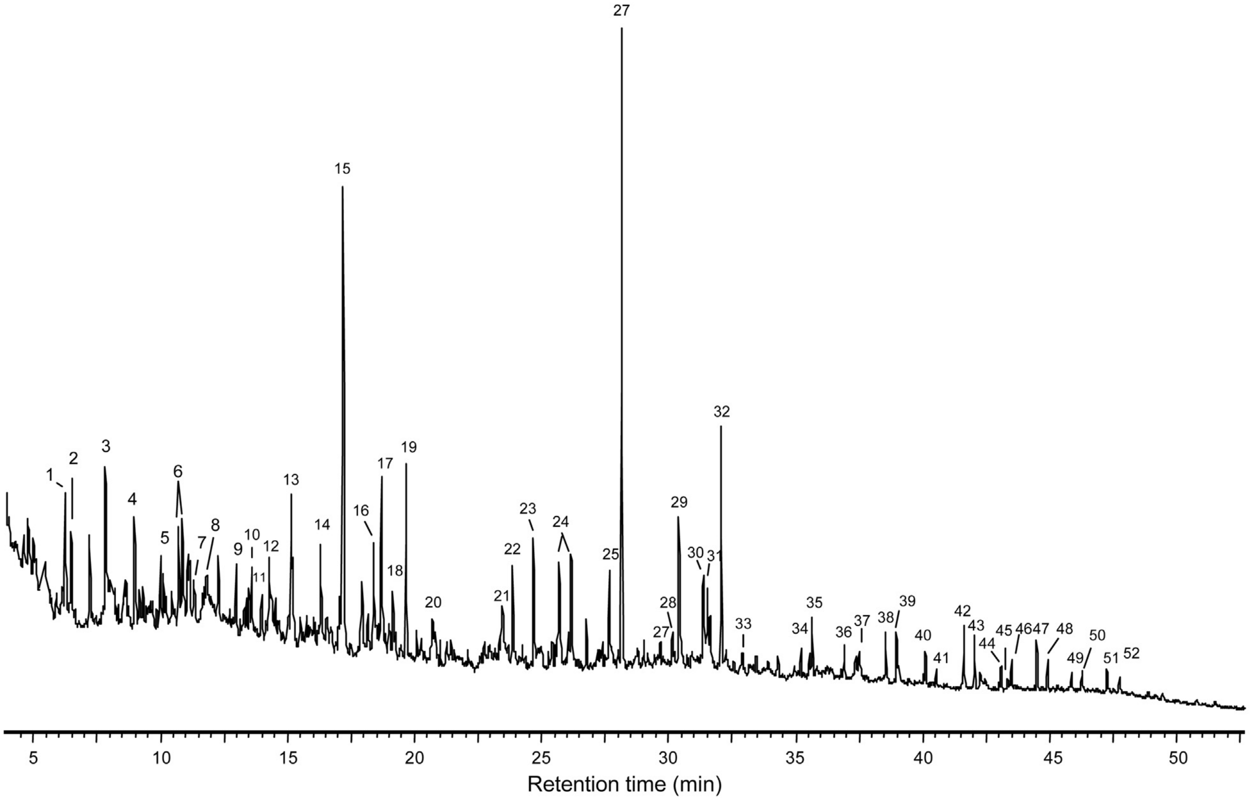

Figure 2. TMAH thermochemolysate of Saint Denis Basilica black crusts. Peaks refer to Table 1.

and alkylcyclohexanes ($C_{11}-C_{18}$) also contributed to this fraction. These have previously been found in aerosols, and their presence has been related with petroleum residues [11]. Pristane and phytane, diagenetic products of phytol and molecular markers of petroleum, were also present.

The α,β-hopane biomarkers were relatively easily detected using ion monitoring at *m/z* 191, in spite of their low contribution to the fraction. The identified hopanes in the studied scan range were 18α(*H*)-22,29,30-trisnorhopane, 17α(*H*)-22,29,30-trisnorhopane, 17α(*H*),21β(*H*)-30-norhopane (31), 17α(*H*),21β(*H*)-hopane, 17α(*H*),21β(*H*)-30-homohopane 22*S* (34), 17α(*H*),21β(*H*)-30-homohopane 22*R*, 17α(*H*),21β(*H*)-30-bishomohopane 22*S*, and 17α(*H*),21β(*H*)-30-bishomohopane 22*R*, although the series probably continued until the pentaquishomohopanes. Interestingly, the pattern of hopanes found in black crusts [2, 5] was similar to those reported for aerosol particles [4, 11], crude oil [12], and automobile and diesel engine exhausts [13]. The presence of isoprenoid hydrocarbons in combination with the alkylcyclohexane series and the hopanes indicates a petroleum source for a major part of the hydrocarbon fraction present in the black crusts.

