

Tobacco Smoke Chemistry. 7. Alkyl- and Alkenyl-Substituted Phenols Found in Cigarette Smoke Condensate

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Phenol and many of its alkyl and alkenyl derivatives are important constituents of cigarette smoke condensate (CSC). Exceeded quantitatively only by the dihydroxybenzenes,¹ they constitute the second largest group of compounds in the semivolatile weak acid portion of the CSC, cf. Fig. 1. These phenolic compounds can derive from lignin, polyphenols and, to some extent, from cellulose and sugars.² Although not proven, it seems likely that virtually every isomer of these low molecular weight phenols is present in CSC.

Our recent studies of CSC, have been focused on certain groups of aroma constituents in the weak acid fraction such as 2-hydroxy-2-cyclopentenones,³ 3-hydroxy-4-pyrones,⁴ 2-hydroxy-2-cyclohexenones⁵ and guaiacols,⁶ while earlier work has concerned the biological effects of the weak acids from CSC and subfractions thereof,^{7a,b} as well as of certain single constituents,^{8a-g} e.g. some of the alkyl- and alkenyl-substituted phenols.

Largely as a result of the biological activity encountered, we have now undertaken a more detailed chemical study of the CSC alkyl- and alkenyl-substituted phenols. The outcome of this work is summarized below.

Material and methods

The collection of the smoke condensate from 100 000 plain cigarettes of American blend type, distillation of this to obtain the semivolatile portion and subsequent fractionation into subfractions, as shown in Fig. 1, has been described in earlier reports.^{3,6}

The reference compounds needed, which were not commercially available, were prepared by conventional synthetic steps and their structures confirmed by ¹H NMR and mass spectra. Thus, application of the Fries rearrangement to *O*-acetylated methylphenols,⁹ furnished, after Clemmensen reduction¹⁰ of the intermediate acetylmethylphenols, compounds **2**, **16** and **20** (Table 1). Phenols **14**, **19** and **37** were prepared by Clemmensen reduction of the corresponding commercially available acetylmethylphenols.

3-Ethylphenol, when subjected to a Reimer–Tiemann reaction,¹¹ gave all three of the expected ethylhydroxybenzaldehydes, which after separation, purification and Clemmensen reduction furnished compounds **21**, **27** and **38**. In the same way **35** was produced from 2,3-dimethylphenol. Compound **34** was prepared from 3-hydroxybenzaldehyde and ethylmagnesium bromide followed by hydrogenolysis of the resulting benzylic alcohol. The vinyl substituted phenols **7**, **25** and **40** were produced from the corresponding hydroxybenzaldehydes by converting the carbonyl function

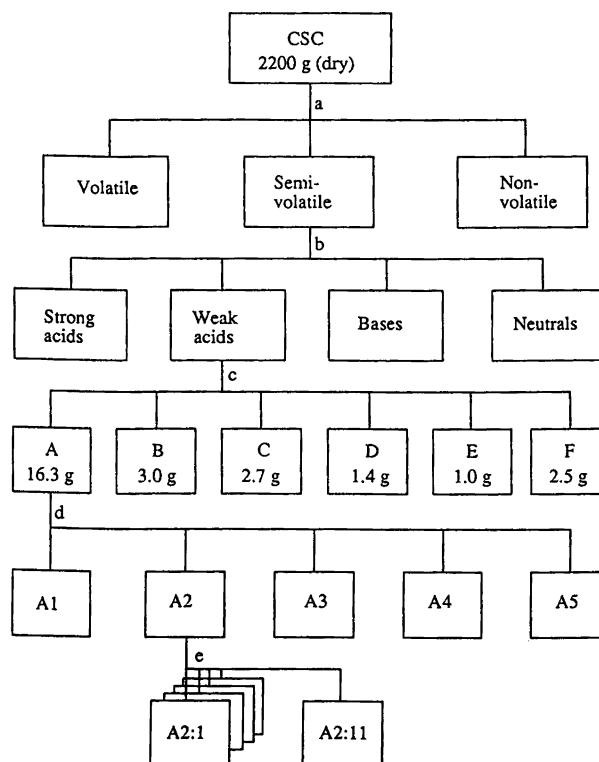


Fig. 1. a, Distillation; b, liquid–liquid extraction; c, silica gel chromatography; d, silica gel chromatography; e, HPLC, CN column.

