Short Communication

Tobacco Smoke Chemistry. 6. Alkyl-Substituted 2-Hydroxy-2-cyclohexenones Found in Cigarette Smoke Condensate

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Our previous studies of cigarette smoke condensate, CSC, have led to the identification of a number of cyclic α-diketones, comprising 26 alkylated 2-hydroxy-2-cyclopentenones[1] and 27 alkylated 3-hydroxy-4-pyrones.[2] These include maltol (3-hydroxy-2-methyl-4-pyrone), a well-known product from the pyrolysis of poly-, oligo- and mono-saccharides, as well as cyclotene (2-hydroxy-3-methyl-2-cyclopentenone), a compound identified in the pyrolysate of sucrose and materials representative of tobacco cell-wall constituents, cellulose, dextrin and lignin.[3]

In some foodstuffs such as roasted coffee, the formation of these compounds seems to be accompanied by the generation of related compounds, e.g. 2.hydroxy-3-methyl-2-cyclohexenone, a six-membered ring homologue of cyclotene. Since these compounds are likely to arise in a similar fashion and all have an aroma reminiscent of burnt sugar, we have examined CSC with respect to the presence of compounds of the latter type. This was accomplished by preparation of a series of mono- and di-alkylated 2-hydroxy-2-cyclohexenones and comparing their electron impact mass spectra and GC retention times with those of the constituents encountered in the CSC fractions examined.

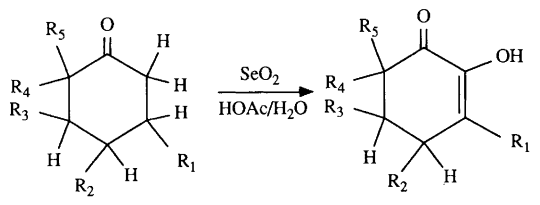
Procedure and discussion

The reference substances were prepared by selenium dioxide oxidation of suitably alkyl-substituted cyclohexanones, Scheme 1, under conditions similar to those described by Hach *et al.*[4] The starting materials which were not available commercially, were prepared either by oxidation of the corresponding cyclohexanol derivative or by alkylation of a suitable cyclohexanone derivative. Although the yields of the selenium dioxide oxidations were usually low, convenient work-up procedures compensate for this drawback. Considerably higher yields have recently been achieved by Horiuchi *et al.*[5] using iodine/copper(II) acetate as the oxidising agent.

The CSC examined was obtained by smoking 100000 commercial plain cigarettes of American blend type. Distillation of the CSC and separation of the components into strong acids, weak acids, bases and neutrals (Scheme 2) have been described previously.[1,6] The weak acid fraction, containing *inter alia* phenols, guaiacols[6] and cyclic α-diketones, was subjected to flash[7] chromatography and the fractions obtained analysed by GC–MS. Fraction A (Scheme 2) was separated further by HPLC and the subfractions examined by GC–MS.

Comparison of GC retention times and mass spectra of the reference substances with the corresponding data for the constituents of the total weak acid fraction revealed the presence of 2-hydroxy-3-methyl-, 2-hydroxy-, 3-ethyl-2-hydroxy and 2-hydroxy-3,6-dimethyl-2-cyclohexenone. Examination of fractions A–F (Scheme 2) showed 2-hydroxy-2-cyclohexenone to occur in fraction B, while the alkyl-substituted derivatives thereof were encountered in fraction A. Further examination of the subfractions derived from fraction A confirmed the presence of the compounds already mentioned and allowed identification of three additional constituents, i.e. 3,4- and 3,5-dimethyl-2-hydroxy-2-cyclohexenone and 2-hydroxy-3-propyl-2-cyclohexenone.

None of these seven 2-hydroxy-2-cyclohexenones, detailed in Table 1 along with their GC retention times and fraction of origin, has to our knowledge been reported present in tobacco smoke condensate. Four of the compounds prepared could not be detected in the CSC, i.e.