Fraction II, eluted with hexane-dichloromethane (1:1), contained a complex mixture of PAH, including some of their alkylated derivatives (**Figure 4**, **Table 3**). Species having from two to five aromatic rings were distributed in this fraction, and the most-abundant compounds were in agreement with those previously identified in the thermochemolysate. The compounds identified were fluoranthene (19), phenanthrene (12), and pyrene (21) as the most abundant, followed by benzonaphthofuran (22), *n*-hexadecanoic acid (17), and triphenylene (31). Peaks with lower intensity corresponded to 3-methylphenanthrene (15), 2-methylphenanthrene (16), benzo[*a*]fluorene (23), methylfluoranthene (24), benzo[*b*]naphtho[2,1-*d*]thiophene (26), benzo[*ghi*]fluoranthene (27), chrysene (30), and $C_{20}H_{12}$ isomers (36). This PAH mixture included sulphur- (peaks 11, 28, and 33), oxygen- (peaks 8, 9, 32, and 37), and nitrogen-containing polycyclic aromatic species (peaks 14 and 20). PAH are ubiquitous, and originate from biomass and fossil fuel combustion [4, 14, 15]

Fraction III, eluted with dichloromethane, was dominated by the series of *n*-fatty acids (C_8-C_{30}), as shown in **Figure 5** and **Table 4**. Their distribution showed a strong even-to-odd C-number predominance (CPI 12), with maximum at C_{16}, which according to Simoneit [11] is characteristic of a biogenic origin. However, further analysis of

Table 1. Major compounds identified in the thermochemolysate of Saint Denis Basilica black crusts.

Peak	Compound	Peak	Compound
1	2-Butenedioic acid	27	*n*-Eicosane
2	1,4-Butanedioic acid	28	*n*-Heptadecanoic acid
3	Benzoic acid	29	Fluoranthene
4	1,5-Pentanedioic acid	30	Pyrene
5	Naphthalene	31	*n*-Heneicosene
6	Methylbenzoic acid	32	*n*-Octadecanoic acid
7	*n*-Nonanoic acid	33	Benzenetetracarboxylic acid
8	1,6-Hexanedioic acid	34	*n*-Tricosane
9	1-Methylnaphthalene	35	*n*-Eicosanoic acid
10	1,3-Methoxy-2-hydroxybenzene	36	*n*-Tetracosane
11	*n*-Decanoic acid	37	Chrysene
12	3-Hydroxybenzoic acid	38	*n*-Pentacosane
13	4-Hydroxybenzoic acid	39	*n*-Docosanoic acid
14	1*H*-Isoindole-1,3(2*H*)-dione	40	*n*-Hexacosane
15	1,2-Benzenedicarboxylic acid	41	*n*-Tricosanoic acid
16	1,4-Benzenedicarboxylic acid	42	*n*-Heptacosane
17	1,3-Benzenedicarboxylic acid	43	*n*-Tetracosanoic acid
18	*n*-Dodecanoic acid	44	*n*-Octacosane
19	1,9-Nonanedioic acid	45	$C_{20}H_{12}$
20	4-Hydroxy-3-methoxybenzoic acid	46	*n*-Pentacosanoic acid
21	Hydroxybenzenedicarboxylic acid	47	*n*-Nonacosane
22	*n*-Tetradecanoic acid	48	*n*-Hexacosanoic acid
23	Phenanthrene	49	*n*-Triacontane
24	Benzenetricarboxylic acid	50	*n*-Heptacosanoic acid
25	*n*-Nonadecane	51	*n*-Hentriacontane
26	*n*-Hexadecanoic acid	52	*n*-Octacosanoic acid

diesel soots obtained from bus exhausts [16] showed a similar pattern, and in this case a biogenic origin could be excluded. In addition, polycyclic aromatic ketones such as fluorenone (9), phenanthrenone (12), methylphenanthrenone (15), and some others coeluted with the *n*-fatty acids (Table 4).

Fraction IV, eluted with methanol, had a yellow colour and was irreversibly adsorbed onto alumina columns in previously tested fractionation schemes. This fraction comprised a complex mixture of compounds, of which a series of *n*-fatty acids (C_{12}–C_{32}) were the most abundant. A few diterpenoids (**Figure 6.a**, **Table 5**) were also identified. Because the benzenecarboxylic acids and other polar compounds found in TMAH thermochemolysis were not identified in Fraction IV, it was thought that these compounds might i) be lost on cold or adsorbing sites, or ii) take part in a macromolecular structure. To resolve this, Fraction IV was subjected to TMAH thermochemolysis at 300°C. The thermochemolysate composition is shown in **Figure 6.b** and Table 5. This analytical approach doubled the number of identified compounds, with methyl-, hydroxy-, hydroxy-methoxy-, and hydroxy-dimethoxy-benzoic acids and benzene(poly)carboxylic acid (BPCA) derivatives being particularly representative. Some of these derivatives corresponded to the three lignin units (*p*-coumaryl, guaiacyl, and syringyl), with the *p*-coumaryl and syringyl derivatives being less abundant than the