Table 1. Alkyl- and alkenyl-substituted phenols detected in cigarette smoke condensate.

No.	Compound	M_r	Rel. t_R^a	Fraction ^b	Rel. am. ^c	Mass spectrum m/z (%) ^d
1	2,6-Dimethylphenol	122	1.000	A2	1	123 (9), 122 (100), 121 (37), 107 (71), 104 (9), 103 (10), 91 (14), 79 (12), 78 (9), 77 (18)
2	2-Ethyl-6-methylphenol	136	1.040	A1	–	137 (3), 136 (36), 122 (8), 121 (100), 117 (3), 115 (3), 107 (3), 91 (14), 77 (11), 65 (3)
3	2,4,6-Trimethylphenol	136	1.078	A2	<0.2	137 (10), 136 (100), 135 (36), 122 (8), 121 (98), 117 (6), 93 (6), 91 (18), 77 (9), 65 (5)
4	2-Methylphenol	108	1.082	A3	28	109 (7), 108 (100), 107 (81), 90 (20), 89 (9), 80 (7), 79 (21), 78 (6), 77 (19), 51 (6)
5	Phenol	94	1.082	A3		95 (6), 94 (100), 66 (15), 65 (13), 63 (3), 55 (4), 51 (2), 47 (2), 40 (3), 39 (6)
6	2,3,6-Trimethylphenol	136	1.114	A1	0.2	137 (8), 136 (91), 135 (24), 122 (9), 121 (100), 117 (9), 103 (6), 93 (8), 91 (20), 77 (11)
7	2-Methyl-6-vinylphenol ^{e,f}	134	1.126	A1	0.02	135 (9), 134 (100), 133 (29), 115 (11), 105 (20), 91 (50), 78 (10), 77 (15), 51 (12), 39 (11)
8	2-Ethylphenol	122	1.138	A3	1.5	123 (4), 122 (40), 121 (3), 108 (8), 107 (100), 103 (4), 91 (4), 79 (8), 78 (4), 77 (16)
9	2,5-Dimethylphenol	122	1.143	A3	1.4	123 (7), 122 (100), 121 (50), 108 (12), 107 (91), 91 (14), 79 (11), 77 (23), 45 (9), 44 (9)
10	2,4-Dimethylphenol	122	1.146	A3	15	123 (8), 122 (100), 121 (55), 108 (6), 107 (80), 91 (12), 79 (9), 78 (6), 77 (16), 51 (5)
11	4-Methylphenol	108	1.146	A3		109 (6), 108 (87), 107 (100), 90 (7), 79 (15), 78 (6), 77 (19), 53 (8), 51 (8), 39 (6)
12	3-Methylphenol	108	1.152	A3	4.6	109 (7), 108 (100), 107 (87), 91 (5), 90 (9), 80 (5), 79 (19), 78 (5), 77 (16), 53 (5)
13	2-Isopropylphenol	136	1.171	A3	<0.05	137 (3), 136 (33), 122 (9), 121 (100), 107 (3), 103 (21), 93 (4), 91 (11), 77 (13), 65 (3)
14	2-Ethyl-4-methylphenol	136	1.193	A2	0.9	137 (4), 136 (43), 135 (5), 122 (9), 121 (100), 115 (3), 107 (7), 93 (4), 91 (14), 77 (11)
15	2-Propylphenol	136	1.193	A2		137 (2), 136 (26), 108 (8), 107 (100), 91 (3), 79 (6), 78 (3), 77 (11), 51 (2), 39 (2)
16	2-Ethyl-5-methylphenol ^f	136	1.196	A2	0.4	137 (3), 136 (32), 135 (2), 122 (8), 121 (100), 115 (3), 93 (3), 91 (11), 77 (9), 65 (3)
17	2,3-Dimethylphenol	122	1.199	A3	1.0	122 (94), 121 (34), 107 (100), 103 (8), 91 (16), 79 (16), 78 (10), 77 (24), 51 (8), 39 (9)
18	2-Allylphenol ^{e,f}	134	1.207	A1	<0.1	134 (100), 133 (39), 132 (13), 131 (26), 119 (30), 115 (28), 107 (20), 105 (18), 91 (27), 77 (22)
19	4-Ethyl-2-methylphenol	136	1.213	A3	2.0	137 (3), 136 (29), 135 (3), 122 (9), 121 (100), 107 (2), 91 (8), 77 (7), 65 (2), 51 (2)
20	2-Ethyl-3-methylphenol ^f	136	1.216	A2		137 (3), 136 (32), 122 (9), 121 (100), 115 (3), 103 (3), 93 (3), 91 (12), 77 (11), 65 (3)
21	5-Ethyl-2-methylphenol ^f	136	1.216	A3		137 (4), 136 (40), 135 (4), 122 (8), 121 (100), 108 (3), 107 (8), 103 (6), 91 (10), 77 (9)
22	3,5-Dimethylphenol	122	1.220	A3	5.2	123 (9), 122 (100), 121 (44), 108 (6), 107 (75), 91 (10), 79 (10), 78 (4), 77 (16), 51 (4)
23	4-Ethylphenol	122	1.220	A3		122 (32), 108 (8), 107 (100), 91 (4), 78 (4), 77 (17), 65 (5), 53 (4), 51 (6), 39 (8)
24	3-Ethylphenol	122	1.225	A3	1.0	123 (4), 122 (47), 121 (6), 108 (7), 107 (100), 94 (6), 91 (4), 79 (4), 78 (3), 77 (14)
25	2-Vinylphenol ^e	120	1.240	A3	0.4	121 (9), 120 (100), 119 (23), 92 (16), 91 (64), 90 (7), 65 (13), 63 (8), 51 (9), 39 (14)
26	2,4,5-Trimethylphenol	136	1.247	A3	<0.5	137 (8), 136 (85), 135 (34), 122 (9), 121 (100), 93 (5), 91 (16), 79 (4), 77 (9), 65 (4)

