Systematic Studies on Structure and Physiological Activity of Cyclic α-Keto Enamines, a Novel Class of "Cooling" Compounds

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3-Methyl- and 5-methyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one were recently identified as intense cooling compounds in roasted dark malt. To gain more insights into the molecular requirements of these compounds for imparting a cooling sensation, 26 cyclic α-keto enamine derivatives were synthesized, and their physiological cooling activities were evaluated. Any modification of the amino moiety, the carbocyclic ring size, or incorporation of additional methyl groups led to a significant increase of the cooling threshold. Insertion of an oxygen atom into the 2-cyclopenten-1-one ring, however, increased the cooling activity, e.g., the cooling threshold of the 5-methyl-4-(1-pyrrolidinyl)-3(2*H*)-furanone was found to be 16-fold below the threshold concentration determined for the 3-methyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one. Shifting the oxygen atom from the 4- into the 5-position of the cyclopentenone ring resulted in a even more drastic increase in cooling activity, e.g., the 4-methyl-3-(1-pyrrolidinyl)-2(5*H*)-furanone exhibited the strongest cooling effect at the low oral threshold concentration of 0.02−0.06 mmol/L, which is 35-fold below the value determined for (−)-menthol. In contrast to the minty smelling (−)-menthol, most of the α-keto enamines were found to be virtually odorless but impart a sensation of "cooling" to the oral cavity as well as to the skin, thus illustrating that there is no physiological link between cooling activity and mint-like odors.

Keywords: *Cooling; malt; Maillard reaction; taste; keto enamines; 4-methyl-3-(1-pyrrolidinyl)-2(5H)-furanone, 5-methyl-4-(1-pyrrolidinyl)-3(2H)-furanone*

INTRODUCTION

(−)-Menthol (**1**) (Figure 1) imparts a "cooling" effect to the skin and the oral cavity and has long been added to food products such as chewing gums or mint sweets, as well as to cigarettes, to give a sensation of "freshness" during consumption. This terpene is added also to pharmaceuticals to alleviate the sensations of inflammation and itch. Furthermore, such cooling agents are used in cosmetic products to satisfy the demand for creating a feeling of "freshness" and "coolness" on the skin.

The sensation of cold on the skin and mucosal surfaces is neither caused by evaporation of the cooling agent, nor by vasodilation; that means there is not an actual temperature change inside the mouth but is due to a specific action on sensory nerve endings (*1*). Physiological experiments showed that, when menthol was administered on a cat's lingual nerve, the cold fibers of that nerve are either provoked into firing or, if already firing, respond with a higher rate of firing (*2*). It is believed that menthol exerts its effect on cold receptors by interfering with the mobility of calcium ions across the cell membrane (*3*). Studies on the discharge activity of cat lingual cold receptors have shown that intravenous application of aqueous menthol solutions induced an increase in the electrical discharge from cold receptors (*4*). This was further strengthened by the fact that the effect of menthol on cold-receptor discharge was abolished by intravenous infusion of calcium ions, thus indicating that menthol causes receptor depolarization

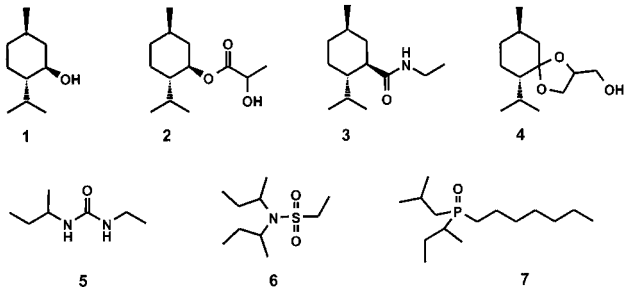

Figure 1. Structural diversity of "cooling" active compounds reported in the literature (*1*).

and increases nervous discharge by inhibiting the efflux of calcium from cold receptors (*4*). Since the neurophysiological bases of the cold response are essentially the same in man (*5*, *6*), it is believed that such coolants act by a common mechanism, which is essentially that of a drug−receptor interaction (*1*).