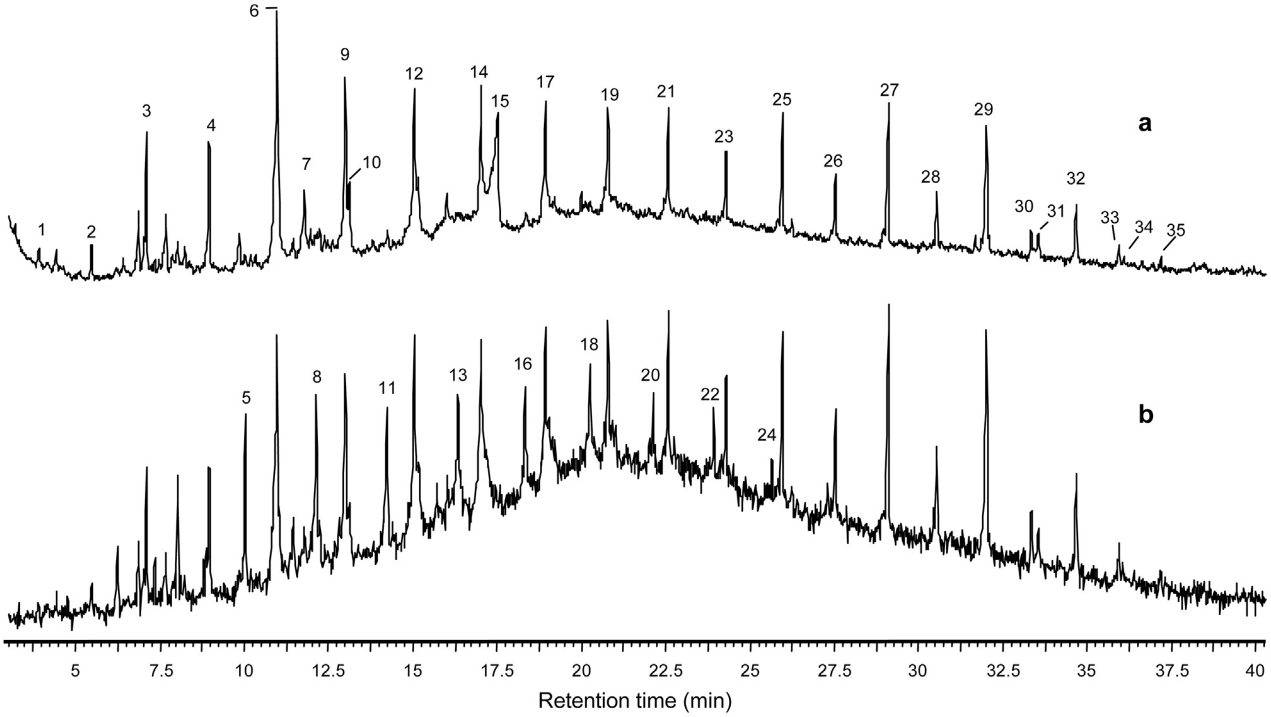

Figure 3. TIC chromatogram of Fraction I from Saint Denis Basilica black crusts (a), and mass fragmentogram (*m/z* 82+83) for alkylcyclohexane series. Peaks refer to Table 2.

Table 2. Major compounds identified in Fraction I from Saint Denis Basilica black crusts.

Peak	Compound	Peak	Compound
1	*n*-Tridecane	19	*n*-Docosane
2	*n*-Tetradecane	20	*n*-Hexadecylcyclohexane
3	*n*-Pentadecane	21	*n*-Tricosane
4	*n*-Hexadecane	22	*n*-Heptadecylcyclohexane
5	*n*-Decylcyclohexane	23	*n*-Tetracosane
6	*n*-Heptadecane	24	*n*-Octadecylcyclohexane
7	Pristane	25	*n*-Pentacosane
8	*n*-Undecylcyclohexane	26	*n*-Hexacosane
9	*n*-Octadecane	27	*n*-Heptacosane
10	Phytane	28	*n*-Octacosane
11	*n*-Dodecylcyclohexane	29	*n*-Nonacosane
12	*n*-Nonadecane	30	*n*-Triacontane
13	*n*-Tridecylcyclohexane	31	17α(*H*),21β(*H*)-30-norhopane
14	*n*-Eicosane	32	*n*-Hentriacontane
15	Cyclic octaatomic sulfur	33	*n*-Dotriacontane
16	*n*-Tetradecylcyclohexane	34	17α(*H*),21β(*H*)-30-homohopane (22*S*)
17	*n*-Heneicosane	35	*n*-Tritriacontane
18	*n*-Pentadecylcyclohexane		