R_1–R_5 = H, CH_3, C_2H_5, $CH(CH_3)_2$, C_3H_7 or C_4H_9

Scheme 1. Preparative route to the compounds detailed in Table 1.

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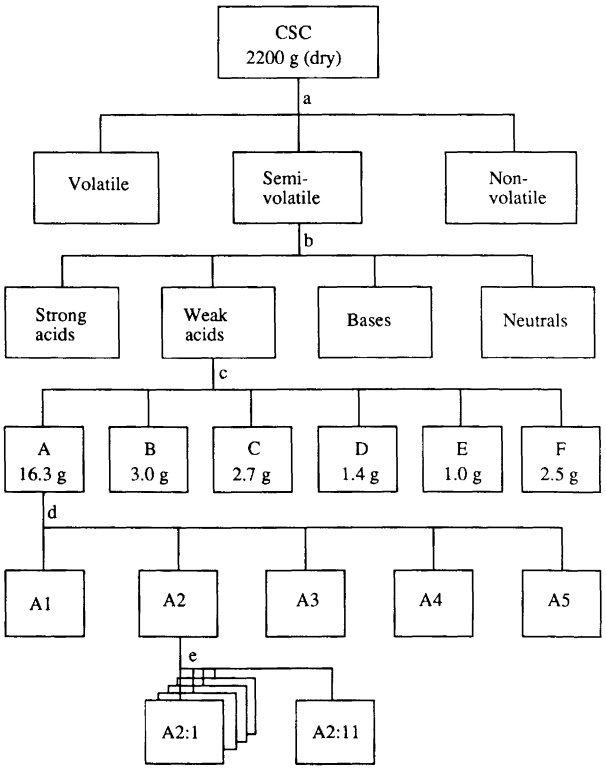

Scheme 2. a, Distillation; b, liquid–liquid extraction; c, silica gel chromatography; d, silica gel chromatography; e, HPLC, CN column.

4-methyl-, 3-isopropyl-, 3,6,6-trimethyl- and 3-butyl-2-hydroxy-2-cyclohexenone.

Although no effort has been made to quantify these constituents, their relative abundances can be estimated from the gas chromatogram of the total weak acid fraction. Thus, expressed as a percentage of the amount of the major component cyclotene,[1,8] 2-hydroxy-3-methyl-, 2-hydroxy-, 3-ethyl-2-hydroxy- and 2-hydroxy-3,6-dimethyl-2-cyclohexenone represent 10, 1, 0.5 and 0.5 %, respectively.

Experimental

General methods. The ^{1}H NMR spectra were recorded on a Varian XL300 spectrometer in $CDCl_3$ using Me_4Si as an internal standard. 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.

Gas chromatography was performed on a Hewlett-Packard model 5880 A instrument, equipped with a flame-ionisation detector. The 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} 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 R401 differential refractometer and a Pharmacia UV-2 Dual Path Monitor (254 nm).

Preparation of cigarette smoke condensate fractions. The procedures for smoking, collecting and fractionating the CSC, as shown in Scheme 2, have been described in earlier reports.[1,6]

General method for the synthesis of C-alkylated 2-hydroxy-2-cyclohexenones. Since the same method was applied for the final oxidation of all compounds, we give only a general description of the methodology followed by the spectroscopic characteristics of each compound. ^{1}H NMR spectral data which are not given agree with literature values.[5]

The ketone (18 mmol) was dissolved in 75 % aqueous acetic acid (50 ml). An equimolar amount of selenium dioxide was added in small portions over 1 h at ambient temperature. After being stirred for 96 h the mixture was filtered through a Celite bed. The bed was washed with ethanol and the combined filtrates evaporated to near dryness. The residue was dissolved in dichloromethane (50 ml), washed with saturated aqueous sodium hydrogencarbonate (50 ml) and extracted with aqueous sodium hydroxide (1 M, 3×20 ml). The alkali-extractable material was washed with dichloromethane, acidified with conc. hydrochloric acid and back-extracted with dichloromethane. Drying over sodium sulfate and evaporation of the solvent gave a crude product. From this the pure compound was isolated by column chromatography on silica gel using an ethyl acetate/hexane mixture as the eluent. The elution was monitored by TLC, using 1 M aqueous ferric chloride for specific detection of the cyclic α-diketones.