To gain more detailed insights into the molecular requirements of ligands interfering with these cold receptors, systematic studies on more than 1200 synthetic cooling compounds were performed between 1971 and 1976 aimed at correlating their structure and their physiological cooling activity (*1*). It was found that the compounds imparting a "cooling" effect spanned a range of different chemical classes, such as, e.g., from menthyl esters (e.g., **2**), carboxamides (e.g., **3**), mentane glycerol ketals (e.g., **4**), alkyl-substituted ureas (e.g., **5**), sulfonamides (e.g., **6**), and phosphine oxides (e.g., **7**). Although most of these compounds exhibited cooling activity, none of these have yet been identified in nature.

Very recently, application of the taste dilution analysis, a novel bioassay-guided screening procedure en-

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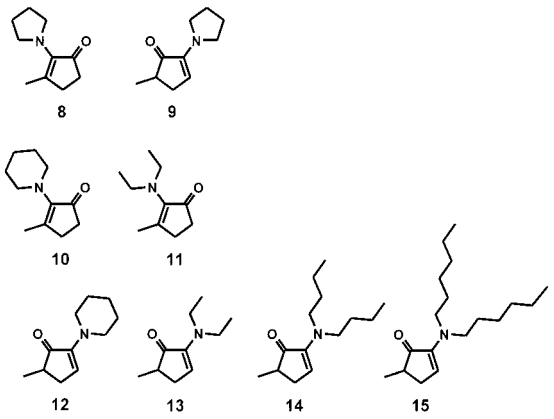

Figure 2. Synthetic α-keto enamines varying in the amino moiety.

abling the identification of taste-active compounds in foods (*7*), succeeded in discovering natural "cooling" compounds in roasted glucose/L-proline mixtures as well as in dark malt (*8*). Mass spectrometric and NMR studies on the structures of the compounds contributing the most to the cooling sensation, followed by synthesis led to their unequivocal identification as 3-methyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one (**8** in Figure 2) and 5-methyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one (**9** in Figure 2). Because these compounds belong to a novel class of cooling agents, it is as yet not known which molecular features of these cyclic α-keto enamines are required to exhibit a strong physiological cooling activity. The purpose of the present investigation was, therefore, to perform structure/activity studies on a series of synthetic α-keto enamines structurally related to the natural cooling agents **8** and **9**.

MATERIALS AND METHODS

Chemicals. The following compounds were obtained commercially: diethylamine, dibutylamine, dihexylamine, morpholine, piperidine, L-proline, L-proline methyl ester, pyrrolidine, acetic acid, diethyl oxalate, ethyl propionate, sodium ethoxide, (−)-menthol, 2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene), 3-hydroxy-2-cyclopenten-1-one, 3-hydroxy-2-methyl-2-cyclopenten-1-one, 2-hydroxy-2-cyclohexen-1-one, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (Sotolon), and 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (Aldrich, Steinheim, Germany). 2-Hydroxy-3-ethyl-2-cyclopenten-1-one, 2-hydroxy-3,5-dimethyl-2-cyclopenten-1-one, 2-hydroxy-3,5-dimethyl-2-cyclopenten-1-one, (*E*)-2-hydroxy-3,4-dimethyl-2-cyclopenten-1-one, 2-hydroxy-3-ethyl-2-cyclohexen-1-one, and 2-hydroxy-3,3,5-trimethyl-2-cyclohexen-1-one were gifts from the Nestle Research Center (Lausanne, Switzerland). Solvents were HPLC-grade (Aldrich, Steinheim, Germany). Deuterated solvents were obtained from Isocom (Landshut, Germany).

3-Methyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one, 5-methyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one, 2,5-dimethyl-4-(1-pyrrolidinyl)-3(2*H*)-furanone (*8*), and 4-hydroxy-5-methyl-3(2*H*)-furanone (*9*) were synthesized following the procedures reported in the literature.

Syntheses of Compounds 10−33 (Names and Structures Are Given in Table 1 and Figures 2−5). *Compounds **10**−**19**, **22**−**28**, **30**, and **33**.* A solution of the cyclic enolone (100 mmol) and the corresponding amino compound (400 mmol) given in Table 1 were refluxed in ethanol (600 mL) and acetic acid (400 mmol) for 3 h. After cooling the sample to room temperature, the solvent was removed in vacuo, the residue was taken up in water (300 mL), and the pH was adjusted to 9 with sodium hydroxide solution (30% in water). The solution was then extracted with diethyl ether (5 × 150 mL), the combined organic layers were washed with an aqueous solution of Na_2CO_3 (200 mL; 0.5 mol/L), dried over Na_2SO_4, and then freed from solvent in vacuo. The residual oil was dissolved in pentane/diethyl ether (8/2, v/v; 10 mL) and then applied onto a column (30 × 500 mm) filled with a slurry of Al_2O_3 (basic activity III−IV, Merck, Darmstadt, Germany) in pentane. Fractionation of the reaction products was performed by chromatography using pentane/diethyl ether mixtures with increasing diethyl ether contents as recently reported (*8*). The fractions collected were freed from solvent under vacuo affording the target compounds as colorless oils.