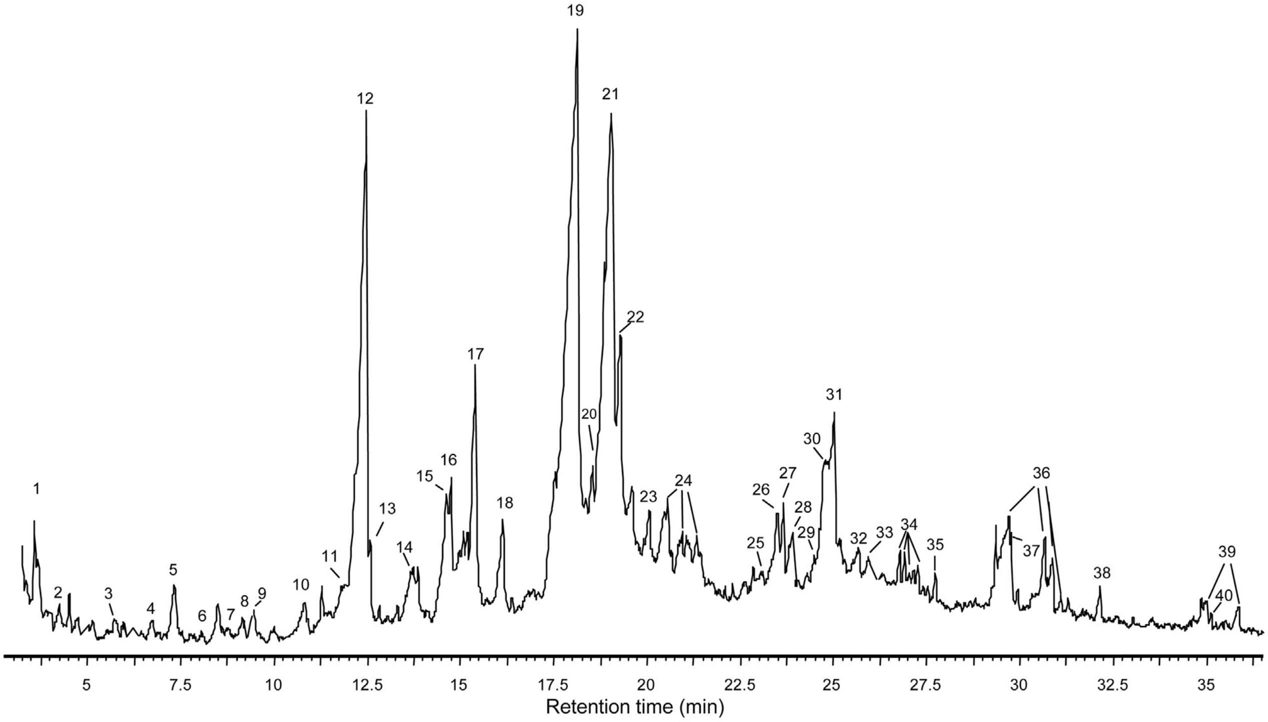

Figure 4. TIC chromatogram of Fraction II from Saint Denis Basilica black crusts. Peaks refer to Table 3.

Table 3. Major compounds identified in Fraction II from Saint Denis Basilica black crusts.

Peak	Compound	Peak	Compound
1	3-Phenoxy-1-propanol	21	Pyrene
2	Biphenyl	22	Benzonaphthofuran
3	Dimethylnaphthalene	23	Benzo[*a*]fluorene
4	Methylbiphenyl	24	Methylfluoranthene
5	*n*-Dodecanoic acid	25	Benzanthrenone
6	Trimethylnaphthalene	26	Benzo[*b*]naphtho[2,1-*d*]thiophene
7	Dimethylbiphenyl	27	Benzo[*ghi*]fluoranthene
8	Xanthene	28	Benzo[*b*]naphtho[1,2-*d*]thiophene
9	Methyldibenzofuran	29	Benz[*a*]anthracene
10	$C_{6:1}$-alkenylnaphthalene	30	Chrysene
11	Dibenzothiophene	31	Triphenylene
12	Phenanthrene	32	Naphtho[2,1,8,7-*klmn*]xanthene
13	Anthracene	33	Methylbenzonaphthothiophene
14	Carbazole	34	Methylbenz[*a*]anthracene
15	3-Methylphenanthrene	35	Binaphthyl
16	2-Methylphenanthrene	36	$C_{20}H_{12}$ isomers
17	*n*-Hexadecanoic acid	37	Dinaphthofuran
18	2-Phenylnaphthalene	38	*n*-Hexacosanoic acid
19	Fluoranthene	39	$C_{22}H_{12}$ isomers
20	Benzo[*c*]carbazole	40	$C_{22}H_{14}$

