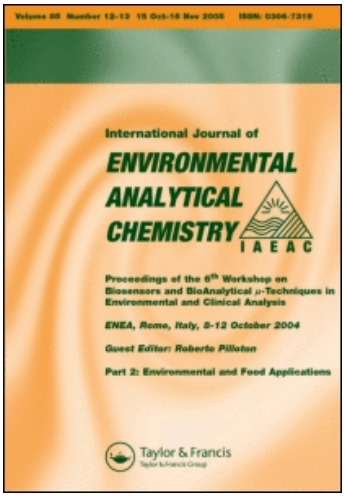

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Urban Aerosol Acids: Analysis of National Institute of Standards and Technology Standard Reference Material 1649

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URBAN AEROSOL ACIDS: ANALYSIS OF NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY STANDARD REFERENCE MATERIAL 1649

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Urban particulate matter, collected from Washington, DC and certified by the National Institute of Standards and Technology (NIST) as Standard Reference Material 1649, was extracted and fractionated into acid, base and neutral fractions. Each fraction was tested for biological activity using a microbial mutagenesis assay system. The organic acid fraction showed unexpectedly high mutagenic activity, and was subjected to chemical characterization studies. Following derivatization, analysis by GC/MS showed the presence of fatty acids, aromatic acids (including phenolic compounds), and a significant number of compounds that could not be identified from mass spectral compendia. Spectroscopic and elemental analysis data supported the characterization of the fraction as predominantly aromatic. Mass spectra from both GC/MS and direct probe analysis showed the presence of a chlorinated substance, subsequently identified as the fungicide Dichlorophen. The compound was shown to comprise over 50% of the mass of the organic acid fraction. A reference standard of Dichlorophen was not mutagenic. The presence of the fungicide in the NIST certified urban aerosol is, in all probability, due to artifactual processes. Attempts to concentrate the observed mutagenic activity by preparative chromatography and acid/base partition experiments were not successful.

KEY WORDS: Aerosol, acids, analysis, bioassay, Dichlorophen, mutagenic.

INTRODUCTION

The extraction, fractionation, and bioassay analyses of organic extractables from Washington, DC urban particulate matter (collected and certified by NIST as Standard Reference Material 1649[1] has been described by Lewtas *et al.*[2] (see previous paper, this issue). The fractionation scheme produced six discrete fractions: organic acids, organic bases, and four fractions of neutral materials, each

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of which was bioassayed using a microbial mutagenesis test system (*Salmonella typhimurium* TA98 plate incorporation). Significant biological activity was associated with the more polar compound class fractions. The type and level of activity exhibited by certain fractions were in general agreement with results reported by other investigators for urban particulate extracts.[3–5] For example, a study[3] of particulate extract fractions from a number of diverse locations (Upland, CA; Lake Charles, LA; Houston, TX; Beaumont, TX; Elizabeth, NJ) showed organic acids comprising approximately 5–30% of the total extract, with all fractions showing slight to moderate mutagenicity. However, the mutagenicity associated with the organic acid fraction of the SRM 1649 extract (DC-acid) was unexpectedly high in terms of specific activity. This fraction was selected for more comprehensive characterization studies in an effort to identify the components or types of components responsible for the observed mutagenicity.

Aerosol organic acids as a class have not been as much the focus of analytical effort as other aerosol fractions (e.g. polynuclear aromatics). Cautreels and Van Cauwenberghe[6] described qualitative and quantitative methods (GC/MS) for urban aerosol acids collected in Belgium. Fatty acids (C_{12}–C_{26}) were the major acidic species identified, with aromatic carboxylic acids and hydroxy aromatic compounds comprising the remainder of the compounds identified. The presence of lower order fatty acids in the part-per-trillion range has been reported for Japanese urban aerosol.[7] Grosjean *et al.*[8] characterized the acid fraction of a metropolitan Los Angeles aerosol sample, also using GC/MS techniques, and unambiguously identified 15 dicarboxylic acids (C_3–C_{10}). Artifact formation during sample collection was deemed an unlikely source for these acids; the presence of the diacids was related to photochemical smog episodes. Wauters *et al.*[9] also identified diacids, as well as other fatty acids and some aromatic carboxylic acids, in aerosol collected in Ghent, Belgium.

Other acidic materials reported as constituents of urban aerosol included phenol and associated compounds. Cautreels and Van Cauwenberghe[6] identified tetra- and pentachlorophenol, as well as monohydroxylated phenanthrene, anthracene and pyrene. Phenol itself was quantitated using HPLC[10] for a Japanese urban air sample.