Spectroscopic data: 3-Methyl-2-(1-piperidinyl)-2-cyclopenten-1-one (**10**): HRMS: $C_{11}H_{17}ON$; MS (EI): 179 (89; $[M]^+$), 178 (54), 150 (100), 137 (21), 136 (39), 124 (30), 123 (34), 122 (52), 108 (53), 96 (24), 94 (29), 42 (28), 41 (58); ^{1}H NMR (360 MHz; $CDCl_3$, COSY, TOCSY): δ 1.50 (m, 2H, CH_2), 1.59 (m, 2 × 2H, CH_2), 2.06 (s, 3H, CH_3), 2.31 (m, 2H, CH_2), 2.42 (m, 2H, CH_2), 3.04 (m, 2 × 2H, CH_2);^{13}C NMR (360 MHz; $CDCl_3$; DEPT; HMQC; HMBC): δ 17.2 [CH_3], 24.2 [CH_2], 26.6 [2 × CH_2], 29.4 [CH_2], 33.7 [CH_2], 50.9 [2 × CH_2], 147.6 [C], 157.7 [C], 207.2 [CO].

3-Methyl-2-diethylamino-2-cyclopenten-1-one (**11**): HRMS: $C_{10}H_{17}ON$; MS (EI): 167 (42; $[M]^+$), 152 (100), 138 (43), 124 (53), 96 (34); ^{1}H NMR (360 MHz; $CDCl_3$, COSY, TOCSY): δ 0.94 (t, 2 × 3H, J = 7.1 Hz, CH_3), 2.04 (s, 3H, CH_3), 2.33 (m, 2H, CH_2), 2.49 (m, 2H, CH_2), 3.03 (q, 2 × 2H, J = 7.1 Hz, CH_2); ^{13}C NMR (360 MHz; $CDCl_3$; DEPT; HMQC; HMBC): δ 13.8 [2 × CH_3], 17.0 [CH_3], 29.5 [CH_2], 33.7 [CH_2], 46.3 [2 × CH_2], 145.6 [C], 166.2 [C], 208.2 [CO].

5-Methyl-2-(1-piperidinyl)-2-cyclopenten-1-one (**12**): HRMS: $C_{11}H_{17}ON$; MS (EI): 179 (100; $[M]^+$), 178 (38), 164 (59), 150 (74), 136 (100), 122 (52), 109 (47), 108 (43), 94 (25), 55 (24), 54 (25), 53 (23), 42 (28), 41 (74); ^{1}H NMR (360 MHz; $CDCl_3$, COSY, TOCSY): δ 1.16 (d, 3H, J = 7.5 Hz, CH_3), 1.53 (m, 2H, CH_2), 1.63 (m, 2 × 2H, CH_2), 2.07 (m, 1H, J = 3.1, 17.7 Hz, CH_2), 2.36 (m, 1H, CH), 2.75 (m, 1H, J = 3.5, 6.6, 17.7 Hz, CH_2), 3.01 (m, 2 × 2H, CH_2), 6.29 (m, 1H, J = 3.1 Hz, CH); ^{13}C NMR (360 MHz; $CDCl_3$; DEPT; HMQC; HMBC): δ 16.2 [CH_3], 23.2 [CH_2], 25.4 [2 × CH_2], 32,4 [CH_2], 40.1 [CH], 49.0 [2 × CH_2], 131.1 [CH], 150.4 [C], 207.6 [CO].