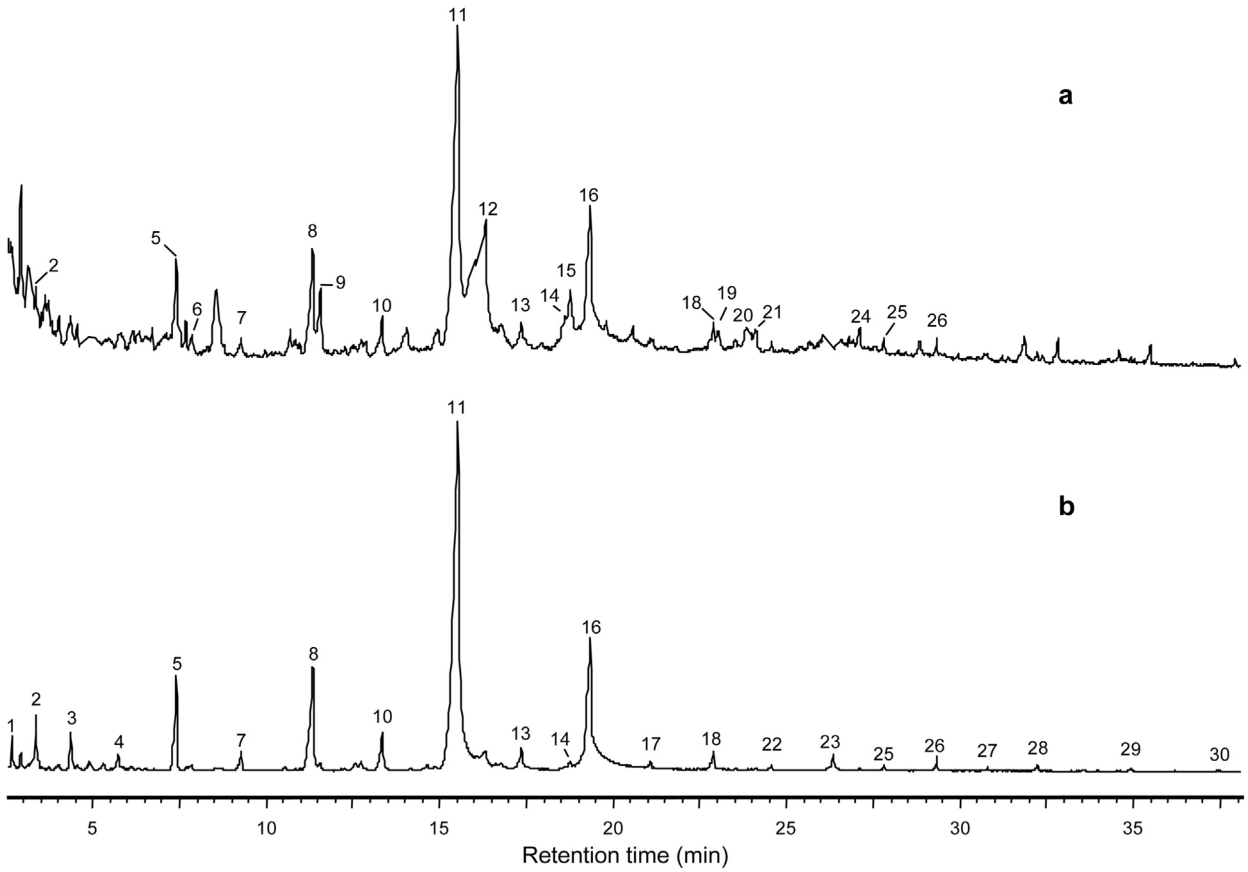

Figure 5. TIC chromatogram of Fraction III from Saint Denis Basilica black crusts (a), and mass fragmentogram (*m/z* 74) for *n*-fatty acids series. Peaks refer to Table 4.

Table 4. Major compounds identified in Fraction III from Saint Denis Basilica black crusts.

Peak	Compound	Peak	Compound
1	*n*-Octanoic acid	16	*n*-Octadecanoic acid
2	*n*-Nonanoic acid	17	*n*-Nonadecanoic acid
3	*n*-Decanoic acid	18	*n*-Eicosanoic acid
4	*n*-Undecanoic acid	19	Benzofluorenone
5	*n*-Dodecanoic acid	20	Benzacridine
6	1,9-Nonanedioic acid	21	Benzanthrenone
7	*n*-Tridecanoic acid	22	*n*-Heneicosanoic acid
8	*n*-Tetradecanoic acid	23	*n*-Docosanoic acid
9	Fluorenone	24	Naphthacenedione
10	*n*-Pentadecanoic acid	25	*n*-Tricosanoic acid
11	*n*-Hexadecanoic acid	26	*n*-Tetracosanoic acid
12	Phenanthrenone	27	*n*-Pentacosanoic acid
13	*n*-Heptadecanoic acid	28	*n*-Hexacosanoic acid
14	*n*-Octadecenoic acid	29	*n*-Octacosanoic acid
15	Methylphenanthrenone	30	*n*-Triacontanoic acid