In characterizing the organic acid fraction of the methylene chloride extract of SRM 1649 (Lewtas *et al.*[2]) several experiments were undertaken in order to obtain as much baseline information as possible. Spectral information, elemental analysis, and thermogravimetric data were obtained to define the chromatographic approaches and procedures to be used for specific compound identification

EXPERIMENTAL

Thermogravimetric Analysis

Thermogravimetric analysis was performed on a Perkin-Elmer TGS-2 thermogravimetric system using a temperature program from 50 °C to 700 °C at 40 °C/minute in a flowing stream of helium (40 mL/min). Approximately 3.0 mg each of derivatized and underivatized sample were analyzed.

Methylation Methods

Methyl ester derivatives were prepared for GC and GC/MS by reaction with Methelute (trimethylanilinium hydroxide—Pierce). Methyl ester derivatives were also prepared by reaction with diazomethane, which was generated *in situ* and bubbled into the sample via. an ultra-pure nitrogen stream from a Diazald (Aldrich Chemical Co.) generator. Sample material was dissolved in diethyl ether/methanol (5:1, v:v) after removal of methylene chloride. After methylation, the sample was concentrated and diluted with methylene chloride for GC/MS analysis.

Gas Chromatography/Mass Spectrometry

Analysis of the Methelute-generated methyl ester derivatives was performed on an LKB 2091 GC/MS system equipped with a Carbowax 20M fused silica capillary column (60 m, 0.25 mm i.d., J & W Scientific). A temperature program of 40 °C (hold for 5 min) to 215 °C at 2 °/min with a linear velocity of 29.3 cm/sec was used for these analyses. The samples were injected (splitless) at 230 °C injector temperature. Mass spectra were collected every 2 sec over a mass range of 40–478 daltons. The ion source temperature was maintained at 210 °C with ionizing voltage of 70 eV and accelerating voltage of 3500 V.

The diazomethane-generated methyl ester derivatives were analyzed on a Finnigan 4000 GC/MS equipped with a modified Carlo–Erba splitless GC injector. The mass range 50–450 daltons was scanned every second. An ionizing voltage of 70 eV was used. The chromatographic separation was achieved using a bonded fused silica capillary column (DB-5, 30 m, 0.25 mm i.d., J & W Scientific). Methane was used as the carrier gas at a linear velocity of 50 cm/sec at 200 °C. The GC was temperature programmed from 140–300 °C at 8 °C/min.

Direct Probe Mass Spectrometry

Direct probe analysis was performed on a VG 7070 E Double Focusing High Resolution Mass Spectrometer at a resolution of 10 000 (M/ΔM). A 70 eV electron beam, emission current 1.5 mamp, was used.

High Performance Liquid Chromatography of DC-Acids

Analytical HPLC separation of the DC-Acid fraction was performed on a small-bore Zorbax-C_8 column (2.1 mm i.d. × 150 mm). The sample was chromatographed using a 40 min continuous gradient from 5% acetonitrile/water (pH 3.5 with acetic acid) to 100% acetonitrile at 0.5 mL/min. The HPLC system consisted of dual Waters Model 6000 pumps, Model 660 solvent programmer, and a U6K injector. The eluents were detected with a Schoeffel SF 770 UV detector operated at 210 nm and a Waters Model 420-E fluorescence detector operated at 340 nm excitation/425 nm emission.

Preparative fractionation was conducted with the same system by reverse-phase liquid chromatography on a Lobar-A RP-8 column (E. Merck) using an

acetonitrile/water gradient at 1.5 mL/min. Fraction collections were made through four regions of solvent composition (water, 0–40% CH_3CN, 40–80% CH_3CN, 80–100% CH_3CN), and were designated LC1, LC2, LC3 and LC4 respectively. The solvent was removed by freeze drying and the fractions were reconstituted in methanol/methylene chloride. Approximately 62 mg of DC-Acid were fractionated.

Dichlorophen Analysis

Dichlorophen (2,2'-methylene-bis-4-chlorophenol, Givaudan Corp.), in the DC-Acid fraction was quantified using external standard quantitation procedures and isocratic reverse phase HPLC conditions (55% acetonitrile, 45% water with 0.1% H_3PO_4) on a Spherisorb ODS-2 (5 μm, 25 cm × 4.6 mm i.d.; Phase Separation Co.) column. The HPLC system used for these analyses was assembled from modular components consisting of an Altex Model 430 gradient system with Model 100A pumps and an LDC Model 1203 UV (254 nm) detector.

