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Investigation of 2-Hydroxy-2-cyclopenten-1-ones in Roasted Coffee

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The weakly acidic volatile components of roasted coffee were analyzed on a GC/MS. Fourteen 2-hydroxy-2-cyclopenten-1-ones were identified. Ten of these compounds are being reported for the first time in roasted coffee. *trans*- and *cis*-2-hydroxy-3,4,5-trimethyl-2-cyclopenten-1-ones are new compounds. Possible precursors of the 2-hydroxy-2-cyclopenten-1-ones are discussed.

To date, 670 compounds have been identified in roasted coffee aroma (Flament and Chevallier, 1988). Four major 2-hydroxy-2-cyclopenten-1-ones were identified in roasted coffee (Gianturco et al., 1963). A mechanism for formation of 2-hydroxy-3-methyl-, 2-hydroxy-3,4-dimethyl-, 2-hydroxy-3,5-dimethyl-, and 3-ethyl-2-hydroxy-2-cyclopenten-1-one (**1**, **2**, **3**, and **8**, respectively) from hydroxy ketones is presented by Shaw et al. (1968). All four 2-hydroxy-2-cyclopenten-1-ones had strong caramel-like odors, **2** being the most powerful. In the present work, we report the identification and quantification of 14 2-hydroxy-2-cyclopenten-1-ones in roasted coffee.

EXPERIMENTAL SECTION

Materials. High-grown Arabica coffee beans of Colombian origin were roasted to a medium roast (205 °C, 12 min) and stored in packages with an excess of air at 4 or 25 °C. 2-Hydroxy-3-methyl-, 2-hydroxy-3,4-dimethyl-, 2-hydroxy-3,5-dimethyl-, and 3-ethyl-2-hydroxy-2-cyclopenten-1-ones (**1**-**3** and **8**) were commercially available (Seimi Chemical Co., Ltd., Hercules Inc., and Pfizer Inc.) All starting chemicals were obtained from reliable commercial sources and used without further purification.

Isolation of 2-Hydroxy-2-cyclopenten-1-ones. A 40-kg portion of roasted coffee was ground and subjected to steam distillation. The distillate (62.5 kg) was extracted with methylene

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Table I. 2-Hydroxy-2-cyclopenten-1-ones Characterized in Roasted Coffee

compd	rel abund, ppm	coffee, mg/kg	I^{ov}	I^{cw}	MS, m/z (%)	odor description	occurence in food
1[d-f]	9400	9.78	1015	1752	112 (M^+, 100), 97 (7), 85 (31), 83 (30), 70 (5), 69 (46), 56 (29), 55 (42), 43 (28), 42 (12), 41 (44)	maple-like burnt	maple syrup[a] coffee[b] tobacco[c]
2[f]	700	0.73	1063	1765	126 (M^+, 100), 111 (94), 98 (43), 97 (18), 83 (81), 70 (22), 69 (22), 56 (11), 55 (72), 43 (61), 42 (10), 41 (29)	sweet burnt	coffee[b] tobacco[c]
3[f]	2100	2.18	1044	1723	126 (M^+, 100), 111 (74), 98 (40), 97 (24), 83 (73), 80 (23), 69 (84), 56 (58), 55 (74), 43 (74), 42 (20), 41 (90)	caramel-like sweet	coffee[b] tobacco[c]
4[f]	20	0.02	1088	1753	140 (M^+, 40), 126 (8), 125 (100), 97 (24), 69 (11), 55 (5), 43 (28), 41 (13)	sweet burnt	
5	100	0.10	1082	1733	140 (M^+, 100), 126 (7), 125 (88), 112 (17), 97 (46), 83 (12), 79 (5), 69 (7), 55 (12), 43 (10), 41 (6)	sweet milky	
6	10	0.01	1138	1826	140 (M^+, 100), 126 (11), 125 (98), 112 (40), 97 (94), 83 (27), 79 (11), 69 (18), 55 (41), 43 (37), 41 (25)	sweet maple-like	
7[f]	200	0.21	1040	1655	140 (M^+, 100), 125 (92), 107 (21), 97 (30), 85 (19), 79 (19), 69 (30), 56 (37), 55 (17), 43 (38), 41 (36)	sweet caramel-like	
8[d-f]	5500	5.72	1100	1816	126 (M^+, 100), 111 (16), 98 (15), 97 (19), 84 (27), 83 (53), 70 (17), 69 (30), 56 (13), 55 (48), 43 (35), 41 (27)	sweet sugary	coffee[b]
9[g]	100	0.10	1143	1812	140 (M^+, 100), 125 (47), 112 (23), 111 (79), 98 (12), 97 (37), 83 (26), 79 (11), 69 (35), 55 (19), 43 (32), 41 (33)	sweet maple-like	
10	60	0.06	1130	1781	140 (M^+, 100), 125 (42), 111 (50), 97 (37), 83 (25), 70 (17), 69 (16), 57 (22), 55 (22), 43 (17), 41 (12)	sweet malty	tobacco[c]
11[h]	200	0.21	1154	1845	140 (M^+, 65), 112 (16), 111 (100), 83 (74), 55 (23), 43 (44), 41 (8)	sweet malty	
12	200	0.21	1135	1799	140 (M^+, 65), 125 (10), 112 (100), 97 (15), 94 (90), 83 (10), 70 (10), 69 (12), 66 (18), 55 (18), 43 (17), 41 (16)	sweet malty	tobacco[c]
13[d,i,j]	400	0.42	1188	1891	140 (M^+, 100), 125 (82), 112 (73), 111 (65), 99 (26), 98 (23), 97 (19), 83 (47), 70 (19), 69 (12), 55 (48), 43 (19), 41 (13)	sweet burnt	tobacco[c]
14[i,j]	20	0.02	1158	1835	140 (M^+, 100), 125 (97), 112 (12), 98 (21), 97 (38), 83 (20), 69 (33), 56 (25), 43 (21), 41 (19)	sweet burnt	