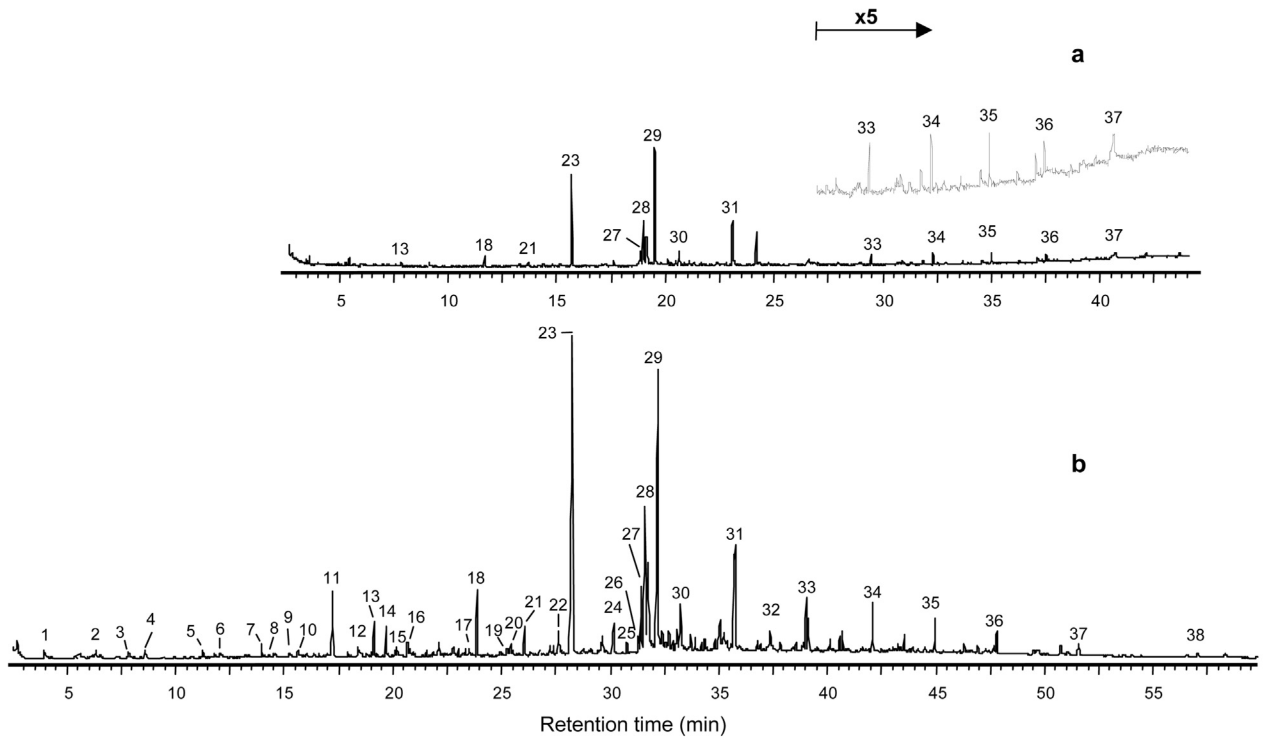

Figure 6. TIC chromatogram of Fraction IV from Saint Denis Basilica black crusts (a), and TMAH thermochemolysate of the same fraction (b). Peaks refer to Table 5.

Table 5. Major compounds identified in Fraction IV from Saint Denis Basilica black crusts. (a) GC-MS injection, (b) TMAH thermochemolysis.

Peak	Compound	a	b	Peak	Compound	a	b
1	Ethenylbenzene	–	+	20	Methyltetradecanoic acid	–	+
2	C_4-alkylcyclohexene	–	+	21	*n*-Pentadecanoic acid	+	+
3	Benzoic acid	–	+	22	Methylpentadecanoic acid	–	+
4	*n*-Octanoic acid	–	+	23	*n*-Hexadecanoic acid	+	+
5	*n*-Nonanoic acid	–	+	24	*n*-Eicosane	–	+
6	4-Hydroxybenzaldehyde	–	+	25	Hydroxyphenanthrene	–	+
7	*n*-Decanoic acid	–	+	26	5β-Podocarpa-8,11,13-trien-16-oic acid	+	+
8	2-Hydroxybenzoic acid	–	+	27	9,12-Octadecadienoic acid	+	+
9	4-Hydroxybenzoic acid	–	+	28	9-Octadecenoic acid	+	+
10	1-Hydroxy-4-nitrobenzene	–	+	29	*n*-Octadecanoic acid	+	+
11	1,2-Benzenedicarboxylic acid	–	+	30	8,15-Isopimaradien-18-oic acid	+	+
12	1,4-Benzenedicarboxylic acid	–	+	31	8,11,13-Abietatrien-18-oic acid	+	+
13	*n*-Dodecanoic acid	+	+	32	*n*-Heneicosanoic acid	+	+
14	1,9-Nonanedioic acid	–	+	33	*n*-Docosanoic acid	+	+
15	3-Methyl-3*H*-benzothiazol-2-one	–	+	34	*n*-Tetracosanoic acid	+	+
16	Methylbenzenedicarboxylic acid	–	+	35	*n*-Hexacosanoic acid	+	+
17	Hydroxybenzenedicarboxylic acid	–	+	36	*n*-Octacosanoic acid	+	+
18	*n*-Tetradecanoic acid	+	+	37	*n*-Triacontanoic acid	+	+
19	Methyltetradecanoic acid	–	+	38	*n*-Dotriacontanoic acid	+	+

guaiacyl ones, denoting a major contribution of conifers. In addition to the series of *n*-fatty acids, a few nitrogen derivatives, *n*-alkanes and aliphatic dicarboxylic acids were also identified. Thus, these data support a likely macromolecular origin for BPCA derivatives.

Of particular importance were a group of diterpenoids, present in either solvent extracts or TMAH thermochemolysis. They were identified as 5β-podocarpa-8,11,13-trien-16-oic acid (26), 8,15-isopimaradien-18-oic acid (30), 8,15-pimaradien-18-oic acid, and 8,11,13-abietatrien-18-oic acid or dehydroabietic acid (31). These compounds were formed from burning plant biomass, and more specifically from conifer woods and lignites [15, 17].