(contd.)

Table 1. Contd.

No.	Compound	M_f	Rel. t_R^a	Fraction ^b	Rel. am. ^c	Mass spectrum m/z (%) ^d
27	3-Ethyl-2-methylphenol ^f	136	1.255	A3	1.6	137 (4), 136 (46), 122 (8), 121 (100), 108 (4), 107 (15), 103 (4), 93 (4), 91 (13), 77 (13)
28	4-Isopropylphenol	136	1.255	A3		136 (27), 122 (9), 121 (100), 120 (3), 107 (3), 103 (11), 93 (4), 91 (10), 77 (12), 65 (4)
29	3,4-Dimethylphenol	122	1.255	A3		123 (7), 122 (83), 121 (50), 108 (7), 107 (100), 91 (12), 79 (8), 77 (20), 51 (8), 39 (8)
30	3-Isopropylphenol	136	1.258	A3		137 (4), 136 (38), 122 (8), 121 (100), 107 (4), 103 (13), 93 (4), 91 (10), 77 (11), 65 (4)
31	2,3,5-Trimethylphenol	136	1.258	A3	<0.3	137 (8), 136 (88), 135 (25), 122 (9), 121 (100), 117 (5), 93 (8), 91 (18), 77 (10), 67 (4)
32	3-Ethyl-5-methylphenol	136	1.286	A3		137 (5), 136 (52), 135 (6), 122 (9), 121 (100), 108 (6), 107 (6), 103 (3), 91 (12), 77 (10)
33	4-Propylphenol	136	1.289	A3	–	137 (2), 136 (20), 108 (8), 107 (100), 78 (2), 77 (7), 65 (2), 53 (1), 51 (2), 39 (1)
34	3-Propylphenol	136	1.299	A3	–	136 (39), 121 (13), 108 (44), 107 (100), 94 (5), 91 (5), 79 (6), 78 (5), 77 (19), 39 (8)
35	2,3,4-Trimethylphenol ^f	136	1.307	A3	0.3	137 (7), 136 (72), 135 (25), 122 (9), 121 (100), 93 (5), 91 (16), 79 (4), 77 (9), 65 (4)
36	2-Propenylphenol ^{e,f}	134	1.316	A3	–	135 (10), 134 (100), 133 (36), 131 (10), 119 (31), 115 (29), 107 (14), 105 (16), 91 (29), 77 (14)
37	4-Ethyl-3-methylphenol ^f	136	1.316	A3	0.5	137 (3), 136 (28), 122 (8), 121 (100), 107 (2), 103 (2), 91 (8), 77 (7), 65 (2), 51 (2)
38	3-Ethyl-4-methylphenol ^f	136	1.316	A3		137 (5), 136 (52), 135 (5), 122 (8), 121 (100), 108 (5), 107 (29), 103 (4), 91 (12), 77 (12)
39	3,4,5-Trimethylphenol	136	1.366	A3	<0.3	137 (6), 136 (68), 135 (29), 122 (9), 121 (100), 91 (13), 79 (3), 77 (7), 67 (3), 65 (3)
40	4-Vinylphenol ^e	120	1.387	A3	2.4	121 (8), 120 (100), 119 (25), 94 (5), 92 (3), 91 (27), 65 (10), 63 (5), 51 (4), 39 (8)
41	3-Propenylphenol ^{e,f}	134	1.465	A3	–	135 (10), 134 (100), 133 (63), 119 (11), 117 (13), 115 (13), 107 (17), 105 (21), 91 (16), 77 (14)
42	4-Propenylphenol ^{e,f}	134	1.476	A3	–	135 (10), 134 (100), 133 (78), 119 (9), 117 (8), 115 (10), 107 (31), 105 (21), 91 (10), 77 (14)