[a] Filipic et al. (1965). [b] Gianturco et al. (1963). [c] Hecht et al. (1975). [d] Ito et al. (1976). [e] Baltes and Bochmann (1987). [f] Gianturco and Friedel (1963). [g] Staudinger and Ruzicka (1924). [h] Ito and Deki (1978). [i] Pattenden and Teague (1982). [j] Maignan and Rouessac (1976).

chloride (16.8 kg) to give 52 g of volatile components (yield 0.13% based on roasted coffee beans) having a strong brown and roasted coffee-like odor. The flavor concentrate (41.7 g) was separated into basic (2.7 g), acidic (1.0 g), weakly acidic (2.9 g), and neutral fractions (10.9 g) by the methods described by Takahashi et al. (1980). The weakly acidic fraction was further fractionated into 13 fractions by preparative TLC (silica gel; eluting with 3:7 acetone–hexane and 3:7 ethyl acetate-hexane). All these fractions were analyzed by GC, GC/MS, and GC/FT-IR.

Synthesis of *trans*- and *cis*-2-Hydroxy-3,4,5-trimethyl-2-cyclopenten-1-one (5 and 6). To the tetrahydro-2*H*-1,4-oxazine (2 g, 23 mmol) was added an ice–water solution (4.5 g) of 16 N hydrogen chloride (2.25 g) to give tetrahydro-2*H*-1,4-oxazine hydrochloride. 2-Hydroxy-3,4-dimethyl-2-cyclopenten-1-one (**2**; 0.75 g, 6 mmol) and 35% formaldehyde (2.25 g, 26 mmol) were then added, and the mixture was stirred for 5 h at 80 °C. To this solution was added water (10 mL). After the unreacted **2** in the organic layer was removed with ether, the aqueous solution was made basic to pH 9 with sodium bicarbonate and extracted with three 20-mL portions of ethyl acetate. To the extract were added zinc dust (1.5 g, 23 mol) and glacial acetic acid (3 mL). The reaction mixture was refluxed for 6 h. After the solid particles were removed by filtration, the solution was then evaporated in vacuo and the residual liquid was extracted with ethyl acetate. The ethyl acetate solution was dried over anhydrous sodium sulfate, and the ethyl acetate was distilled off. The crude oil obtained (120 mg) was separated by preparative TLC (eluting with 3:7 ethyl acetate–hexane) and preparative GLC to give **5** (65 mg) and **6** (5.6 mg).