The black crust residue after solvent extraction was also subjected to TMAH thermochemolysis. The thermochemolysate pattern (not shown) was very similar to that obtained for Fraction IV. The major compounds were the homologous series of *n*-fatty acids (C_{14} to C_{18}), aliphatic dicarboxylic acids (C_4 to C_9), and the BPCA from benzoic to tricarboxylic acids. Hydroxy-, hydroxy-methoxy-, and hydroxy-dimethoxy-benzenes and -benzoic acids, as well as a few PAH (phenanthrene, pyrene, fluoranthene), were also identified. The presence of these compounds in the residue indicated that for a complete extraction, after dichloromethane-methanol (2:1), the black crusts should be extracted with methanol, and the two extracts combined, as a completely extracted polar fraction, before column fractionation.

4 Discussion

The organic fraction of black crusts from Saint Denis Basilica, France, is composed of a complex mixture of aliphatic and aromatic compounds. These belong to the classes of aliphatic hydrocarbons (*n*-alkanes, *n*-alkenes), aliphatic and aromatic carboxylic acids (*n*-fatty acids, α,ω-dicarboxylic acids, BPCA), PAH, and molecular biomarkers (isoprenoid hydrocarbons, diterpenoids, and triterpenoids), which have been identified previously in aerosols and particulate matter [11, 14, 15, 17] and in black crusts from other European monuments [5].

Series of compounds such as *n*-alkanes, *n*-alkenes, *n*-fatty acids, PAH, etc., are not source-specific, as they are generally found in every combustion emission [4, 16]. However, a small number of organic compounds are considered molecular biomarkers, showing little or no change in chemical structure from their parent organic molecule. According to Oros and Simoneit [14], these biomarkers are characterized by their restricted occurrence, source specificity, molecular stability, and suitable concentration for analytical detection. In fact, compounds – identified in the black crusts – having the abietane and pimarane skeletons are the major diterpenoids produced by conifers in the northern hemisphere, and represent some of the major thermal alteration products in conifer and coal smokes [14, 17]. Lignin thermochemolysis products were also found [8], particularly the most oxidized units (*p*-hydroxybenzoic acid, vanillic acid, and syringic acid). The hopane series is a precise indicator of an input source from fossil fuel use (e.g. vehicular traffic), and its C_{27} to C_{35} members are found in all urban aerosols [13].

While the presence of diterpenoids in the yellow fraction, together with the finding of lignin thermochemolysis products [8, 9], indicates that a source of these compounds was wood and/or lignite combustion, triterpenoids and isoprenoid hydrocarbons point to a contribution from petroleum derivatives. The particulate matter derived from all these combustion processes probably contributed to the formation of black crusts in Saint Denis Basilica.

The identification of BPCA in the black crusts is of interest because, as discussed by a number of authors [18–22], it supports the presence of humic-acid-like substances in the polar fractions of atmospheric particulate matter. Terrestrial and aquatic soluble humic substances (humic and fulvic acids) are macromolecular materials whose building blocks are constituted by BPCA, lignin derivatives, and aliphatic materials [8, 9]. Yokouchi and Ambe [18] were the first authors to report the presence of BPCA in the methanolic fraction of airborne particulate matter. Havers et al. [19] extracted urban particulate matter with NaOH, and isolated a humic-acid-like fraction, concluding that this is an important constituent of the organic carbon present in atmospheric particulates. Attempts to characterize these humic-acid-like fractions were not very successful, partly because the inadequacy of the chosen methods excluded the achieving of a clear molecular structure and the individualization of the building blocks [20–22].

The finding of water-soluble organic compounds formed by oxidation of hexane soot has recently been reported [23]. This water-soluble fraction has been claimed to contain polycarboxylic acids, and it has been suggested that the structure of these compounds is analogous to that of naturally occurring macromolecular humic and/or fulvic acids. Decesari et al. [23] considered that the identification of macromolecular polyacid compounds was consistent with previous findings concerning the presence of water-soluble macromolecular humic-like substances in atmospheric aerosol samples [20–22]. However, the presence of polycarboxylic acids was deduced from the H-NMR spectra, and no building blocks or structural units were identified. Graham et al. [24] used GC-MS to study aerosols from burning biomass. The identified compounds gave a clear pattern of lignocellulose combustion products, as denoted by the finding of anhydrosugars and lignin pyrolysis products [25]. However, only one benzenedicarboxylic acid (phthalic acid) was identified.

In our laboratory, BPCA has been found in diesel particulate matter directly extracted from exhausts [16], and in black crusts from monuments. In both materials there were major components of a polar, yellow fraction. Therefore, BPCA could have a dual origin. In fact, although a few BPCA can be found in aerosols from burning biomass, when BPCA are found in black crusts from monuments (in urban environments), their origin is probably the combustion of fossil fuels and, in particular, petroleum derivatives, as suggested by the identification of benzenedi-, tri-, and tetracarboxylic acids in diesel soots.