5-Methyl-2-diethylamino-2-cyclopenten-1-one (**13**): HRMS: $C_{10}H_{17}ON$; MS (EI): 167 (40; $[M]^+$), 152 (100), 138 (21), 124 (56), 82 (32), 54 (23); ^{1}H NMR (360 MHz; $CDCl_3$, COSY, TOCSY): δ 1.03 (t, 2 × 3H, J = 7.1 Hz, CH_3), 1.14 (d, 3H, J = 7.5 Hz, CH_3), 2.05 (m, 1H, J = 2.7, 17.7 Hz, CH_2), 2.34 (m, 1H, CH), 2.73 (m, 1H, J = 3.5, 6.6, 17.7 Hz, CH_2), 3.22 (m, 2 × 2H, CH_2), 6.03 (dd, 1H, J = 3.1 Hz, CH); ^{13}C NMR (360 MHz; $CDCl_3$; DEPT; HMQC; HMBC): δ 12.3 [2 × CH_3], 16.6 [CH_3], 32.5 [CH_2], 40.4 [CH], 43.2 [2 × CH_2], 126.6 [CH], 147.1 [C], 207.4 [CO].

5-Methyl-2-dibutylamino-2-cyclopenten-1-one (**14**): HRMS: $C_{14}H_{25}ON$; MS (EI): 223 (55; $[M]^+$), 181 (26), 180 (97), 139 (25), 138 (100), 124 (72), 82 (28), 68 (24), 55 (21), 41 (39); ^{1}H NMR (360 MHz; $CDCl_3$, COSY, TOCSY): δ 0.91 (t, 2 × 3H, J = 7.5 Hz, CH_3), 1.15 (d, 3H, J = 7.5 Hz, CH_3), 1.28 (m, 2 × 2H, CH_2), 1.44 (m, 2 × 2H, CH_2), 2.05 (m, 1H, J = 3.1, 17.7 Hz, CH_2), 2.34 (m, 1H, CH), 2.71 (m, 1H, J = 3.5, 6.6, 17.7 Hz, CH_2), 3.17 (m, 2 × 2H, CH_2), 5.95 (dd, 1H, J = 3.1, 3.5 Hz, CH); ^{13}C NMR (360 MHz; $CDCl_3$; DEPT; HMQC; HMBC): δ 13.7 [2 × CH_3], 16.2 [CH_3], 20.0 [2 × CH_2], 29.2 [2 × CH_2], 32.1 [CH_2], 40.2 [CH], 49.3 [2 × CH_2], 125.2 [CH], 147.0 [C], 207.2 [CO].

5-Methyl-2-dihexylamino-2-cyclopenten-1-one (**15**): HRMS: $C_{18}H_{33}ON$; MS (EI): 279 (39; $[M]^+$), 209 (42), 208 (100), 139 (37), 138 (100), 124 (63), 82 (24), 52 (28), 43 (53);^{1}H NMR (360 MHz; $CDCl_3$, COSY, TOCSY): δ 0.88 (t, 2 × 3H, J = 7.1 Hz, CH_3), 1.16 (d, 3H, J = 7.1 Hz, CH_3), 1.27 (m, 6 × 2H, CH_2), 1.43 (m, 4 × 2H, CH_2), 2.06 (m, 1H, J = 2.6, 17.7 Hz, CH_2), 2.36 (m, 1H, CH), 2.71 (m, 1H, J = 3.5, 6.6, 17.7 Hz, CH_2), 3.16 (m, 2 × 2H, CH_2), 5.95 (dd, 1H, J = 3.5 Hz, CH); ^{13}C NMR (360 MHz; $CDCl_3$; DEPT; HMQC; HMBC): δ 14.1 [2 × CH_3], 16.6 [CH_3], 22.7 [2 × CH_2], 26.9 [2 × CH_2], 27.3 [2 × CH_2], 31.8 [2 × CH_2], 32.4 [CH_2], 40.6 [CH_2], 49.9 [2 × CH_2], 125.8 [CH], 147.4 [C], 207.8 [CO].

5-Ethyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one (**16**): HRMS: $C_{11}H_{17}ON$; MS (EI): 179 (89; $[M]^+$), 178 (29), 164 (99),