Microbial Mutagenicity Method

Subfractions of DC-Acid were assayed for mutagenicity toward *Salmonella typhimurium* tester strain TA98 according to the procedures described by Ames *et al.*[11] The samples were assayed at six doses with triplicate plates at each dose. The plates were counted electronically using an Artek Model 880 automatic colony counter. The studies reported here for TA98 included positive controls both with activation (2-anthramine) and without activation (2-nitrofluorene). Non-linear model slopes were determined using the method of Stead *et al.*[12]

RESULTS AND DISCUSSION

Routine spectroscopic studies (IR, UV, Fl) were conducted on the DC-Acid fraction. The IR spectrum, obtained from a thin film of sample, was complex, and showed intense absorbances in regions corresponding to the presence of hydroxyl and carbonyl groups. The UV spectrum was characterized by intense end absorbance, and a λ_{max} at 286 nm, an absorption pattern consistent with an "aromatic" sample. Fluorescence spectroscopy showed intense emission maxima at 530 nm (460 nm excitation) and 440 nm (340 nm excitation), values that also are consistent with an "aromatic" sample.

The aromatic nature of the sample was further confirmed by results from elemental analysis. Relative sample composition based on analysis of carbon, hydrogen, oxygen, nitrogen and sulfur was: carbon, 57.02%; hydrogen, 4.6%; oxygen, 15.51%; nitrogen, 0.67%; and sulfur, 2.12%. Although it is recognized that the presence of other elements, e.g., halogens or metals, may substantially alter the absolute contribution of each, the relative abundances can be informative. The carbon/hydrogen percentage ratio (12.3) is indicative of a sample that is predominantly aromatic in character. Such ratios are generally less than 10 for nonaromatic systems (e.g., adipic acid, 7.2; stearic acid, 6.0; linoleic acid, 6.7) and are *ca.* 10–

20 for aromatic systems (e.g., phenol, 13.0; hydroxyphenanthrene, 16.8; pyrene carboxylic acid, 19.2).

The relative amount of organic sulfur is high, although later experiments disclosed the presence of significant quantities of dimethyl sulfoxide (DMSO) in this acid fraction.

Analysis of a portion of the acid fraction using energy dispersive X-ray analysis revealed no significant quantities of any metal species.

Thermogravimetric Analysis (TGA)

A determination of general sample volatility was deemed useful for assessing the applicability of gas chromatography as a comprehensive means of analyzing this sample. A dual TGA experiment was conducted in order to determine the relative amounts of material volatilized, as a function of temperature, for an underivatized DC-Acid sample and for a methylated (diazomethane) sample. Each sample was heated in an inert gas stream from ambient to 700 °C. Weight loss curves were obtained from which a bar graph representation of the results was prepared (Figure 1).

The results indicated, as expected, a greater volatility for the derivatized sample than for the underivatized sample. This is particularly true for those components volatilizing at temperatures less than *ca.* 215 °C, where derivatization more than tripled the amount volatilized. The results indicated that a significant portion of the methylated sample should be amenable to analysis by gas chromatography. Although this conclusion is somewhat tentative without knowledge of the specific sample constituents, the fact that *ca.* 93% of the derivatized sample was volatilized at temperatures up to 320 °C indicated that GC and GC/MS would be useful analytical approaches.

Detection and Quantification of Dichlorophen

Analysis of the diazomethane-generated methyl ester derivatives of the DC-Acid fraction by EI GC/MS on a non-polar GC column led to detection of a dichlorinated compound and a bromodichlorinated compound, both present in relatively large quantities. The dichlorinated compound has been identified as Dichlorophen (2,2′-methylene-bis-4-chlorophenol, $C_{13}H_{10}O_2Cl_2$, CAS No. 97-23-4, MW 268), a commercial fungicide. The identification was based on comparison of mass spectra and GC retention of authentic Dichlorophen with values obtained from the DC-Acid sample. Both samples were derivatized and analyzed under identical conditions and both samples exhibited spectra consistent with monomethyl (*m/z* 282) and dimethyl (*m/z* 296) derivatives of Dichlorophen. The bromodichlorinated compound corresponds, by analogous fragmentation pattern and molecular weight, to a bromo-Dichlorophen isomer. Both monomethyl (*m/z* 360) and dimethyl (*m/z* 374) derivatives of this compound were observed in DC-Acid. The EI mass spectra of the dimethyl derivatives of Dichlorophen and bromo-Dichlorophen are shown in Figure 2.