5 Conclusions

The use of TMAH thermochemolysis is the first-option approach for the study of organic compounds present in black crusts. The technique provides an almost complete picture of the main classes of compounds entrapped in the mineral matrix.

The thermochemolysis technique also provides clues revealing the presence of BPCA that either form part of a macromolecular matrix or are linked to other compounds (e.g. hydrogen bonds, C–O and C–C bonds, etc.), as revealed in the analysis of the yellow Fraction IV. These compounds are also present in fulvic acids (a yellow, water-soluble humic fraction) [9], in airborne particulate matter [18], and, apparently, in the water-soluble fraction from fogwater [22].

Solvent extraction and column fractionation provides a complete picture of the classes of compounds present in black crusts. The advantage of this protocol is that molecular biomarkers and compounds usually present in low amounts can be extracted from large samples (typically 20 g) of black crusts, enabling separation and identification, and complementing the data obtained from thermochemolysis. In such a case, a source apportionment can be provided.

Acknowledgements

This work was supported by the European Commission (project EV4K-CT2000-00029), the French Ministry of Culture (subvention de recherche chapitre 6698 20, exercice 2001), and the MCyT, project BTE2001-1277.

References

[1] C. Saiz-Jimenez, B. Hermosin, J.J. Ortega-Calvo, G. Gomez-Alarcon, *J. Anal. Appl. Pyrol.* **1991**, *20*, 239–251.

[2] C. Saiz-Jimenez, in: *Science, Technology and European Cultural Heritage,* N.S. Baer, C. Sabbioni, A.I. Sors (Eds.), Butterworth-Heinemann, Oxford 1991, pp. 523–526.

[3] S. Machill, K. Althaus, W.E. Krumbein, W.E. Steger, *Org. Geochem.* **1997**, *27*, 79–97.

[4] B.R.T. Simoneit, M.A. Mazurek, *CRC Crit. Rev. Environ. Control* **1981**, *11*, 219–276.

[5] C. Saiz-Jimenez, *Atmos. Environ.* **1993**, *27B*, 77–85.

[6] C. Saiz-Jimenez, B. Hermosin, J.J. Ortega-Calvo, *Int. J. Environ. Anal. Chem.* **1994**, *56*, 63–71.

[7] C. Saiz-Jimenez, *Org. Geochem.* **1995**, *23*, 81–85.

[8] C. Saiz-Jimenez, *Environ. Sci. Technol.* **1994**, *28*, 1173–1780.

[9] C. Saiz-Jimenez, *Environ. Sci. Technol.* **1994**, *28*, 197–200.

[10] T. Aoyama, T. Shioiri, *Tetrahedron Lett.* **1990**, *29*, 5507–5508.

[11] B.R.T. Simoneit, *Int. J. Environ. Anal. Chem.* **1986**, *23*, 207–237.

[12] R.P. Philp, *Mass Spectrom. Rev.* **1985**, *4*, 1–54.

[13] M.P. Fraser, G.R. Cass, B.R.T. Simoneit, *Atmos. Environ.* **1999**, *33*, 2715–2724.

[14] D.R. Oros, B.R.T. Simoneit, *Appl. Geochem.* **2001**, *16*, 1513–1455.

[15] B.R.T. Simoneit, *Appl. Geochem.* **2002**, *17*, 129–162.

[16] J. Reyes, B. Hermosin, C. Saiz-Jimenez, in*: Air Pollution and Cultural Heritage*, C. Saiz-Jimenez (Ed.), Balkema, Lisse 2004, in press.

[17] D.R. Oros, B.R.T. Simoneit, *Fuel* **2001**, *79*, 515–536.

[18] Y. Yokouchi, Y. Ambe, *Atmos. Environ.* **1986**, *9*, 1727–1734.

[19] N. Havers, P. Burba, J. Lambert, D. Klockow, *J. Atmos. Chem.* **1998**, *29*, 45–54.

[20] S. Decesari, M.C. Facchini, S. Fuzzi, *J. Geophys. Res.* **2000**, *105*, 1481–1489.

[21] B. Varga, G. Kiss, I. Ganszky, A. Gelencsér, Z. Krivácsy, *Talanta* **2001**, *55*, 561–572.

[22] G. Kiss, B. Varga, A. Gelencsér, Z. Krivácsy, A. Molnár, T. Alsberg, L. Persson, H.-C. Hasson, M.C. Facchini, *Atmos. Environ.* **2001**, *35*, 2193–2200.

[23] S. Decesari, M.C. Facchini, E. Matta, M. Mircea, S. Fuzzi, A.R. Chughtai, D.M. Smith, *Atmos. Environ.* **2002**, *36*, 1827–1832.

[24] B. Graham, O.L. Mayol-Bracero, P. Guyon, G.C. Roberts, S. Decesari, M.C. Facchini, P. Artaxo, W. Maenhaut, P. Köll, M.O. Andreae, *J. Geophys. Res.* **2002**, *107 (D20),* 8047, doi:10.1029/2001JD000336.

[25] C. Saiz-Jimenez, J.W. de Leeuw, *Org. Geochem.* **1986**, *10*, 869–876.