Tobacco Smoke Chemistry 3. Aromatic Acids of Cigarette Smoke Condensate

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A fraction containing mainly aromatic acids has been isolated from cigarette smoke condensate. Gas chromatographic and mass spectral analysis of the corresponding methyl esters and comparison with reference compounds, many of which were synthesized for this purpose, made possible the identification of 27 constituents (Table 1). Eighteen of these have not been detected in tobacco smoke condensate before.

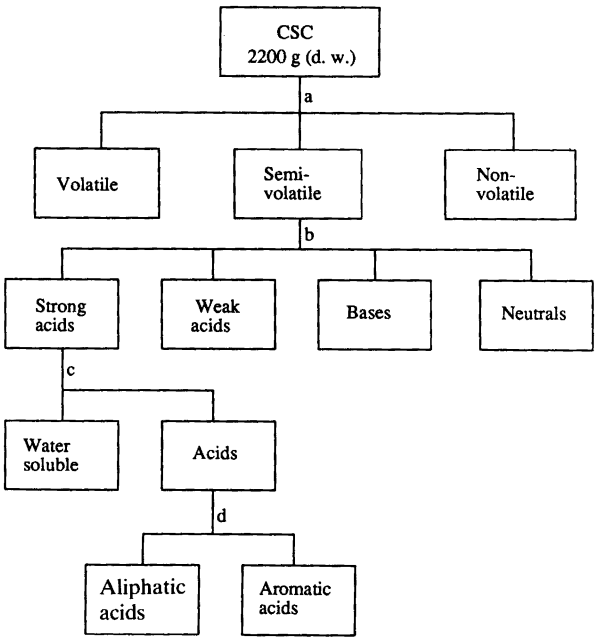
On-going cyto- and geno-toxic studies of cigarette smoke condensate, CSC, in our laboratory have revealed that most of the biological activity of the strong acids present in the semivolatile material resides in a fraction containing mainly aromatic acids. It was therefore of considerable interest to try to identify the major constituents of this fraction.

Numerous publications dealing with the chemical composition of tobacco smoke condensate have appeared, but the information on the aromatic acids present is quite limited. Johnstone and Plimmer[1] have, in a review 1959, reported benzoic and furoic acids as constituents of both tobacco and tobacco smoke, and Osman and Barson[2] have, somewhat later, disclosed the presence of phenylacetic, 2-phenylpropanoic, 3-methylbenzoic and 4-methylbenzoic acids in cigar smoke condensate. The list of aromatic acids in tobacco smoke has since been expanded to include 5-methyl-2-furoic, 3-hydroxybenzoic, 4-hydroxybenzoic and 2-methylbenzoic acids by Ishiguro *et al.*,[3,4] and 3-furoic and 3-phenylpropanoic acids by Schumacher *et al.*[5] It is also evident from the literature that the origin of the aromatic acids in smoke is unclear. Ishiguro *et al.*[3] have shown that the amount of acids in the smoke exceeds that in the leaves from which the smoke is derived, so transfer from the tobacco during smoking does obviously not constitute a major pathway.

The minute quantity and complexity of the material required the use of GC and GC–MS techniques and access to adequate reference compounds. A detailed account of the outcome of this approach, which involved conversion into methyl esters and synthesis of the reference compounds, is given below. The acids identified are summarized in Table 1.

Procedure and discussion

The CSC was obtained from the smoking of 100 000 commercial plain cigarettes of American blend type (23 mg tar, 11 mg CO, 1.8 mg nicotine per cigarette) and the condensate was collected using procedures described previously.[6,7] The CSC was distilled *in vacuo* using carbon dioxide as the carrier gas[8] to give a volatile, a semivolatile and a non-



Scheme 1. (a) Distillation; (b) Liquid–liquid extraction; (c) Ion-exchange chromatography, Amberlite IR-45; (d) Chromatography, LH 20.