Table 1. Synthetic α-Keto Enamine Derivative

cyclic enolone	amine	target compounds[a]	yield [%]
2-hydroxy-3-methyl-2-cyclopenten-1-one	pyrrolidine	3-methyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one (**8**)[b]	8
2-hydroxy-3-methyl-2-cyclopenten-1-one	pyrrolidine	5-methyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one (**9**)[b]	10
2-hydroxy-3-methyl-2-cyclopenten-1-one	piperidine	3-methyl-2-(1-piperidinyl)-2-cyclopenten-1-one (**10**)	7
2-hydroxy-3-methyl-2-cyclopenten-1-one	diethylamine	3-methyl-2-diethylamino-2-cyclopenten-1-one (**11**)	7
2-hydroxy-3-methyl-2-cyclopenten-1-one	piperidine	5-methyl-2-(1-piperidinyl)-2-cyclopenten-1-one (**12**)	8
2-hydroxy-3-methyl-2-cyclopenten-1-one	diethylamine	5-methyl-2-diethylamino-2-cyclopenten-1-one (**13**)	7
2-hydroxy-3-methyl-2-cyclopenten-1-one	dibutylamine	5-methyl-2-dibutylamino-2-cyclopenten-1-one (**14**)	10
2-hydroxy-3-methyl-2-cyclopenten-1-one	dihexylamine	5-methyl-2-dihexylamino-2-cyclopenten-1-one (**15**)	6
2-hydroxy-3-ethyl-2-cyclopenten-1-one	pyrrolidine	5-ethyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one (**16**)	25
(*E*)-2-hydroxy-3,4-dimethyl-2-cyclo-penten-1-one	pyrrolidine	(*E*)-4,5-dimethyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one (**17**)	18
2-hydroxy-3,5-dimethyl-2-cyclopenten-1-one	pyrrolidine	3,5-dimethyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one (**18**)	59
(*E*)-2-hydroxy-3,4-dimethyl-2-cyclo-penten-1-one	pyrrolidine	3,4-dimethyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one (**19**)	16
3-hydroxy-2-cyclo-penten-1-one	pyrrolidine	3-(1-pyrrolidinyl)-2-cyclopenten-1-one (**20**)	50
3-hydroxy-2-methyl-2-cyclopenten-1-one	pyrrolidine	2-methyl-3-(1-pyrrolidinyl)-2-cyclopenten-1-one (**21**)	5
2-hydroxy-2-cyclohexen-1-one	pyrrolidine	2-(1-pyrrolidinyl)-2-cyclohexen-1-one (**22**)	16
2-hydroxy-3-methyl-2-cyclohexen-1-one	pyrrolidine	3-methyl-2-(1-pyrrolidinyl)-2-cyclohexen-1-one (**23**)	6
2-hydroxy-3,5,5-trimethyl-2-cyclo-hexen-1-one	pyrrolidine	3,5,5-trimethyl-2-(1-pyrrolidinyl)-2-cyclohexen-1-one (**24**)	1
2-hydroxy-3-methyl-2-cyclohexen-1-one	pyrrolidine	6-methyl-2-(1-pyrrolidinyl)-2-cyclohexen-1-one (**25**)	10
2-hydroxy-3,5,5-trimethyl-2-cyclo-hexen-1-one	pyrrolidine	4,4,6-trimethyl-2-(1-pyrrolidinyl)-2-cyclohexen-1-one (**26**)	3
2-hydroxy-3-methyl-2-cyclopenten-1-one	morpholine	5-methyl-2-(1-morpholino)-2-cyclopenten-1-one (**27**)	18
2-hydroxy-3-methyl-2-cyclopenten-1-one	L-proline methylester	(*S*)−5-methyl-2-(2-methoxycarbonyl-1-pyrrolidinyl)-2-cyclopenten-1-one (**28**)	9
2-hydroxy-3-methyl-2-cyclopenten-1-one	L-proline	(*S*)−3-methyl-2-(2-carboxy-1-pyrrolidinyl)-2-cyclopenten-1-one (**29**)	7
4-hydroxy-5-methyl-3(2*H*)-furanone	pyrrolidine	5-methyl-4-(1-pyrrolidinyl)-3(2*H*)-furanone (**30**)	5
2,5-dimethyl-4-hydroxy-3(2*H*)-furanone	pyrrolidine	2,5-dimethyl-4-(1-pyrrolidinyl)-3(2*H*)-furanone (**31**)[b]	14
3-hydroxy-4-methyl-2(5*H*)-furanone	pyrrolidine	4-methyl-3-(1-pyrrolidinyl)-2(5*H*)-furanone (**32**)	14
4,5-dimethyl-3-hydroxy-2(5*H*)-furanone	pyrrolidine	4,5-dimethyl-3-(1-pyrrolidinyl)-2(5*H*)-furanone (**33**)	36

[a] Chemical structures and abbreviations are given in Figures 2−5. [b] Synthesized as reported earlier in the literature (*8*).