trans-2-Hydroxy-3,4,5-trimethyl-2-cyclopenten-1-one (**5**): IR (ν_{max}, cm^{-1}; neat) 3300, 2950, 2910, 2850, 1745, 1690, 1645, 1445, 1405, 1350, 1305, 1260, 1200, 1165, 1125, 1090, 1050, 1020, 970, 945, 905, 765; ^1H NMR (δ, $CDCl_3$, Me_4Si; *J*, Hz) 1.18 (3 H, d, *J* = 7.0), 1.19 (3 H, d, *J* = 7.5), 1.94 (1 H, qdq, *J* = 7.5, 2.8, 0.75), 1.96 (3 H, dd, *J* = 1.4, 0.75), 2.23 (1 H, qdq, *J* = 7.0, 2.8, 1.4), 5.99 (1 H, br s); ^{13}C NMR (δ, $CDCl_3$, Me_4Si) 11.77, 14.82, 17.61, 42.63, 46.60, 146.07, 147.24, 204.40; MS, Table I.

cis-2-Hydroxy-3,4,5-trimethyl-2-cyclopenten-1-one (**6**): IR (ν_{max}, cm^{-1}; neat) 3300, 2960, 2900, 2850, 1750, 1695, 1645, 1445, 1405, 1355, 1205, 1150, 1090, 1040, 1010, 970, 940, 760; ^1H NMR (δ, $CDCl_3$, Me_4Si, *J*, Hz) 1.08 (3 H, d, *J* = 7.3), 1.10 (3 H, d, *J* = 7.7), 1.96 (3 H, dd, *J* = 1.2, 0.8), 2.55 (1 H, qdq, *J* = 7.7, 6.2, 0.8), 2.79 (1 H, qdq, *J* = 7.3, 6.2, 1.2), 5.49 (1 H, br s); ^{13}C NMR (δ, $CDCl_3$, Me_4Si) 11.21, 12.13, 14.68, 37.44, 41.46, 146.82, 147.29, 204.81; MS, Table I.

Synthesis of 3-Ethyl-2-hydroxy-5-methyl- and 5-Ethyl-2-hydroxy-3-methyl-2-cyclopenten-1-one (10 and 12). These compounds were prepared by the same methods used to prepare **5** and **6** (Tonari et al., 1970).

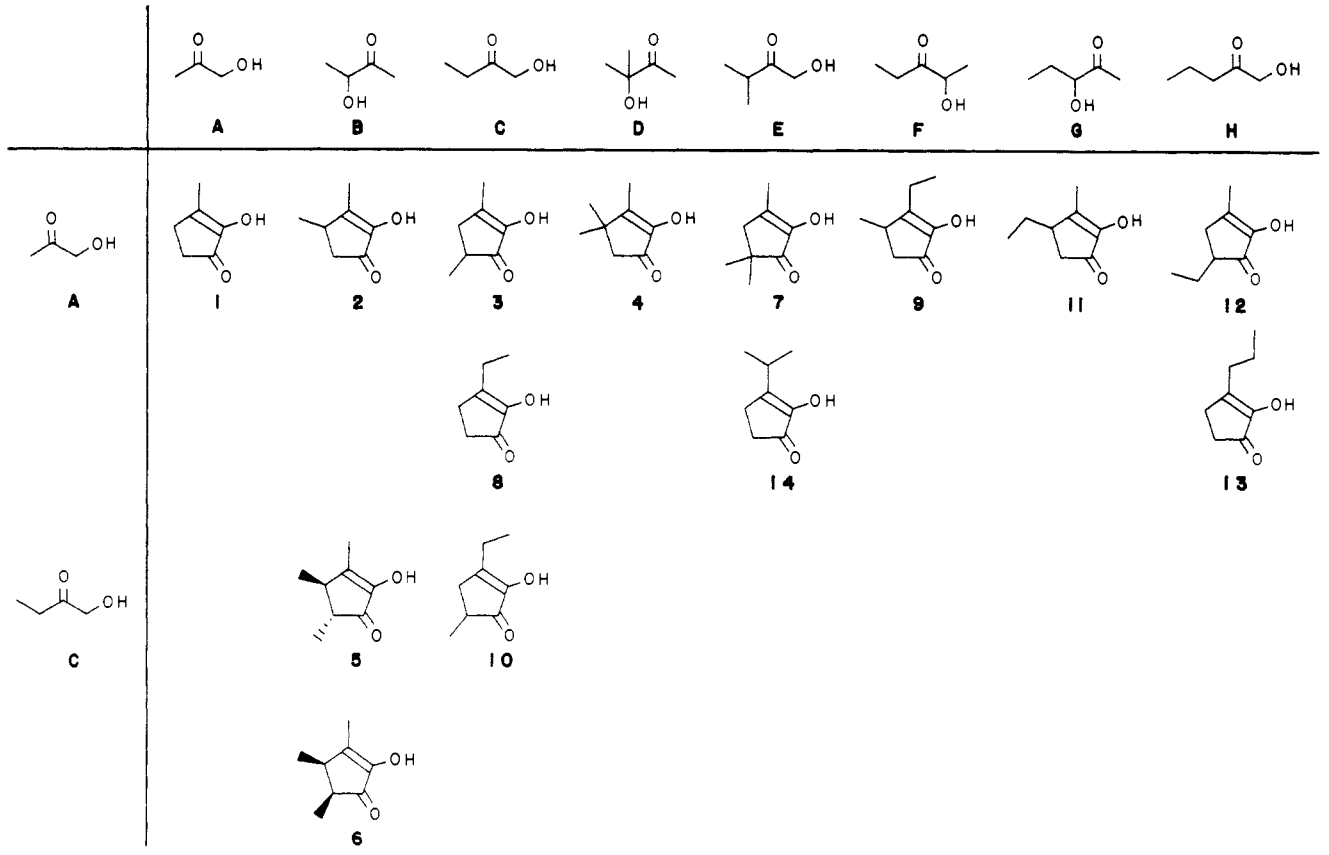
Synthesis of 2-Hydroxy-3,4,4-trimethyl-, 3-Ethyl-2-hydroxy-4-methyl-, 2-Hydroxy-3-propyl-, and 2-Hydroxy-3-isopropyl-2-cyclopenten-1-one (4, 9, 13, and 14). The procedure used for the synthesis of these compounds was the same as that previously described (Ito et al., 1976).