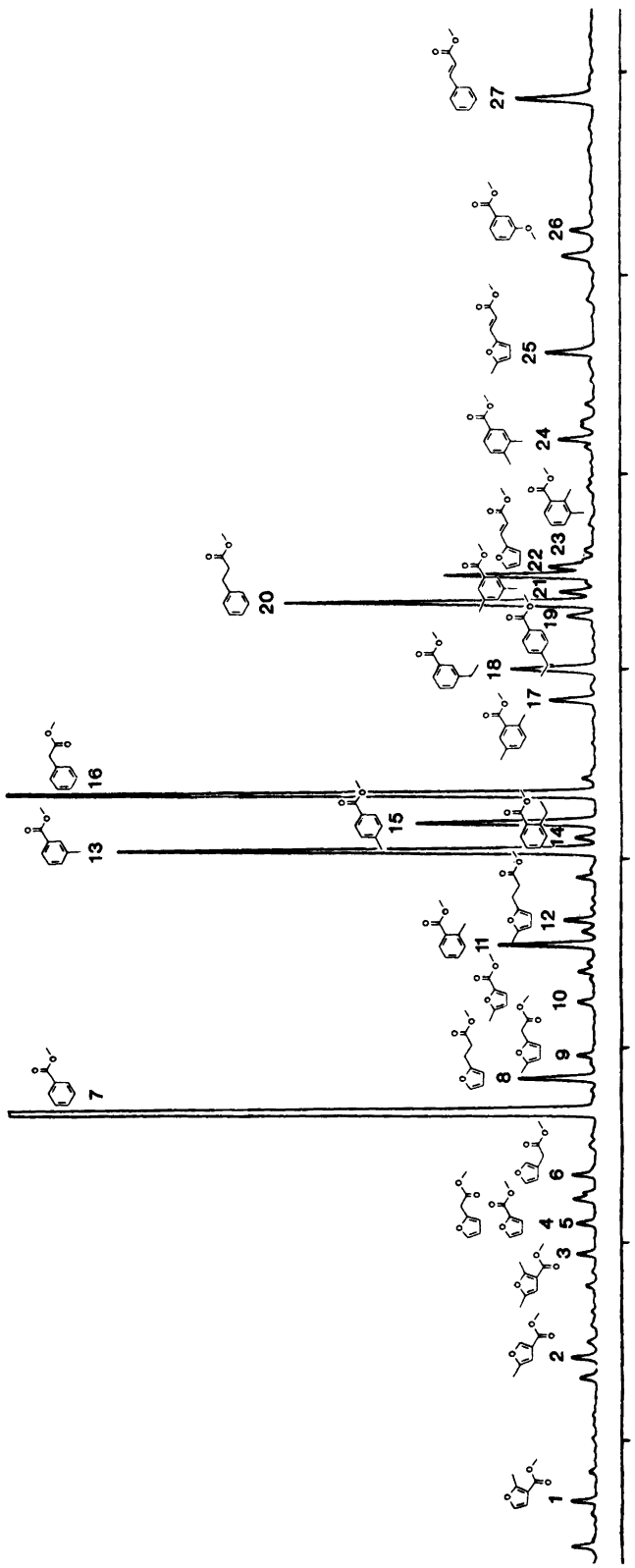


Fig. 1. GC trace of the methyl esters derived from the aromatic acid fraction, see Scheme 1.

volatile fraction. Subsequent liquid–liquid extraction of the semivolatiles[9] using, consecutively, 1 M aqueous solutions of sodium hydrogen carbonate, sodium hydroxide and hydrochloric acid, furnished four dichloromethane-soluble fractions, see Scheme 1. As a result of the extraction procedure, the sodium hydrogen carbonate extractable fraction contained both strong acids and other partly water-soluble substances. To isolate the strong acids, this fraction was subjected to ion-exchange chromatography. The acidic fraction obtained in this way was chromatographed on a Sephadex LH 20 column to give two fractions, one of which contained mainly aliphatic acids and the other mainly aromatic acids.

In order to simplify the GC–MS analysis of the aromatic acids, they were converted into the corresponding methyl esters. A GC-trace of the esterified fraction is depicted in Fig. 1. In addition to the easily recognizable methyl esters of benzoic, methylbenzoic and furoic acids, the GC–MS study furnished a number of spectra which suggested the presence of more heavily substituted benzoic or furoic acids. There were also spectra which we tentatively assigned to furyl- and phenyl-substituted acetic, propanoic and propenoic acids. Appropriate reference compounds were therefore needed for comparison of GC retention times and mass spectra – those not available by other means were synthesized as discussed below.