150 (31), 136 (100), 122 (35), 108 (40), 94 (32), 70 (20), 67 (20), 55 (20), 41 (28); ^{1}H NMR (360 MHz; $CDCl_3$, COSY, TOCSY): δ 0.93 (t, 3H, J = 7.5 Hz, CH_3), 1.37 (m, 1H, CH_2), 1.79 (m, 2 × 2H + 1H, 2 × CH_2 + CH_2), 2.14 (m, 1H, CH_2), 2.23 (m, 1H, CH), 2.63 (m, 1H, CH_2), 3.26 (m, 2 × 2H, CH_2), 5.79 (t, 1H, J = 7.5 Hz, CH); ^{13}C NMR (360 MHz; $CDCl_3$; DEPT, HMQC, HMBC): δ 11.3 [CH_3], 24.7 [CH_2], 25.0 [2 × CH_2], 30.1 [CH_2], 47.0 [CH], 48.3 [2 × CH_2], 123.3 [CH], 147.3 [C], 205.9 [CO].

(*E*)-4,5-Dimethyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one (**17**): HRMS: $C_{11}H_{17}ON$; MS (EI): 179 (82; $[M]^+$), 165 (20), 164 (100), 136 (93), 122 (21), 108 (31), 95 (21), 94 (26), 70 (38), 68 (25), 67 (25), 55 (23), 53 (23), 41 (34); ^{1}H NMR (360 MHz; $CDCl_3$, COSY, TOCSY): δ 1.14 (d, 3H, J = 6.6 Hz, CH_3), 1.16 (d, 3H, J = 7.1 Hz, CH_3), 1.83 (m, 2 × 2H, CH_2), 1.89 (m, 1H, CH), 2.35 (m, 1H, J = 7.1 Hz, CH), 3.20 (m, 2H, CH_2), 3.34 (m, 2H, CH_2), 5.70 (d, 1H, J = 3.1 Hz, CH); ^{13}C NMR (360 MHz; $CDCl_3$; DEPT, HMQC, HMBC): δ 14.3 [CH_3], 20.8 [CH_3], 24.9 [2 × CH_2], 39.6 [CH], 48.3 [2 × CH_2], 49.5 [CH], 128.8 [CH], 146.1 [C], 206.7 [CO].

3,5-Dimethyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one (**18**): HRMS: $C_{11}H_{17}ON$; MS (EI): 179 (100; $[M]^+$), 178 (45), 164 (24), 151 (28), 150 (32), 136 (70), 122 (21), 109 (31), 108 (33), 70 (27), 41 (32); ^{1}H NMR (360 MHz; $CDCl_3$, COSY, TOCSY): δ 1.14 (dd, 3H, J = 7.5 Hz, CH_3), 1.79 (m, 2 × 2H, CH_2), 2.02 (dd, 1H, J = 17.7 HZ, CH_2), 2.11 (s, 3H, CH_3), 2.30 (m, 1H, J = 7.1, 7.5 Hz, CH), 2.65 (dd, 1H, J = 7.1, 17.7 Hz, CH_2), 3.36 (m, 2H, CH_2), 3.52 (m, 2H, CH_2); ^{13}C NMR (360 MHz; $CDCl_3$; DEPT, HMQC, HMBC): δ 16.4 [CH_3], 17.4 [CH_3], 24.8 [2 × CH_2], 38.7 [CH_2], 39.2 [CH], 49.3 [2 × CH_2], 142.4 [C], 143.2 [C], 208.0 [CO].

3,4-Dimethyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one (**19**): HRMS: $C_{11}H_{17}ON$; MS (EI): 179 (45; $[M]^+$), 164 (60), 136 (95), 110 (30), 109 (22), 108 (21), 97 (53), 96 (33), 95 (24), 70 (100), 69 (41), 41 (33); ^{1}H NMR (360 MHz; $CDCl_3$, COSY, TOCSY): δ 1.13 (d, 3H, J = 6.6 Hz, CH_3), 1.79 (m, 2 × 2H, CH_2), 1.93 (m, 1H, CH), 2.07 (s, 3H, CH_3), 2.57 (dd, 2H, J = 2.7, 16.8 Hz, CH_2), 3.37 (m, 2H, CH_2), 3.47 (m, 2H, CH_2); ^{13}C NMR (360 MHz; $CDCl_3$; DEPT, HMQC, HMBC): δ 15.4 [CH_3], 20.2 [CH_3], 25.2 [2 × CH_2], 35.3 [CH], 43.0 [CH_2], 49.7 [2 × CH_2], 128.9 [C], 150.4 [C], 205.0 [CO].