2-Hydroxy-2-cyclohexenone is commercially available. MS *m/z* (%): 112 (100, $M^{+\cdot}$), 55 (81), 56 (60), 70 (51), 84 (46), 43 (45), 71 (32), 42 (30), 41 (28), 39 (26).

2-Hydroxy-3-methyl-2-cyclohexenone was prepared from 2-methylcyclohexanone. MS *m/z* (%): 126 (100, $M^{+\cdot}$), 84 (45), 55 (41), 41 (37), 43 (37), 83 (34), 70 (30), 39 (27), 97 (18), 69 (17).

3-Ethyl-2-hydroxy-2-cyclohexenone. 2-Ethylcyclohexanol was oxidised with potassium permanganate[9] in acidic solution to 2-ethylcyclohexanone. Selenium dioxide oxidation of this ketone gave the title compound. MS *m/z* (%): 140 (100, $M^{+\cdot}$), 55 (49), 97 (46), 41 (38), 83 (36), 111 (33), 43 (29), 84 (27), 39 (26), 125 (22).

2-Hydroxy-3-propyl-2-cyclohexenone. Cyclohexanone (10 ml), 1-iodopropane (9.5 ml) and sodium hydride (2.4 g) in diethyl ether (50 ml) were boiled under reflux overnight. After addition of hydrochloric acid (1 M, 50 ml), the aqueous phase was separated and extracted with diethyl ether (2×25 ml). The combined diethyl ether phase was dried over sodium sulfate and the diethyl ether evaporated off to give a crude product (15 g) which on distillation gave

Table 1. 2-Hydroxy-2-cyclohexenones used in this study.

Alkyl substituent(s) on 2-hydroxy-2-cyclohexenone	Rel. t_R[a]	Fraction[b]
None	0.746	Tot. weak acids
3-Methyl	0.788	Tot. weak acids
4-Methyl and 5-methyl	0.762	N.d.
3,4-Dimethyl	0.832	A2
3,5-Dimethyl	0.805	A2
3,6-Dimethyl	0.773	A2
3,6,6-Trimethyl	0.726	N.d.
3-Ethyl	0.840	A2
3-Isopropyl	0.858	N.d.
3-Propyl	0.917	A2:6
3-Butyl	1.012	N.d.

[a]GC retention time relative to that of 2,6-dimethylphenol on Supelcowax 10 fused silica column. [b]The fraction in which the identification was made, cf. Scheme 1. N.d. = not detected.

2-propylcyclohexanone (5 g). This compound was oxidised with selenium dioxide as described above to give the title compound. MS *m/z* (%): 154 (100, $M^{+\cdot}$), 97 (73), 55 (61), 125 (56), 41 (49), 79 (47), 139 (46), 112 (45), 43 (37), 126 (36). ^{1}H NMR: δ 6.05 (br s, 1 H), 2.49, 2.36, 2.29, 1.96 and 1.53, a series of incompletely resolved multiplets, each containing 2 H, 0.95 (t, J = 7 Hz, 3 H).

2-Hydroxy-3-isopropyl-2-cyclohexenone was prepared as described for 2-hydroxy-3-propyl-2-cyclohexenone but using 2-iodopropane in the alkylating step. MS *m/z* (%): 111 (100), 154 (70, $M^{+\cdot}$), 55 (66), 139 (55), 41 (47), 43 (44), 83 (37), 39 (31), 82 (29), 67 (23). ^{1}H NMR: δ 6.11 (br s, 1 H), 3.16 (septet, J = 6.9, 1 H), 2.48, 2.32 and 1.95, incompletely resolved multiplets, each containing 2 H, 1.06 (d, J = 6.9 Hz, 6 H).