Synthesis of 2-Hydroxy-3,5,5-trimethyl-2-cyclopenten-1-one (7). The procedure used for the synthesis of this compound was the same as that previously described (Naoshima et al., 1974).

Degradation of Sucrose. A mixture of sucrose (14 g, 0.04 mol) and an aqueous solution (3 mL) containing sodium hydroxide (1.7 g, 0.04 mol) was heated at 115–160 °C for 30 min. To this solution was added water (100 mL). The resulting mixture was acidified (pH 5) with 6 N hydrochloric acid solution and then extracted with 25 mL of ethyl acetate. The ethyl acetate extracts were dried over anhydrous $MgSO_4$ and concentrated with a Kuderna–Danish evaporative concentrator to 0.2 mL. The sample was subjected to GC/MS analysis.

Instruments. *Gas Chromatography (GC).* A Hewlett-Packard Model 5710A gas chromatograph (carrier gas N_2) with a FID was used. Two types of wall-coated open tubular (WCOT) fused silica capillary columns prepared in our laboratory were used in the experiments: 50 m × 0.23 mm (i.d.) coated with Carbowax 20M and 50 m × 0.23 mm (i.d.) coated with OV-101. The column temperature was programmed from 80 to 200 °C at a rate of 2 °C/min, and the flow rate of the carrier gas was 0.67 mL/min. Preparative gas chromatography was performed on a Hitachi 5201 gas chromatograph equipped with a thermal conductivity detector and a glass column (2-m length × 4-mm i.d.) packed with 5% Carbowax 20M on Chromosorb WAW DMCS (60/80). The oven temperature was programmed from 170 to 200 °C at 5 °C/min.

Chart I



GC/MS Conditions. A Hitachi Model M-80 mass spectrometer was used under the following conditions: ionizing voltage, 70 eV; accelerating voltage, 3100 V; ion source temperature, 200 °C; carrier gas, helium. The gas chromatographic column and oven conditions were as described for the Hewlett-Packard gas chromatograph. Identification of all the peaks was made by comparison of their mass spectra and Kovats indices to those of authentic compounds.

IR and NMR Spectral Analysis. Infrared spectra were recorded on either a Jasco IR-S or a Hewlett-Packard 5965A GC/FT-IR system. NMR spectra were measured in $CDCl_3$ with a Bruker AM-400 with tetramethylsilane as the internal standard.

Sensory Evaluation. Odor thresholds (T) for the 2-hydroxy-2-cyclopenten-1-ones were determined by the 2/5 test employed by Amoore (1970). The odor description for each compound was carried out at the level of 1 ppm in water.