Quantification of Dichlorophen showed that this compound, and additionally

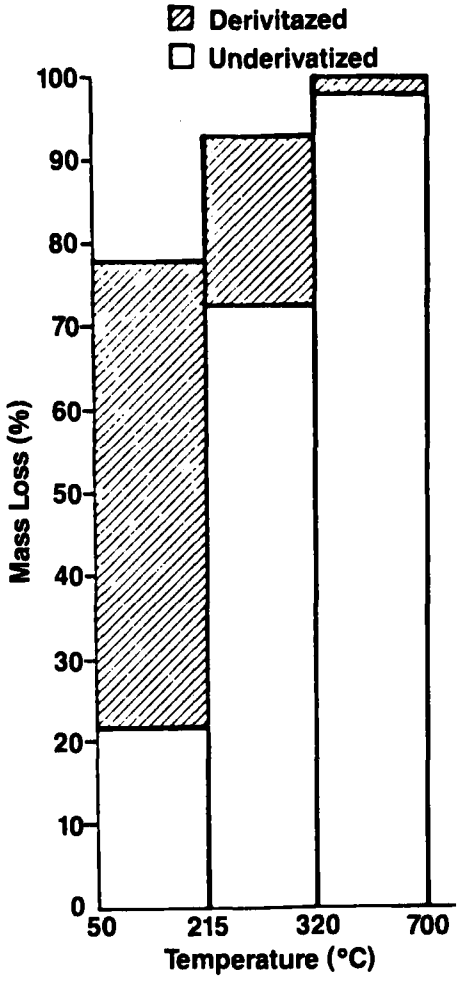

Figure 1 Thermogravimetric analysis of Washington, DC-Acids. Diazomethane was used for sample derivatization.

the bromo-Dichlorophen, accounted for 61% of the total DC-Acid fraction mass. Dichlorophen was assayed for mutagenicity toward *Salmonella typhimurium* TA98 and was found to be non-mutagenic with and without S9.

The probe high resolution mass spectra of both underivatized and diazomethane-derivatized DC-Acid fraction material supported the above identifications. The spectrum of underivatized DC-Acid showed prominent ions at *m/z* 346, 268, 233, 231, 141 and 128. The elemental composition and mass measurement accuracy of these ions are shown in Table 1. The mass spectrum of derivatized DC-Acid showed prominent ions at *m/z* 404, 374, 360 and 296, which are also recorded in the table. The elemental compositions assigned are consistent with

Table 1 Elemental composition of major ions detected by high resolution mass spectrometry

Nominal mass	*Elemental composition*	*Mass measurement accuracy (ppm)*	*Tentative structure*
Underivatized DC-Acid			
346	$C_{13}H_9O_2Cl_2Br$	1	Br-Dichlorophen
268	$C_{13}H_{10}O_2Cl_2$	6	Dichlorophen
233	$C_{13}H_{10}O_2Cl$	−11	Dichlorophen-Cl
231	$C_{13}H_8O_2Cl$	−38	Br-Dichlorophen-HClBr
141	C_7H_6OCl	75[a]	Fragment ion
128	C_6H_5OCl	5	Fragment ion
Derivatized DC-Acid			
404	$C_{16}H_{15}O_3Cl_2Br$	−38	OH-Br-Dichlorophen 3-methylated OH
374	$C_{15}H_{13}O_2Cl_2Br$	−5	Br-Dichlorophen 2-methylated OH
360	$C_{14}H_{11}O_2Cl_2Br$	−3	Br-Dichlorophen 1-methylated OH
296	$C_{15}H_{14}O_2Cl_2$	−25	Dichlorophen 2-methylated OH

[a]Centroid of mass peak not detected accurately due to other *m/z* 141 ions in sample.

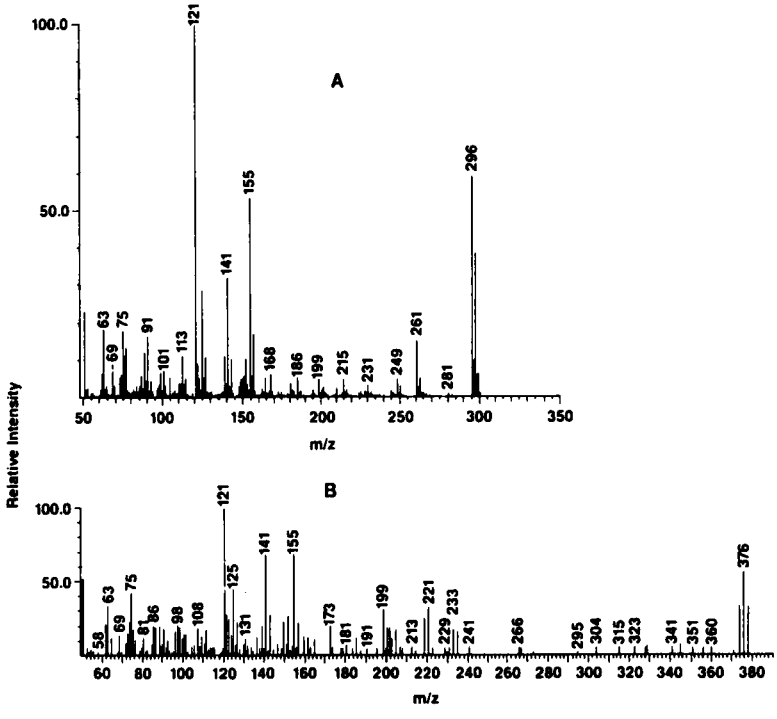

Figure 2 Mass spectrum of methylated Dichlorophen (A) and methylated bromo-Dichlorophen (B).