3-Butyl-2-hydroxy-2-cyclohexenone was prepared as described for 2-hydroxy-3-propyl-2-cyclohexenone but using 1-iodobutane in the alkylating step. MS *m/z* (%): 126 (100), 139 (51), 113 (48), 168 (40, $M^{+\cdot}$), 97 (36), 112 (29), 125 (28), 79 (20), 111 (18), 55 (17). ^{1}H NMR: δ 6.05 (br s, 1 H), 2.50, 2.38, 2.32, 1.96, 1.48, and 1.38, a series of incompletely resolved multiplets, each containing 2 H, 0.95 (t, J = 8 Hz, 3 H).

2-Hydroxy-4-methyl-2-cyclohexenone (and its tautomeric form 2-hydroxy-5-methyl-2-cyclohexenone) was prepared from 4-methylcyclohexanone. The two forms were formed in equal amounts and did not separate on the GC column. Since we used GC–MS for the identification of CSC constituents, no attempt was made to characterize each form separately, and the spectroscopic data refers to the mixture. MS *m/z* (%): 56 (100), 84 (96), 55 (92), 126 (76, $M^{+\cdot}$), 83 (71), 43 (65), 41 (53), 39 (46), 111 (38), 71 (29). ^{1}H NMR: δ 6.12 and 6.00 (dd, one for each tautomer), 5.95 (br s), 2.7–1.5 (a large number of unresolved multiplets), 1.18 and 1.09 (d, one for each tautomer).

2-Hydroxy-3,4-dimethyl-2-cyclohexenone was prepared as described for 3-ethyl-2-hydroxy-2-cyclohexenone replacing 2-ethylcyclohexanol by 2,3-dimethylcyclohexanol. MS *m/z* (%): 125 (100), 97 (96), 140 (86, $M^{+\cdot}$), 98 (57), 43 (53), 41 (52), 55 (51), 69 (35), 39 (28), 83 (22). ^{1}H NMR: δ 6.07 (br s, 1 H), 2.60, 2.45, 2.12 and 1.70, a series of multiplets from the ring protons, 1.90 (d, J = 1 Hz, 3 H), 1.19 (d, J = 7 Hz, 3 H).

2-Hydroxy-3,5-dimethyl-2-cyclohexenone was prepared from 3,5-dimethylcyclohexanone. MS *m/z* (%): 140 (100, $M^{+\cdot}$), 41 (90), 69 (86), 70 (83), 43 (65), 39 (55), 55 (54), 98 (50), 97 (45), 111 (36).

2-Hydroxy-3,6-dimethyl-2-cyclohexenone. Alkylation of 3-methylcyclohexanone gave, after distillation, a mixture of 2,5-dimethylcyclohexanone and 2,2,5-trimethylcyclohexanone. Oxidation of this mixture with selenium dioxide gave, after chromatography, the title compound. MS *m/z* (%): 111 (100), 140 (71, $M^{+\cdot}$), 70 (65), 69 (62), 41 (58), 55 (48), 43 (42), 97 (38), 39 (32), 71 (31).

2-Hydroxy-3,6,6-trimethyl-2-cyclohexenone was prepared as described for 2-hydroxy-3,6-dimethyl-2-cyclohexenone. MS *m/z* (%): 111 (100), 70 (73), 41 (48), 154 (40, $M^{+\cdot}$), 43 (33), 69 (32), 55 (29), 39 (26), 85 (25), 109 (19). ^{1}H NMR: δ 6.1 (br s, 1 H), 2.33 (t, J = 6 Hz further coupled into q, J = 1.2 Hz, 2 H) 1.88 (t, J = 1.2 Hz, 3 H), 1.79 (t, J = 6 Hz, 2 H), 1.13 (s, 6 H).

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