Preparation of reference compounds. Most of the alkyl substituted benzoic acids needed are commercially available as the free acids and were easily converted into the required methyl esters. There are two exceptions, however, 2-ethyl- and 3-ethyl-benzoic acid. The former was conveniently prepared from 2-acetylbenzoic acid by catalytic hydrogenation, and the latter was obtained from 3-bromoacetophenone by a Clemmensen reduction followed by conversion of the resulting 3-bromoethylbenzene into the corresponding Grignard compound and treatment of this with solid carbon dioxide to introduce the carboxy group.

Four of the desired acids containing a furyl group were purchased either as methyl esters or as the free acids (peaks **1**, **3**, **4** and **22** in Fig. 1). Of the remaining eight compounds of this type, six were synthesized. Since the major purpose was to obtain reference substances, conventional synthetic steps were used throughout although the yields in some cases were low.

5-Methyl-2-furoic acid (peak **10** in Fig. 1) was prepared by oxidation of 5-methylfurfural. The acetic acid derivatives **5** and **6**, where obtained from 2- and 3-furfuryl alcohol by conversion into the chlorides and subsequent treatment with potassium cyanide to give the corresponding cyano derivatives which on being hydrolysed gave the desired acids. Attempts to synthesize 5-methyl-2-furylacetic acid (**5**) in the same way failed because the required chloride could not be obtained. The propanoic acid derivatives **8** and **12** were produced by reduction of the corresponding propenoic acids.

Table 1. Methyl esters of aromatic acids found in cigarette smoke condensate.