^aGC retention time relative to that of 2,6-dimethylphenol on Supelcowax 10 fused silica column. ^bRefers to Fig. 1. ^cAmount in the total weak acid fraction relative to that of 2,6-dimethylphenol. The determinations were made by GC; the < sign designates the presence of co-eluting minor compounds, while the – sign designates that no determination was made because of the presence of co-eluting major non-phenolic compounds. ^d10 strongest peaks in the mass spectra of the reference substances. ^eThe amount of the alkenyl-substituted phenols is somewhat higher in the freshly prepared weak acid fraction. ^fPresence not previously demonstrated in CSC.

into a vinyl group through sequential treatment with trimethylsilylmethylmagnesium chloride and acetyl chloride.^{12,6} The 1-propenyl derivatives **41** and **42** were prepared by treating 3- and 4-hydroxybenzaldehyde with ethylmagnesium bromide and subsequently eliminating the hydroxy group formed by overnight refluxing in acetic acid.

The GC retention times and mass spectra were recorded individually for each alkyl and alkenyl phenol available (Table 1). A mixture of all of them was prepared to evaluate the possibilities of detecting and identifying them solely by GC–MS analysis.

Gas chromatography was performed on a Hewlett–Packard model 5880 A instrument, equipped with a flame-ionisation detector. The GC retention times relative to that

of 2,6-dimethylphenol were measured on a Supelcowax 10 fused silica column (0.32 mm i.d., 60 m) programmed from 60 to 250 °C at 2 K min^{–1}.

Mass spectra were obtained by means of a Kratos MS25RFA mass spectrometer interfaced to a Data General Eclipse S/280 computer operated by DS90 software and connected to a Carlo Erba Mega 5160 gas chromatograph equipped with a 60 m Supelcowax 10 fused silica capillary column. The mass spectrometric conditions were: acceleration voltage 4 kV, electron energy 70 eV, electron beam 250 μ A, source temperature 250 °C.

A Siemens Sichromat 2 was used in the heartcut experiments. The first oven, programmed as mentioned above, was equipped with a 30 m Supelcowax 10 fused silica

column connected to a flame-ionisation detector and via a live T switch to the second oven. After heartcutting, the latter was programmed from 60 to 260 °C at 2 K min⁻¹. It was equipped with a SPB-35 fused silica column (0.32 mm i.d., 30 m), which was connected to a Kratos MS50 mass spectrometer in turn interfaced to the data system mentioned above. Mass spectra were recorded using an acceleration voltage of 8 kV, an electron energy of 70 eV, electron beam 500 µA, source temperature 250 °C.