2-(1-Pyrrolidinyl)-2-cyclohexen-1-one (**22**): HRMS: $C_{10}H_{15}ON$; MS (EI): 165 (92; $[M]^+$), 164 (71), 150 (59), 137 (51), 136 (100), 122 (41), 109 (54), 108 (66), 94 (31), 81 (54), 80 (40), 70 (58), 68 (22), 67 (37), 55 (33), 54 (48), 53 (23), 41 (49); ^{1}H NMR (360 MHz; $CDCl_3$; COSY) δ 1.84 (m, 2 × 2H, CH_2), 1.93 (m, J = 6.9 Hz, 2H, CH_2), 2.39 (m, 2H, CH_2), 2.47 (dd, J = 6.9 Hz, 2H, CH_2), 3.08 (m, 2 × 2H, CH_2), 5.64 (t, J = 4.7 Hz, 1H, CH); ^{13}C NMR (360 MHz; $CDCl_3$; HMQC, HMBC): δ 23.5 [CH_2], 24.4 [2 × CH_2], 25.4 [CH_2], 39.9 [CH_2], 49.5 [2 × CH_2], 119.5 [CH], 144.5 [C], 197.0 [CO].

3-Methyl-2-(1-pyrrolidinyl)-2-cyclohexen-1-one (**23**): HRMS: $C_{11}H_{17}ON$; MS (EI): 179 (100; $[M]^+$), 178 (37), 150 (91), 136 (90), 122 (24), 95 (20), 70 (68), 68 (23), 41 (38); ^{1}H NMR (360 MHz; $CDCl_3$, COSY, TOCSY): δ 1.85 (m, 2 × 2H, CH_2), 1.91 (m, 2H, CH_2), 1.98 (s, 3H, CH_3), 2.37 (m, 2 × 2H, CH_2), 2.94 (m, 2 × 2H, CH_2); ^{13}C NMR (360 MHz; $CDCl_3$; DEPT, HMQC, HMBC): δ 20.0 [CH_3], 22.3 [CH_2], 26.1 [2 × CH_2], 32.3 [CH_2], 39.8 [CH_2], 50.7 [2 × CH_2], 141.2 [C], 156.0 [C], 197.1 [CO].

3,5,5-Trimethyl-2-(1-pyrrolidinyl)-2-cyclohexen-1-one (**24**): HRMS: $C_{13}H_{21}ON$; MS (EI): 207 (100; $[M]^+$), 206 (25), 192 (20), 164 (35), 123 (79), 122 (77), 108 (46), 95 (36), 70 (49), 68 (25), 55 (20), 41 (38); ^{1}H NMR (360 MHz; $CDCl_3$, COSY, TOCSY): 1.02 (s, 2 × 3H, CH_3), 1.86 (m, 2 × 2H, CH_2), 1.96 (s, 3H, CH_3), 2.26 (bs, 2 × 2H, CH_2), 2.96 (m, 2 × 2H, CH_2); ^{13}C NMR (360 MHz; $CDCl_3$; DEPT, HMQC, HMBC): δ 20.2 [CH_3], 26.1 [2 × CH_2], 28.2 [2 × CH_3], 32.9 [C], 46.5 [CH_2], 50.8 [CH_2], 53.3 [2 × CH_2], 141.2 [C], 156.0 [C], 197.3 [CO].