No.[a]	Methyl ester of	Rel. R_T[b]	M_r	Mass spectrum *m/z* (%)[c]
1	2-Methyl-3-furoic acid[d,e]	0.782	140	140 (51), 125 (22), 110 (6), 109 (100), 108 (18), 80 (9), 53 (8), 52 (6), 51 (8), 43 (18)
2	5-Methyl-3-furoic acid[e,f]	0.851	140	140 (48), 110 (7), 109 (100), 81 (15), 53 (30), 52 (9), 51 (12), 50 (10), 44 (5), 43 (14)
3	2,5-Dimethyl-3-furoic acid[d,e]	0.919	154	154 (65), 139 (62), 123 (76), 122 (12), 95 (10), 94 (17), 81 (13), 53 (14), 51 (11), 43 (100)
4	2-Furoic acid[d]	0.936	126	127 (2), 126 (36), 96 (7), 95 (100), 81 (1), 68 (4), 67 (3), 66 (1), 53 (1), 44 (2)
5	2-Furylacetic acid[e,g]	0.936	140	141 (2), 140 (24), 98 (2), 82 (6), 81 (100), 59 (6), 53 (26), 52 (5), 51 (6), 50 (3)
6	3-Furylacetic acid[e,g]	0.964	140	141 (2), 140 (28), 112 (19), 82 (7), 81 (100), 59 (8), 53 (27), 52 (5), 51 (8), 50 (5)
7	Benzoic acid[d]	1.000	136	137 (3), 136 (35), 106 (8), 105 (100), 78 (6), 77 (55), 76 (4), 74 (3), 51 (22), 50 (10)
8	3-(2-Furyl)propanoic acid[e,g]	1.018	154	154 (29), 123 (9), 97 (7), 95 (14), 94 (84), 81 (100), 67 (7), 65 (7), 53 (14), 41 (8)
9	5-Methyl-2-furylacetic acid[e,f]	1.031	154	154 (17), 96 (6), 95 (100), 94 (5), 67 (4), 52 (5), 51 (8), 44 (4), 43 (20), 41 (8)
10	5-Methyl-2-furoic acid[g]	1.061	140	140 (45), 110 (7), 109 (100), 82 (5), 81 (4), 53 (20), 52 (6), 51 (6), 50 (4), 43 (6)
11	2-Methylbenzoic acid[d]	1.095	150	150 (47), 120 (9), 119 (100), 118 (59), 91 (80), 90 (24), 89 (14), 65 (31), 63 (15), 51 (9)
12	5-Methyl-3-(2-furyl)propanoic acid[e,g]	1.109	168	168 (19), 109 (5), 108 (27), 107 (5), 96 (6), 95 (100), 55 (3), 53 (3), 43 (14), 41 (3)
13	3-Methylbenzoic acid[d]	1.150	150	150 (36), 120 (9), 119 (100), 92 (5), 91 (60), 90 (5), 89 (8), 65 (21), 63 (9), 51 (5)
14	2-Ethylbenzoic acid[e,g]	1.158	164	164 (55), 133 (69), 132 (100), 131 (33), 105 (31), 104 (32), 103 (26), 91 (15), 77 (30), 51 (14)
15	4-Methylbenzoic acid[d]	1.166	150	150 (33), 120 (8), 119 (100), 92 (4), 91 (45), 90 (4), 89 (7), 65 (16), 63 (7), 51 (4)
16	Phenylacetic acid[d]	1.183	150	151 (2), 150 (26), 92 (8), 91 (100), 90 (3), 89 (5), 65 (15), 63 (6), 59 (7), 51 (4)
17	2,5-Dimethylbenzoic acid[d,e]	1.238	164	164 (73), 149 (14), 134 (9), 133 (100), 132 (93), 105 (58), 104 (35), 103 (14), 79 (12), 77 (23)
18	3-Ethylbenzoic acid[e,g]	1.255	164	164 (51), 149 (24), 134 (9), 133 (100), 119 (6), 105 (51), 103 (10), 79 (12), 77 (16), 51 (6)
19	4-Ethylbenzoic acid[d,e]	1.285	164	165 (4), 164 (39), 149 (11), 134 (9), 133 (100), 105 (29), 103 (6), 79 (7), 77 (10), 51 (4)
20	3-Phenylpropanoic acid[d]	1.293	164	164 (32), 133 (11), 105 (35), 104 (100), 103 (12), 91 (63), 79 (8), 78 (12), 77 (15), 51 (11)
21	3,5-Dimethylbenzoic acid[d,e]	1.301	164	165 (5), 164 (45), 134 (10), 133 (100), 105 (33), 103 (6), 79 (6), 78 (3), 77 (9), 51 (4)
22	3-(2-Furyl)propenoic acid[d,e]	1.314	152	152 (57), 122 (8), 121 (100), 109 (8), 93 (9), 92 (7), 65 (49), 64 (6), 63 (14), 59 (8)
23	2,3-Dimethylbenzoic acid[d,e]	1.317	164	164 (63), 133 (100), 132 (87), 105 (56), 104 (27), 103 (15), 79 (12), 78 (10), 77 (23), 51 (10)
24	3,4-Dimethylbenzoic acid[d,e]	1.386	164	165 (4), 164 (38), 134 (10), 133 (100), 105 (26), 103 (6), 79 (7), 78 (3), 77 (10), 51 (4)
25	5-Methyl-3-(2-furyl)propenoic acid[e,g]	1.433	166	166 (77), 151 (9), 136 (9), 135 (100), 108 (8), 107 (13), 106 (8), 77 (15), 53 (8), 43 (16)
26	3-Methoxybenzoic acid[d]	1.499	166	166 (61), 136 (9), 135 (100), 107 (37), 92 (23), 77 (31), 76 (6), 64 (18), 63 (16), 50 (7)
27	3-Phenylpropenoic acid[d,e]	1.571	162	162 (50), 161 (17), 132 (9), 131 (100), 104 (8), 103 (61), 102 (17), 77 (38), 51 (27), 50 (9)

[a]Refers to Fig. 1. [b]GC retention time relative to methylbenzoate on Supelcowax 10 fused silica column. [c]Mass spectra (10 strongest peaks) from reference substances except Nos. 2 and 9. [d]Identified by comparison with a commercially available reference. [e]Presence in CSC not previously demonstrated. [f]Tentatively identified. [g]Identified by comparison with a synthesized reference.

Aromatic acids found in CSC. Comparison of the GC retention times and mass spectra of the reference compounds with the corresponding data obtained from the CSC fraction allowed the identification of 25 constituents. Another two constituents were tentatively identified from their mass spectra, namely, methyl 5-methyl-3-furanoate (**2**) and methyl 5-methyl-2-furylacetate (**9**). These 27 compounds are detailed in Table 1 along with their relative GC retention times and mass spectral data. Except for constituents **2** and **9**, the MS data quoted is derived from the reference substances.