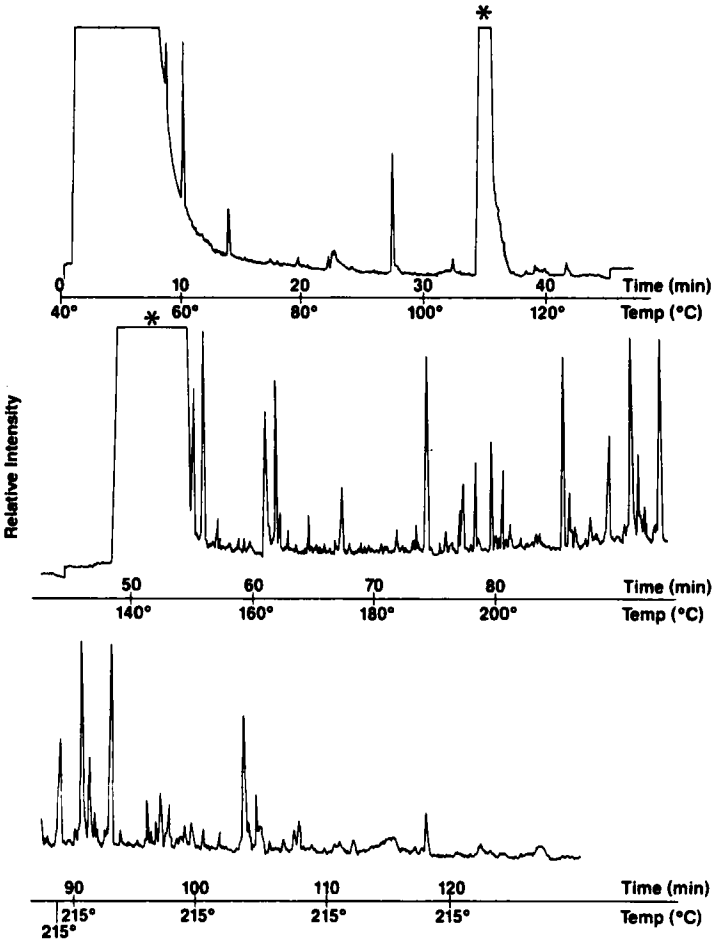

Figure 3 Total ion current profile of derivatized Washington, DC-Acids. Peaks associated with the side products of the derivatization reaction are denoted by asterisks.

structures identified as Dichlorophen, bromo-Dichlorophen, and a hydroxylated-bromo-Dichlorophen isomer.

Detection of Additional Organic Species

The total ion current chromatogram for the EI GC/MS analysis of the Methelute-generated methyl ester derivatives of the DC-Acid fraction on a polar column (Carbowax 20M) is shown in Figure 3. Tentatively identified compounds are listed in Table 2. The mass spectra of discrete peaks were manually interpreted and compared to spectra in data compendia.[13,14] In general, a minimum of five masses and intensities were matched between the unknown and the library spectrum. This level of identification does not utilize further information such as retention times since, for many components, the authentic compound was not available for

Table 2 Compounds tentatively identified (GC/MS) from methylated Washington, DC-Acid fraction

