

Tobacco Smoke Chemistry. 5. Alkyl Substituted 3-Hydroxy-4-pyrones Found in Cigarette Smoke Condensate

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In the course of studies of cigarette smoke condensate a fraction was obtained which contained many alkyl substituted 3-hydroxy-4-pyrones. The structures of these constituents were determined either by comparison with reference compounds or tentatively by interpretation of their electron impact mass spectra. In all, 27 alkyl substituted 3-hydroxy-4-pyrones were identified in this way, 25 of which are new to tobacco smoke.

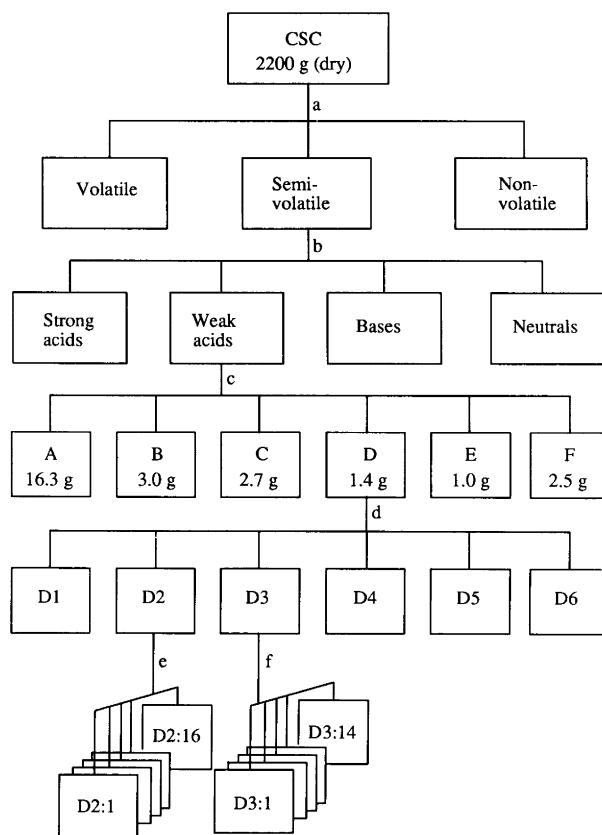
The presence of 3-hydroxy-2-methyl-4-pyrone (maltol) in cigarette smoke condensate, CSC, was first reported by Elmenhorst¹ in 1971. Other studies have confirmed this finding and have also demonstrated the presence of 5-hydroxy-2-methyl-4-pyrone (allomaltol) and 3-hydroxy-4-pyrone.^{2a-d}

In an on-going study of CSC we have come across a series of such compounds. Since most of them are present in low concentration, gas chromatography–mass spectrometry (GC–MS) was used to detect them. In order to improve the accessibility of structural information contained in the mass spectra, we have in an earlier study³ prepared a set of mono-, di- and tri-alkylated 3-hydroxy-4-pyrones and examined their mass spectrometric fragmentation. Using this information, the identifications made here were accomplished either by comparison of both retention times and mass spectra of reference substances with those derived from the CSC or, as discussed below, by interpretation of the mass spectra obtained.

Procedure and discussion

The CSC was obtained by trapping the smoke from 100 000 commercial plain cigarettes of American blend type (23 mg tar, 11 mg carbon monoxide, 1.8 mg nicotine per cigarette) using a Borgwaldt smoking machine. This condensate was distilled and the distillate separated into fractions (Scheme 1) employing procedures described previously.⁴ The weak acid fraction was subjected to flash chromatography⁵ on silica gel, utilizing a stepwise gradient to give six main subfractions, which were analysed by GC–MS. It followed from these results that fraction D contained maltol and several other constituents apparently possessing a 3-hydroxy-4-pyrone skeleton. Many of these compounds were, however, present in minute quantities and their mass spec-

tra often suffered from interference from various CSC components. In order to obtain interpretable mass spectra, we separated a part of fraction D by HPLC on a silica gel



Scheme 1. (a) Distillation; (b) liquid–liquid extraction; (c) silica gel chromatography; (d) HPLC, silica gel column; (e) and (f) HPLC, C₁₈ column.

Table 1. Alkyl substituted 3-hydroxy-4-pyrones found in cigarette smoke condensate. Substituent numbering refers to Fig. 1.