Since none of these esters could be detected in the CSC fraction before methylation we conclude that the corresponding acids are present in CSC. It should be noted, however, that methyl 3-methoxybenzoate probably originates from 3-hydroxybenzoic acid. To our knowledge, 18 of these acids have not previously been identified in tobacco smoke condensate.

In addition to the reference substances mentioned above, we had access to and recorded retention data and mass spectra of the methyl esters of 2,4-dimethyl- and 2,6-dimethyl-benzoic acid, 4-methoxybenzoic acid, 2-phenylpropanoic, 3- furoic and 3-methyl-2-furoic acid. However, none of these could be detected in the fraction investigated.

Experimental

General methods. ^{1}H NMR spectra were recorded in $CDCl_3$ at 300 MHz on a Varian XL-300 instrument using $SiMe_4$ as an internal standard. Mass spectra (EI, 70 eV) were obtained on a Kratos MS 50 mass spectrometer, the intensities of peaks (*m/z* 40 to *M*+1) are given as a percentage of the base peak.

Chromatography. Gas chromatography was performed on a Hewlett-Packard model 5880 A instrument, equipped with a flame-ionisation detector. The relative retention times were measured on a Supelcowax 10 fused silica column (0.32 mm i.d., 60 m) programmed from 60–250 °C at 2 K min^{-1} using helium as the carrier gas. Merck Kieselgel 60 (230–400 mesh) and Sephadex LH-20 (30–100 μm) were used for column chromatography. Amberlite resin IR-45 (OAc) was used for the ion-exchange chromatography.

Preparation of cigarette smoke condensate fractions. The CSC was prepared from 100000 plain cigarettes of American blend type (23 mg tar, 11 mg CO and 1.8 mg nicotine per cigarette). The cigarettes were smoked according to a standard procedure[6] using a Borgwaldt smoking machine (Type R 09.012, Heinr. Borgwaldt, Hamburg, F.R.G.). The CSC was condensed in an Elmenhorst trap cooled in a solid carbon dioxide–ethanol mixture.[7] The CSC was distilled *in vacuo* with carbon dioxide as the carrier gas[8] giving three fractions – volatiles, semivolatiles and non-volatiles. The semivolatiles were divided into four dichloromethane soluble fractions – acids, weak acids, bases and neutrals – utilising an extraction procedure described earlier.[9]

From a part of the first fraction (8.0 g), the acids were separated from other, partly water-soluble components by ion-exchange chromatography (Amberlite IR-45, OAc, 6×15 cm, methanol 500 ml, 0–0.5 M acetic acid in methanol as a gradient 800 ml, 0.5 M acetic acid in methanol 500 ml). The eluate was collected in 25 ml tubes and after GC evalutation (OV 351) combined into 2 fractions, i.e. a non-acidic fraction (including phenolic compounds, about 5 g) and one comprising acidic compounds (about 3 g). A portion of the acidic part (1.8 g) was further separated into aliphatic and aromatic acids by column chromatography (Sephadex LH 20, 4×35 cm, methanol). The eluate was collected in 25 ml tubes and combined after GC evaluation (OV 351), to give 2 main fractions (1.0 and 0.5 g, respectively).

Methylation of the acids. 200 mg of the fraction containing the aromatic acids was stirred with aqueous 0.5 M tetrabutylammonium hydroxide solution for 30 min, extracted with dichloromethane and dried (sodium sulfate). Methyl iodide (1.5 ml) was added and the mixture was refluxed for 30 min. The solvent was evaporated, the solid residue was triturated with boiling diethyl ether and the insoluble tetrabutylammonium iodide was filtered off. After being concentrated, the sample was subjected to GC–MS analysis.

Methyl esters of the acids, whether available commercially or by synthesis, were prepared either by the above method or by treatment with thionyl chloride in dichloromethane followed by dissolution of the resulting acid chloride in methanol.

Synthesis of reference compounds. Conventional synthetic steps were used throughout and no attempts were made to optimize the yields. We therefore give only a general description of the steps involved and include spectroscopic data for the acids. ^{2}H NMR and mass spectra of isolated intermediates served to confirm the structures proposed.