Elution time (min)	*Elution temp. (°C)*	*Compound*	*Elution time*	*Elution temp.*	*Compound*
2.5	45	Unknown	52.0	144	Methyl phenyl acetate
2.5	45	Siloxane	53.0	146	Methyl salicylate
3.5	47	Dimethyl octanoic or dimethyl nonanoic acid	53.0	146	Methyl benzyl sulfide (tent.)
3.5	47	Formaldehyde dimethyl acetal or trimethylsilanol	53.5	147	Phthalate (tent.)
4.0	48	Unknown (*m/z* 224, 193)	54.0	148	Unknown (*m/z* 141, 151, 109, 107-possibly contains S)
7.0	54	Methyl pentanoate	55.0	150	Siloxane
12.0	64	Unknown (*m/z* 59, 43)	55.0	150	Unknown (*m/z* 105, 133, 157, 164)
14.5	69	Methyl hexanoate	55.0	150	Methyl laurate
14.5	69	Methyl hepanoate	55.5	151	Unknown (*m/z* 113, 153)
15.0	70	Methyl octanoate	56.5	153	Unknown (*m/z* 106, 177, 134, 166)
19.0	78	Unknown (*m/z* 91, 61, 45, 75)	56.5	153	4-Phenyl butyric acid or methyl phenyl propionate
23.5	87	Fatty acid methyl ester	57.0	154	Unknown (*m/z* 133, 106, 164)
23.5	87	Fatty acid methyl ester	57.0	154	Methyl beta-hydroxy octanoate
24.5	89	Anisole	58.0	156	C_7H_{10} isomer (cyclo compound)
28.5	97	Unknown (*m/z* 165, 135)	59.0	158	$C_{18}H_{38}$ isomer
31.5	103	Trimethyl amine (deriv. artifact)	59.0	158	2,2,4-trimethyl penta-1,3-diol di-isobutyrate (artifact)
31.5	103	DMSO	59.5	159	Unknown (*m/z* 105, 193)
42.5	125	Dimethyl aniline (artifact)	60.5	161	2,4-dimethyl-methylbenzoate
44.0	128	1,4-Dimethoxytetramethylbenzene	60.5	161	Unknown (*m/z* 71, 171)
44.0	128	Methyl caproate	60.5	161	Silane
45.0	130	$C_{16}H_{34}$ isomer	61.0	162	2,4-*t*-butyl-6-methyl phenol
45.0	130	Hippuric acid (tent.)	61.0	162	Phthalate
45.0	130	N,N-dimethyl-4-methyl aniline (artifact)	62.0	164	Unknown (*m/z* 140)
46.0	132	Diisopropyl ether	62.0	164	Siloxane
46.0	132	Siloxane	62.5	165	$C_{10}H_{22}O_2$ (tent.)
47.5	135	Unknown (*m/z* 55, 83, 101, 177)	62.5	165	Unknown (*m/z* 103, 148, 180)
49.5	139	Methyl undecanoate	63.5	167	3,4-dimethyoxy chlorobenzene
50.0	140	N-methyl aniline (artifact)	63.5	167	Methyoxychloro compound (tent.)
50.5	141	Unknown (*m/z* 97, 57, 69, 81, 83, 179, 180)	64.5	169	$C_{11}H_8S_3$ (tent.)
50.5	141	Methyl *m*-methyl benzoate	65.0	170	4-Methoxybenzaldehyde
51.5	143	Methyl *p*-methyl benzoate	66.0	172	Methyl tetradecanoate

Table 2 (continued)

Elution time (min)	*Elution temp. (°C)*	*Compound*
66.5	173	Aldehyde (tent.)
66.5	173	Dimethyoxy methyl chlorobenzene isomer (tent.)
67.0	174	*o*-Methoxy benzoic acid methyl ester
67.0	174	*p*-Methoxy benzoic acid methyl ester
69.0	178	Siloxane
71.0	182	Methyl pentadecanoate
71.0	182	Methyl nonane-1,9-dioate or dimethyl acelate
71.5	183	Siloxane
72.0	184	$C_{17}H_{14}O_3$ isomer (tent.)
73.0	186	Methyl ester (tent.)
73.0	186	Unknown (*m/z* 98)
74.0	188	Unknown (*m/z* 153, 331, 346)
74.0	188	2-Bromo-S-methyoxy benzenesulfonyl chloride (tent.)
75.0	190	Siloxane
76.0	192	Methyl hexadecanoate
76.5	193	Dimethyl phthalate
76.5	193	3-Chloro-4'-methoxy acetophenone
76.5	193	Unknown (*m/z* 153, 123, 92)
78.0	196	Dimethyl terephthalate
78.0	196	Bromo-chloro compound (*m/z* 169, 167, 171, 200, 202)
79.0	198	Methyl ester (tent.)
80.5	201	Bromo compound (*m/z* 227, 229)
80.5	201	Unknown (*m/z* 167, 91, 137, 220)
81.0	202	Unknown (*m/z* 147, 177, 248)
81.5	203	Methyl heptadecanoate
81.5	203	Dimethyl-2-methyl terephthalate
82.0	204	Diethyl phthalate
82.0	204	Bromo-chloro compound (tent.)
83.0	206	Di-sec-butylphenyl isomer (tent.)
83.0	206	Unknown (*m/z* 155, 125, 214, 218, 127)
83.5	207	Siloxane
83.5	207	Unknown (*m/z* 182, 127, 141, 212)
84.0	208	Unknown (*m/z* 147, 119, 91, 150)
85.0	210	2,4,4,7-Tetramethyl-5,6,7,8-tetrahydro-1,4-benzopyran (tent)
85.5	211	Methyl 4-hydroxy-2-methoxy-3,5,6-trimethylbenzoate (tent).
86.0	212	Methyl 3,4-dimethoxy benzoate
87.5	215	Methyl ester (tent.)
89.0	215	Methyl stearate
90.5	215	Unknown
91.5	215	Unknown (*m/z* 264)
92.0	215	Naphthalene carboxylic acid methyl ester (tent.)
93.0	215	$C_6H_8O_4$ (tent.)
94.0	215	Unknown
96.0	215	Unknown (*m/z* 127, 128, 226)
97.0	215	Unknown (*m/z* 161, 133, 192)
97.5	215	Linoleic acid methyl ester
98.0	215	Unknown (*m/z* 179, 210, 123, 181, 212)
99.0	215	Unknown (*m/z* 136, 165)
99.5	215	Unknown (*m/z* 89, 125, 153, 212)
100.5	215	Siloxane
102.0	215	Siloxane
104.0	215	Unknown
104.5	215	4,4'-bis-(N, N-dimethyl amino phenyl) methane (possible artifact)
105.0	215	Unknown (*m/z* 191, 221, 292)
106.5	215	Unknown (*m/z* 57, 71, 175, 221)
107.5	215	Unknown (*m/z* 213, 89)
108.0	215	Unknown (*m/z* 134, 254, 118, 91, 239)
111.0	215	Unknown (*m/z* 161)
112.5	215	Unknown (*m/z* 208)
115.5	215	Phthalate
118.0	215	Unknown (*m/z* 397, 189, 149)