RESULTS AND DISCUSSION

Fourteen 2-hydroxy-2-cyclopenten-1-ones detected in roasted coffee, their retention indices on OV-101 (I^{ov}) and Carbowax 20M (I^{cw}), mass spectral data, and odor descriptions are presented in Table I. Ten of these compounds are being reported for the first time in the aroma of roasted coffee. Seven compounds were newly identified as naturally occurring flavor compounds. **5** and **6** are new compounds. 2-Hydroxy-3,4,5-trimethyl-2-cyclopenten-1-ones (**5** and **6**; **5**/**6** = 11/1) were prepared from 2-hydroxy-3,4-dimethyl-2-cyclopenten-1-one by the reduction of their Mannich derivatives (Tonari et al., 1970). The ^1H NMR spectrum of **5** and **6** showed that the coupling constant values for $J_{4,5}$ were 2.8 and 6.2 Hz for **5** and **6**, respectively. These values established that **5** and **6** were therefore assigned the trans and cis configurations, respectively. In order to confirm their relative stereochemistry, the investigation of the steric compression effect of the chemical shift in the ^{13}C NMR spectra was carried out. The signals of the two methyl carbons attached at the alkyl carbons of **6** (δ 12.13, 14.68) indicated a higher magnetic field compared to those of **5** (δ 14.82, 17.61). These differences were considered to be due to the steric compression effect caused by the cis form of **6**.

Sensory evaluations showed the odor thresholds of **5** and **6** to be 0.4 and 0.015 ppm in water, respectively. Regarding the odor thresholds of **1** (0.3 ppm) and **2** (0.02 ppm), the threshold value of **6** is nearly equal to that of **2**. **5** has a sweet and maple-like odor. On the other hand, **6** possesses a sweet and milky odor.

Shaw et al. (1968) suggested that combinations of the hydroxy ketones through base-catalyzed condensation could lead to 2-hydroxy-2-cyclopenten-1-ones. The eight hydroxy ketones **A**–**H** can lead to all fourteen 2-hydroxy-2-cyclopenten-1-ones **1**–**14**, as illustrated in Chart I. For example, 2-hydroxy-3,5,5-trimethyl- and 2-hydroxy-3-isopropyl-2-cyclopenten-1-one (**7** and **14**) would be obtained by the condensation of 1-hydroxypropan-2-one (**A**) and 1-hydroxy-3-methylbutan-2-one (**E**). Among the previously mentioned eight hydroxy ketones, **A**, 3-hydroxybutan-2-one (**B**), 1-hydroxybutan-2-one (**C**), 2-hydroxypentan-3-one (**F**), and 3-hydroxypentan-2-one (**G**) have been identified in roasted coffee aroma (Walter and Weidemann, 1969). Sucrose is a major component in green coffee and is quickly destroyed during roasting (Feldman et al., 1969). When sucrose was heated with alkali in aqueous solution, 2-hydroxy-2-cyclopenten-1-ones **1**–**14** were identified by GC/MS. This model study suggests that sucrose is a precursor for 2-hydroxy-2-cyclopenten-1-ones in coffee.

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Two-Dimensional GC-DCCC Analysis of the Glycoconjugates of Monoterpenes, Norisoprenoids, and Shikimate-Derived Metabolites from Riesling Wine

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Two-dimensional mapping of glycoconjugates from Riesling wine was achieved by using droplet countercurrent chromatography to separate the glycosides followed by enzymic hydrolysis and GC–MS to characterize the volatile aglycons. This technique has allowed observation of 27 monoterpenes, 20 shikimate metabolites, and 40 norisoprenoids, with compounds in all classes apparently conjugated with mono- and disaccharides. The presence of conjugates more polar than disaccharides was also evident. New wine aglycons identified in this work include the uroterpenols (*p*-menth-1-ene-8,9-diols), four isomeric 3,4-dihydro-3-hydroxyactinidols, 9-hydroxymegastigma-5,7-dien-4-one, 9-hydroxymegastigm-5-en-4-one, and (tentatively) 4-hydroxy-3-methoxyphenylacetic acid ethyl ester, 2-(4-hydroxy-3-methoxyphenyl)ethyl acetate, and 8,9-dehydrotheaspirone. The relative abundance of norisoprenoid glycosides emphasizes the need to further study these compounds in relation to wine aroma.

Recent studies in these laboratories have shown that volatile secondary metabolites of grapes, including mevalonate- and shikimate-derived compounds, accumulate in these fruits as nonvolatile conjugates (Williams et al., 1989). The work also demonstrated the sensory significance of compounds released from the conjugates and indicated the benefits in analyzing these flavor precursors for determining varietal differences. In an analogous approach to this last aspect, Versini et al. (1988) have also attempted to relate bound monoterpenes and norisoprenoids to varietal and clonal differences among grapes.

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