Compound	R ₁	R ₂	R ₃	M _r	Rel. t _r ^a	Fraction ^b	Ident. ^c
1	H	H	H	112	0.990	Strong acids	GC,MS
2	Me	H	H	126	1.000	D3	GC,MS
3 ^d	Et	H	H	140	1.059	D3	GC,MS
4 ^d	Me	Me	H	140	0.974	D3	GC,MS
5 ^d	Me	H	Me	140	1.088	D3	GC,MS
6 ^d	H	Me	Me	140	1.075	D3:7	MS
7 ^d	1-Me-Et	H	H	154	1.064	D2:9	GC,MS
8 ^d	Pr	H	H	154	1.144	D2:8	GC,MS
9 ^d	Et	Me	H	154	1.034	D2:8	MS
10 ^d	Et	H	Me	154	1.124	D3	GC,MS
11 ^d	Me	Me	Me	154	1.097	D2:8	GC,MS
12 ^d	Me	H	Et	154	1.149	D3:13	GC,MS
13 ^d	1-Me-Pr	H	H	168	1.149	D2:12	GC,MS
14 ^d	2-Me-Pr	H	H	168	1.175	D2:13	GC,MS
15 ^d	Bu	H	H	168	1.260	D2:13	GC,MS
16 ^d	1-Me-Et	H	Me	168	1.110	D2:12	MS
17 ^d	Pr	H	Me	168	1.208	D2:12	MS
18 ^d	Et	Me	Me	168	1.136	D2:12	MS
19 ^d	Et	H	Et	168	1.187	D2:12	MS
20 ^d	Me	H	Pr	168	1.244	D2:12	MS
21 ^d	2-Me-Bu	H	H	182	1.304	D2:15	MS
22 ^d	3-Me-Bu	H	H	182	1.316	D2:15	MS
23 ^d	Pe	H	H	182	1.367	D2:15	MS
24 ^d	1-Me-Pr	H	Me	182	1.189	D2:15	MS
25 ^d	2-Me-Pr	H	Me	182	1.227	D2:15	MS
26 ^d	Bu	H	Me	182	1.323	D2:15	MS
27 ^d	Pr	H	Et	182	1.268	D2:15	MS

^aGC retention time relative to maltol (2) on OV351 fused silica column. ^bThe fraction in which the identification was made, cf. Scheme 1. ^cThe identification was made either by comparison with a reference compound (GC,MS) or tentatively by interpretation of the mass spectrum (MS). ^dPresence in cigarette smoke condensate not previously demonstrated.

column into six subfractions. Of these subfractions D2 and D3 contained the compounds of interest and were separated further by HPLC on a C₁₈ column, cf. Scheme 1.

Comparison of GC retention times and mass spectra of the assumed 3-hydroxy-4-pyrones with those of available references led to the identification of 13 alkyl-substituted 3-hydroxy-4-pyrones. These are given in Table 1, where GC, MS denotes the means of identification.

Of the remaining large number of unidentified constituents in these fractions, there were some that could be tentatively identified as alkylated 3-hydroxy-4-pyrones, since this groups of substances, as discussed in an earlier

paper,³ undergoes some diagnostically important ring and side chain cleavage reactions.

The ring cleavages of importance to the subsequent discussion are summarised in Fig. 1, where, for the compounds under consideration, route A leads to important and prominent ions if R₁ ≠ H. Route D gives significant ions only if R₁ = H, while routes B and C furnish informative ions as long as the substituents are short (methyl or ethyl).

In the aforementioned mass spectrometric study,³ most homologues with R₂ = R₃ = H and R₁ ranging from methyl to butyl were examined. All of these show a significant m/z 71 ion formally derived by route A. Depending on the nature of R₁, they also furnish ions arising by cleavage of the β and γ bonds in R₁, of which cleavage of the latter is the major process when R₁ is a straight chain. When R₁ possesses three or more consecutive carbons and a γ-hydrogen, prominent ions of even mass are formed by a McLafferty reaction. Moreover, when R₁ has a branchpoint at the α-carbon other ions of even mass arise by an alkyl rearrangement reaction. These cleavages are exemplified in Fig. 2.