5-Methyl-2-furoic acid.[10] Sodium hydroxide (200 ml, 1 M) was added to a solution of potassium permanganate (0.5 g) in water (60 ml) and the resulting mixture was poured into a stirred dispersion of 5-methylfurfural (0.5 g) in water (400 ml). Manganese dioxide was filtered off after 1 h and the remaining aldehyde was extracted with dichloromethane. The aqueous phase was acidified, saturated with sodium chloride and 5-methylfuroic acid was extracted with diethyl ether. ^{2}H NMR: δ 7.25 (d, 1 H), 6.18 (d, 1 H), 2.42 (s, 3 H).

3-Furylacetic acid.[11] 3-Furoic acid was reduced with lithium aluminium hydride and the resulting alcohol was treated with thionyl chloride in diethyl ether. The solvent was evaporated and the crude chloride dissolved in *N,N*-dimethylformamide. A large excess of potassium cyanide was added and the mixture was stirred at 90 °C for 4 h, diluted with water and extracted with dichloromethane. The crude 3-furylacetonitrile was purified by column chromatography

(silica gel, cyclohexane–ethyl acetate 3:1). The purified compound was hydrolysed with aqueous potassium hydroxide (20 %) to give 3-furylacetic acid. ^{1}H NMR: δ 7.41 (d, 2 H), 6.41 (dd, 1 H), 3.54 (s, 2 H).

2-Furylacetic acid. Furfuryl alcohol was treated with thionyl chloride in diethyl ether, the solvent was evaporated, and the residue was dissolved in dichloromethane. To convert the chloride obtained into 2-furylacetonitrile, a large excess of tetrabutylammonium cyanide was added and the mixture was boiled under reflux for 20 h, diluted with water and extracted with dichloromethane. The nitrile, purified by column chromatography (silica gel, cyclohexane–ethyl acetate 19:1), was hydrolysed with aqueous sodium hydroxide (20 %) at 80 °C (until the evolution of ammonia ceased) to give 2-furylacetic acid in very poor yield. ^{1}H NMR: δ 7.39 (d, 1 H), 6.36 (dd, 1 H), 6.27 (d, 1 H), 3.76 (s, 2 H).

3-(2-Furyl)propanoic acid was obtained by reduction of commercially available 3-(2-furyl)propenoic acid with sodium amalgam in water–ethanol 1:1 (16 h, 20 °C).[12] ^{1}H NMR: δ 7.32 (d, 1 H), 6.29 (dd, 1 H), 6.05 (d, 1 H), 2.98 (t, 2 H), 2.72 (t, 2 H).

3-(5-Methyl-2-furyl)propenoic acid was obtained by condensation of 5-methylfurfural with malonic acid in pyridine (2 h, 100 °C).[13] ^{1}H NMR: δ 7.44 (d, 1 H), 6.56 (d, 1 H), 6.23 (d, 1 H), 6.11 (d, 1 H), 2.36 (s, 3 H).

3-(5-Methyl-2-furyl)propanoic acid was prepared from 3-(5-methyl-2-furyl)propenoic acid as described for 3-(2-furyl)propanoic acid above. ^{1}H NMR: δ 5.91 (d, 1 H), 5.85 (dd, 1 H), 2.93 (t, 2 H), 2.70 (dt, 2 H), 2.25 (s, 3 H).

2-Ethylbenzoic acid was prepared from 2-acetylbenzoic acid by catalytic reduction (Pd–C, ethanol) and showed ^{1}H NMR: δ 8.05 (dd, 1 H), 7.50 (dt, 1 H), 7.32 (dd, 1 H), 7.29 (dt, 1 H), 3.08 (q, 2 H), 1.28 (t, 3 H).

3-Ethylbenzoic acid. 3-Bromoacetophenone was reduced by a Clemmensen reaction. The resulting 3-ethylbromobenzene was treated with magnesium and the Grignard reagent thus formed, was treated with solid carbon dioxide to give the desired acid. ^{1}H NMR: δ 7.97 (t, 1 H), 7.96 (dt, 1 H), 7.46 (dt, 1 H), 7.40 (dd, 1 H), 2.73 (q, 2 H), 1.28 (t, 3 H).

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