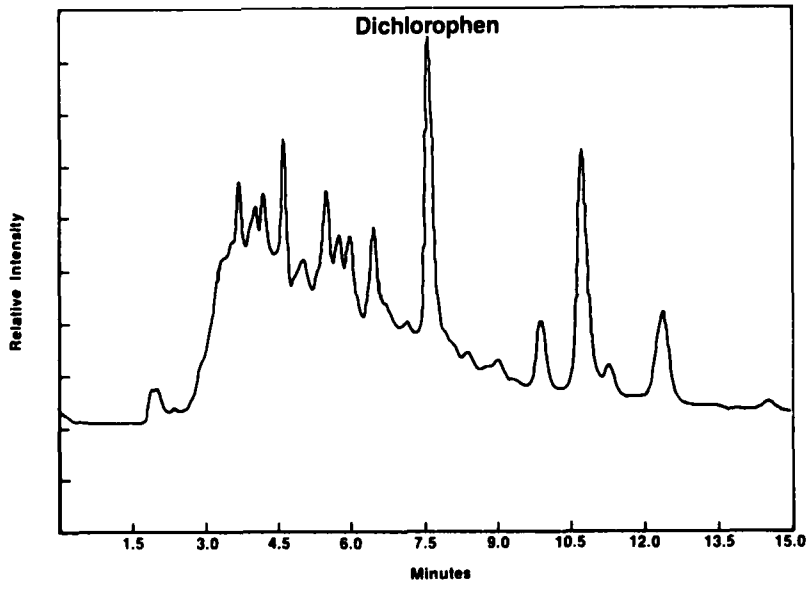

Figure 4 Gradient reverse phase HPLC of Washington, DC-acids. UV detection (210 nm).

establishing retention times. Those compounds listed in Table 2 as "(tent)" have *ca.* 3–5 masses and intensities (±50% SD) that match library spectra: interferences were the usual reason for lack of a more acceptable match.

Mutagenicity of DC-Acid Subfractions

The complex nature of the DC-Acid fraction as revealed by the reverse phase HPLC chromatogram (see Figure 4), and the presence of very high levels of Dichlorophen and related compounds, indicated that subfractionation would be necessary to detect the mutagenic species. The use of low wavelength UV detection (210 nm) represents, for this type of sample, a nearly universal detector since all sample components contain heteroatoms, and hence, possess UV end absorption. Using this chromatogram as a guide, the DC-Acid sample was fractionated as described earlier by preparative chromatography. Due to the low mass associated with fractions LC1 and LC4, only LC2 and LC3 were assayed for mutagenicity. Bioassay test results are shown in Table 3.

The two HPLC fractions assayed were mutagenic, with LC3, the less polar fraction, being more mutagenic both with and without S9 (6.5 and 8.4 rev/μg). LC2 was twice as mutagenic in the absence of S9 (1.8 rev/μg) as in the presence of S9 (0.9 rev/μg). Most of the mutagenicity (82–88%) and mass (68.6%) was in fraction LC3.