In the CSC fractions, there are three unidentified constituents, the mass spectra of which suggest that they possess a 3-hydroxy-4-pyrone skeleton where R₂ = R₃ = H (m/z 71 ion, cleavage A, Fig. 1). All three have molecular weights of 182, and exhibit only one prominent ion of even mass, the

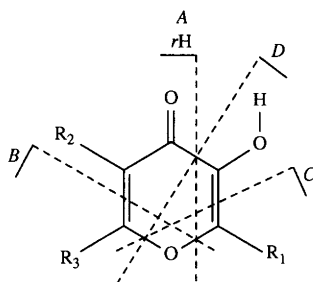


Fig. 1. Major fragmentation reactions of the 3-hydroxy-4-pyrone ring under EI conditions (70 eV).

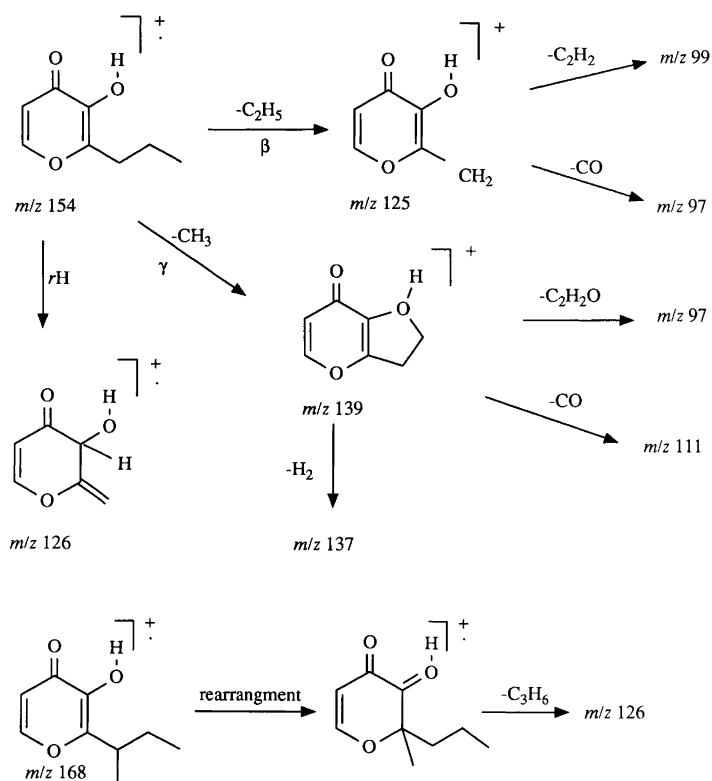


Fig. 2. Major fragmentation reactions involving the substituent of 2-alkyl-3-hydroxy-4-pyrone under EI conditions (70 eV).

m/z 126 ion, which constitutes the base peak. The fragment ions arising on cleavage of the side chains allow differentiation between the possible isomers. Thus the first constituent, which displays $(M-15)^+$ and $(M-29)^+$ ions, but no $(M-43)^+$ ion, must have a 2-methylbutyl substituent. The second constituent, which gives rise to $(M-15)^+$ and $(M-43)^+$ ions, but no $(M-29)^+$ ion, is bound to have a 3-methylbutyl substituent, while the third, which exhibits a very weak $(M-15)^+$ ion, a prominent $(M-43)^+$ ion and a $(M-29)^+$ as well as a $(M-57)^+$ ion must carry a pentyl substituent. Accordingly, these were identified as **21**, **22** and **23** (Table 1).

Because of the dominating influence of R_1 on the fragmentation, compounds having the same R_1 substituent as our reference substances are readily recognised, as is also the case when an additional methyl group is present at position 5 or 6 (R_2 or R_3). Thus, the ions in the upper half of the spectra are shifted 14 units towards higher mass and the ion generated by cleavage *A* now appears at m/z 85. When $R_3 = \text{methyl}$, a prominent ion at m/z 43 is generated by way of cleavage *B*. Compounds **16**, **17**, **24**, **25** and **26** were identified in this way.

Compounds where R_1 is an ethyl group give a very intense $(M-1)^+$ ion as a result of a γ -cleavage, but do not give any prominent ions of even mass. Apart from the compounds of this type identified on comparison with the reference substances (**3** and **10**), there are three mass spectra derived from the CSC fractions that have both the fragment ions expected of a 3-hydroxy-4-pyrone skeleton and also a strong $(M-1)^+$ ion. In this case, the most impor-