Result and discussion

Examination of the aforementioned mixture of reference substances by GC-MS indicated that most of the phenols should be identifiable either through their unique retention times or by combining retention time and mass spectral data. Less readily distinguished compounds encountered as inadequately separated groups were: (I) **14**, **15** and **16**; (II) **20** and **21**; (III) **22** and **23**; (IV) **27**, **28**, **29**, **30** and **31**; (V) **37** and **38**.

The total weak acid fraction (WA) and subfraction A (Fig. 1) were subjected to GC-MS analysis under conditions identical with those used for the reference mixture. Most of the phenols could be identified in these fractions (Table 1) although some co-eluted with various minor constituents of different structures. However, single ion monitoring and comparison with the mass spectra obtained from the subfractions A1, A2 and A3 provided adequate confirmatory evidence. Critical overlap in the GC-MS analysis of the fractions were observed only in the same areas as those of the reference mixture. Thus, in the WA and A fractions it was only possible to detect **16** while **14** and **15** appeared as a mixture. In fraction A2, however, the latter two compounds are present in ca. equal amounts with GC peaks separate enough to allow the identification of them as constituents of CSC. The mass spectra of **20** and **21** are almost identical so we cannot conclude from the above GC-MS result whether one or both are present. However, in an earlier study of guaiacols we have separated the A2 fraction further (Fig. 1) by HPLC and examination of these subfractions shows that **20** is present in A2:8 while **21** (and **19**) appears in A2:10. The amount of **23** is considerably greater than that of **22**, and, therefore, only **23** can be detected in the WA and A fractions. Most of **23** occurred in fraction A4 which made it possible to detect and identify **22** in fraction A3. To be able to ensure the presence of **27**–**31**, we used a GC heartcut technique which gave the result presented in Fig. 2. The same approach also made it possible to conclude that both **37** and **38** are present in the CSC.

In all, 42 alkyl- or alkenyl-substituted phenols were identified and, to the best of our knowledge, 12 of these have not previously been reported as constituents of cigarette smoke condensate. All these phenols are detailed in Table 1 along with their GC retention times and mass spectral data as well as information on which of the subfractions A1–A3 that has the largest amount of a given compound.

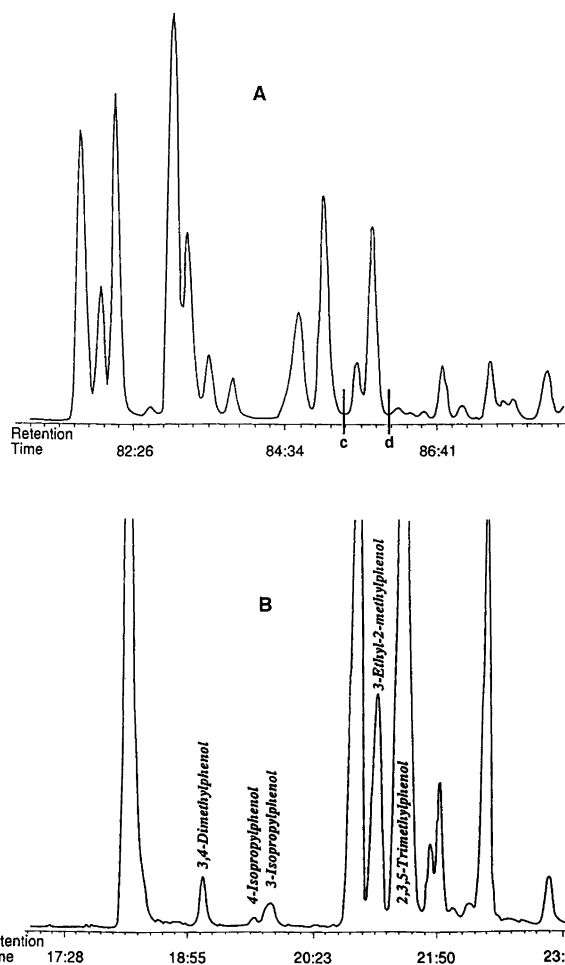


Fig. 2. A shows a part of the total ion curve from fraction A3, and the section of this (c to d) transferred to the second column in the heartcutting experiment giving the curve shown in B.

Estimated values of their abundance in the total weak acid fraction, relative that of 2,6-dimethylphenol and based on the GC results, are also given in Table 1.

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