Separation of the DC-Acid fraction into weak and strong acid fractions using a solvent partitioning procedure validated for separation of saturated and unsaturated aliphatic carboxylic acids from phenols,[15] provides contradictory evidence

Table 3 Mutagenicity of DC-acid subfractions

Fraction	*Mass (mg)*	*Percent mass*	*Model slopes (rev/μg)*		*Weighted slopes (rev/μg)*		*Percent mutagenicity*	
			−S9	*+S9*	*−S9*	*+S9*	*−S9*	*+S9*
HPLC fractionation								
LC1	—	—	NT	NT	—	—	—	—
LC2	22.0	28.5	6.4	3.2	1.8	0.9	17.8	12.3
LC3	53.0	68.6	12.2	9.5	8.4	6.5	82.2	87.7
LC4	—	—	NT	NT	—	—	—	—
				Sum	10.2	7.4		
Liquid/liquid partitioning								
Weak acids	8.7	17.5	4.0	0.8	0.7	0.1	5.6	1.3
Strong acids	18.5	82.5	14.3	9.2	11.8	7.6	94.4	98.7
				Sum	12.5	7.7		

regarding the nature of the most mutagenic material. As shown in Table 3, strong acids were substantially more mutagenic (9.2 rev/μg + S9; 14.3 rev/μg − S9) than the weak acids (0.8 rev/μg + S9; 4.0 rev/μg − S9). The strong acids represented 82.5% of the recovered mass and contained 78–92% of the mutagenic activity. The weak acids contained 17.5% of the recovered mass and 8–22% of the mutagenic activity.

The mutagenicity of underivatized and diazomethane-derivatized DC-Acid samples were very similar. The methylated material produced TA98 mutagenic potency of 11.3 (+S9) and 15.5 (−S9) rev/μg. These results argue against the direct involvement of the carboxyl and, probably, the aromatic hydroxyl moieties in the cellular binding process. Both of these functional groups are methylated with diazomethane. It seems probable that a molecule with mixed functionality (e.g. nitro-hydroxyl aromatic) is responsible for at least some significant portion of the total observed activity.

CONCLUSIONS

As shown by the results of these studies, the comprehensive characterization of urban aerosol organic acids is a formidable challenge, and is best met by a combination of techniques. The following conclusions can be drawn from the work presented here:

1) The acids represent a significant portion (15%) of the total aerosol organics.
2) The acids are responsible for a significant proportion (*ca.* 50%) of the total mutagenic activity.
3) The fraction is predominantly aromatic in nature.

4) Most of the fraction components display, after derivatization, volatilities within a range that is compatible with GC analysis.
5) The major compounds identified by EI GC/MS include fatty acids, aromatic acids, and a fungicidal agent (Dichlorophen).
6) The compound(s) responsible for the observed biological activity are not discernible based on the approaches used here.

Although the derivatized fraction was, from the TGA results, amenable to GC analysis, the results from the GC/MS studies were somewhat disappointing. From Table 2, it can be seen that some 35–40 compounds were not identified, even though they produced clean, full scan mass spectra with well-defined fragmentation patterns. These compounds represent materials for which library spectra are not recorded in the two *ca.* 35000 compound compendia used[13,14] for spectral matching. This is a major limitation to the use of such approaches and emphasizes the difficulty in obtaining comprehensive qualitative information for this type of sample.

A significant and unexpected result from the GC/MS studies was the identification of Dichlorophen in the sample, and its presence in such large amounts. The fact that the compound comprised over half of the mass of this organic acid fraction, and that it had not been previously reported as a component of ambient air aerosol, led to some suspicion as to its source. Further information was obtained upon examination of aerosol collected in St. Louis, MO (NIST SRM 1648), using the same bag houses used for collection of SRM 1649. The acid fraction was isolated and analyzed by GC/MS and HPLC. Dichlorophen was found and quantitated at levels corresponding to *ca.* 23% of the acid fraction of this urban particulate extract. To check the possibility that bag material may have been treated with the fungicide, a sample of the material used by NBS for bag house filters was obtained and analyzed. The material analyzed was not obtained from the bags actually used for sampling. The acid fraction of the bag extract was derivatized (diazomethane) and analyzed by GC/MS. Dichlorophen was not detected. Although the compound's existence in aerosol from two geographically disparate sites suggests an artificial origin, the source remains unknown. Other investigators and users of the Washington, DC dust and St. Louis particulate as obtained from NIST (SRM 1649 and 1648, respectively) should be aware of the fungicide's presence.

Neither the fractionation of the acids into weak and strong acids or on the basis of polarity by HPLC was very successful in concentrating most of the mutagenic activity into a smaller mass to simplify identification of the mutagens. It is possible that the acid fraction contains a wide spectrum of acidic mutagens rather than one narrow class.

The most promising area of activity for future work would appear to involve further fractionation of the acid fraction using liquid chromatographic procedures. The collection of a relative large number of fractions (10–20), each of which could be tested for mutagenicity, would identify those fractions deemed important for further study. Each fraction should be examined by high resolution MS techniques to provide compositional information on individual compounds. The use of LC/

MS might provide data on compounds that do not chromatograph under conventional GC/MS conditions.

The ultimate goal of correlation of mutagenicity with specific aerosol organic component(s) depends not only on identification and quantitation of such components, but also on their availability in sizeable amounts for confirmation and further mutagenicity testing.

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Disclaimer

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