tant information for establishing the position(s) of the remaining substituents comes from the ring cleavage reactions. One of the constituents has a molecular weight of 154 and this, together with the fact that the fragment ion arising by route *A* appears at m/z 85, indicates, that either R_2 or R_3 is a methyl group. Since there is no prominent m/z 43 ion (route *B*), which suggests that $R_3 = \text{H}$, this compound could be identified as **9**, consistent with the fact that the other isomer, having $R_1 = \text{ethyl}$ and $R_3 = \text{methyl}$, is one of our reference substances (**10**). The other two constituents have molecular weights of 168 and both give an ion at m/z 99 by way of route *A*, which implies, that $R_2 = R_3 = \text{methyl}$ or that one of them is an ethyl group. Since one of them has a prominent ion at m/z 43, indicative of $R_2 = R_3 = \text{methyl}$ substitution, it could be assigned structure **18**. The other constituent lacks a prominent m/z 43 ion, but exhibits a comparatively intense ion at m/z 57 explicable by cleavages *B* and *C* when R_3 is an ethyl group, and could therefore be attributed structure **19**.

The presence of an ion at m/z 58 derived by cleavage *D* is typical of 3-hydroxy-4-pyrones in which $R_1 = \text{H}$. One of the unidentified constituents, of molecular weight 140, furnishes this ion as well as a prominent m/z 43 ion (route *B*), which indicates that $R_2 = R_3 = \text{methyl}$. Since, in addition, only low-abundant ions corresponding to the extrusion of a hydrogen or a methyl radical followed by loss of carbon monoxide are present in the spectrum, it is consistent with a 3-hydroxy-4-pyrone possessing no substituents heavier than methyl groups. Hence structure **6** was assigned to this compound.

One of the constituents, which has a molecular weight of 182, displays a fragmentation pattern in the upper part of the spectrum typical of 3-hydroxy-4-pyrone in which $R_1 =$ propyl. Since there is also an m/z 99 ion derived by way of cleavage *A* and a prominent ion at m/z 57, which indicates that $R_3 =$ ethyl (cleavage *B*), this compound could be identified as **27**.

Although there were some compounds in which $R_1 = R_2 =$ H among the reference substances prepared, no alkylated components of this type were found in the CSC fractions mentioned above. Comparison of the mass spectra of these reference substances with those derived from the CSC fraction was, nevertheless, helpful. One constituent, with a molecular weight of 168, shows a mass spectrum in agreement with that expected for $R_3 =$ propyl and R_1 or $R_2 =$ methyl. Both the fact that the ion at m/z 58 (route *D*) is absent and the presence of a prominent ion at m/z 43 (route *C*) indicate that $R_1 =$ methyl. This constituent was then identified as **20**.

Hence a total of 27 3-hydroxy-4-pyrone derivatives were identified, 25 of which are, to the best of our knowledge, reported for the first time as constituents of cigarette smoke condensate. These are detailed in Table 1 along with data on their GC retention times and the CSC fractions in which they were identified. Most of them are present in very small quantities and even an estimate of their relative abundance would be meaningless due to chromatographically coinciding major CSC constituents of known and/or unknown structure.

Experimental

General methods. Mass spectra (70 eV) were measured on a Kratos MS 25 mass spectrometer, intensities of ions (m/z 39 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 OV 351 fused silica column (0.32 mm i.d., 30 m) programmed from 60 to 220 °C at 3 K min⁻¹ using helium as the carrier gas. Merck Kieselgel 60 (230–400 mesh) was used for column chromatography. HPLC was performed on a Varian 5000 instrument, equipped with a Waters Differential Refractometer R401 and a Pharmacia Dual Path Monitor UV-2 (254 nm).

Preparation of cigarette smoke condensate fractions. The CSC was prepared from 100 000 plain cigarettes of American blend type (23 mg tar, 11 mg CO and 1.8 mg nicotine per cigarette) which were smoked according to a standard procedure⁶ using a Borgwaldt automatic smoking machine (Type R 09.012, Heinr. Borgwaldt, Hamburg, F.R.G.). The CSC was condensed in an Elmenhorst trap cooled in a dry ice/ethanol mixture.⁷ The CSC was distilled *in vacuo* with carbon dioxide as the carrier gas⁸ to give three fractions – volatiles, semi-volatiles and non-volatiles.⁹ The semi-vola-

tiles were divided into four dichloromethane soluble fractions – acids, weak acids, bases and neutrals – utilizing an extraction procedure described earlier.¹⁰ Portions (8–9 g) of the weak acids were subjected to flash chromatography⁵ on silica gel. The column was eluted with 1300 ml batches of 6 different mixtures of cyclohexane, ethyl acetate and methanol (6:1:0; 3:1:0; 1:1:0; 1:3:0; 0:3:1; 0:0:1). The eluate was collected in 25 ml tubes and, after TLC evaluation, combined into 6 fractions, A–F. Fraction A contained, according to GC–MS results, most of the alkylphenols and alkyl-

Table 2. Mass spectra of tentatively identified alkyl substituted 3-hydroxy-4-pyrone. Compound numbering refers to Table 1.

Compound	M_r	Mass spectrum [m/z (%)]
6	140	140 (100), 55 (64), 43 (64), 83 (64), 39 (48), 53 (38), 66 (27), 41 (26), 97 (23), 111 (20), 58 (18), 139 (12), 125 (9)
9	154	154 (100), 153 (70), 139 (27), 85 (22), 111 (21), 39 (14), 43 (9), 41 (9), 55 (9), 57 (5), 125 (4), 126 (4)
16	168	153 (100), 140 (43), 43 (36), 168 (35), 85 (23), 125 (22), 39 (18), 167 (14), 83 (14), 55 (12)
17	168	140 (100), 153 (70), 168 (32), 139 (30), 111 (28), 43 (28), 85 (12), 167 (12), 125 (6), 151 (3), 83 (2)
18	168	168 (100), 167 (72), 153 (40), 125 (33), 43 (32), 99 (25), 39 (10), 55 (9), 83 (8), 139 (8), 57 (7)
19^a	168	168 (100), 167 (82), 153 (56), 99 (26), 125 (25), 69 (17), 57 (15), 39 (12), 43 (10), 139 (10), 83 (4)
20^a	168	168 (100), 139 (48), 43 (29), 111 (25), 140 (16), 153 (12), 113 (9), 97 (8), 69 (7), 55 (6)
21^a	182	126 (100), 153 (18), 41 (18), 55 (17), 43 (12), 125 (10), 97 (10), 71 (9), 167 (6), 182 (3)
22^a	182	126 (100), 139 (21), 125 (11), 41 (10), 43 (9), 71 (8), 97 (7), 39 (7), 55 (6), 167 (5), 182 (1)
23^a	182	126 (100), 139 (29), 153 (13), 125 (10), 71 (10), 182 (9), 97 (9), 43 (9), 41 (9), 55 (7), 167 (1)
24^a	182	153 (100), 167 (76), 154 (56), 43 (47), 140 (38), 125 (29), 41 (27), 182 (26), 85 (15), 151 (12)
25^a	182	140 (100), 139 (42), 167 (38), 43 (37), 41 (33), 111 (26), 55 (23), 39 (22), 182 (16), 85 (14)
26^a	182	140 (100), 153 (27), 43 (15), 139 (14), 111 (13), 167 (9), 85 (9), 182 (7), 39 (7), 125 (3), 151 (3)
27^a	182	154 (100), 167 (84), 182 (62), 153 (36), 43 (34), 126 (32), 57 (29), 125 (28), 181 (27), 139 (25), 99 (12)

^aBackground-subtracted mass spectrum.

guaiacols,¹¹ fractions B and C included the alkyl-substituted 2-hydroxy-2-cyclopentenones⁴ and fractions D and E comprised compounds such as the alkyl-substituted 3-hydroxy-4-pyrones (3-hydroxy-2-methyl-4-pyrone being the major constituent in E according to GC). A part of fraction D was fractionated by HPLC (silica column, cyclohexane-chloroform-methanol 160:40:2) into 6 subfractions, D1-D6. GC-MS evaluation of these showed that D2 and D3 contained most of the alkyl-substituted 3-hydroxy-4-pyrones but also some other compounds that in some cases made the interpretation of the mass spectra uncertain. To overcome this problem both fractions were chromatographed on an HPLC C₁₈ column (methanol-0.05 M aqueous hydrochloric acid 1:1 over 5 min followed by a gradient to 100% methanol over 15 min). Rather small fractions were collected and subjected to GC-MS examination. Acceptable mass spectra were obtained in most cases, requiring, when components were of low abundance, background subtraction. The mass spectral data given in Table 2 comprise, in addition to the *m/z* values and intensities of the eight most abundant ions, the corresponding values for some ions of importance in the structural elucidation of the tentatively identified alkyl-substituted 3-hydroxy-4-pyrones.

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