6-Methyl-2-(1-pyrrolidinyl)-2-cyclohexen-1-one (**25**): HRMS: $C_{11}H_{17}ON$; MS (EI): 179 (100; $[M]^+$), 178 (39), 164 (88), 151 (28), 150 (84), 136 (48), 122 (26), 109 (21, 108 (37), 81 (30), 70 (46), 67 (20), 55 (20), 54 (33), 41 (38); ^{1}H NMR (360 MHz; $CDCl_3$, COSY, TOCSY): δ 1.14 (d, 3H, J = 6.6 Hz, CH_3), 1.68 (m, 1H, J = 13.3 Hz, CH_2), 1.84 (m, 2 × 2H, CH_2), 2.00 (m, 1H, J = 13.3 Hz, CH_2), 2.45 (m, 1H + 2H, CH + CH_2), 2.98 (m, 2H, CH_2), 3.14 (m, 2H, CH_2), 5.57 (dd, 1H, J = 4.4, 4.9 Hz, CH); ^{13}C NMR (360 MHz; $CDCl_3$; DEPT, HMQC, HMBC): δ 15.5 [CH_3], 24.4 [CH_2], 24.5 [2 × CH_2], 31.6 [CH_2], 42.9 [CH], 49.5 [2 × CH_2], 118.3 [CH], 144.1 [C], 199.9 [CO].

4,4,6-Trimethyl-2-(1-pyrrolidinyl)-2-cyclohexen-1-one (**26**): HRMS: $C_{13}H_{21}ON$; MS (EI): 207 (52; $[M]^+$), 193 (41), 192 (100), 164 (35), 136 (32), 70 (29), 55 (20), 41 (26);^{1}H NMR (360 MHz; $CDCl_3$, COSY, TOCSY): δ 1.11 (d, 3H, J = 6.6 Hz, CH_3), 1.12 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 1.62 (m, 1H, J = 13.3 Hz, CH_2), 1.75 (m, 1H, J = 1.8, 13.3 Hz, CH_2), 2.66 (m, 1H, J = 1.8, 6.6 Hz, CH), 2.91 (m, 2H, CH_2), 3.16 (m, 2H, CH_2), 5.22 (s, 1H, CH);^{13}C NMR (360 MHz; $CDCl_3$; DEPT, HMQC, HMBC): δ

15.5 [CH_3], 24.5 [2 × CH_2], 27.3 [CH_3], 32.5 [CH_2], 32.7 [CH_3], 38.7 [CH_2], 45.6 [CH], 49.4 [2 × CH_2], 128.5 [CH], 141.7 [C], 199.6 [CO].

5-Methyl-2-(1-morpholino)-2-cyclopenten-1-one (**27**): HRMS: $C_{10}H_{15}O_2N$; MS (EI): 181 (88; $[M]^+$), 163 (22), 150 (100), 138 (89), 137 (67), 124 (57), 123 (78), 122 (44), 108 (50), 96 (38), 95 (54), 94 (65), 81 (23), 80 (31), 68 (36), 67 (93), 55 (29), 54 (47), 53 (38), 41 (38); ^{1}H NMR (250 MHz; $CDCl_3$; COSY) δ 1.16 (d, J = 7.3 Hz, 3H, CH_3), 2.09 (m, J = 18.3, 2.4 Hz, 1H, CH_aH), 2.39 (m, J = 7.3, 2.4 Hz, 1H, CH), 2.77 (m, J = 18.3, 3.4 Hz, 1H, CH_bH), 2.99 (m, 2H, CH_2), 3.18 (m, 2H, CH_2), 3.77 (m, 2 × 2H, CH_2), 6.32 (dd, J = 3.4 Hz, 1H, CH); ^{13}C NMR (250 MHz; $CDCl_3$; HMQC, HMBC): δ 16.4 [CH_3], 32.5 [CH_2], 40.3 [CH], 48.3 [2 × CH_2], 66.5 [2 × CH_2], 131.6 [CH], 149.6 [C], 207.4 [CO].

(*S*)-5-Methyl-2-(2-methoxycarbonyl-1-pyrrolidinyl)-2-cyclopenten-1-one (**28**) (two diastereomers in ratio of 3:2): *dias. A:* HRMS: $C_{12}H_{17}O_3N$; MS (EI): 223 (43; $[M]^+$), 164 (100), 136 (34), 134 (23), 108 (25), 106 (22), 94 (31), 70 (36), 67 (28), 55 (30), 41 (30); ^{1}H NMR (360 MHz; $CDCl_3$, COSY, TOCSY): δ 1.14 (d, 3H, J = 2.2 Hz, CH_3), 1.92 (m, 2 × 2H, CH_2), 1.98−2.25 (m, 4 × 2H, CH_2), 2.75 (m, 2 × 2H, CH_2), 3.39 (m, 2 × 2H, CH_2), 3.71 (s, 3H, CH_3), 4.77 (dd, 1H, J = 2.7 + 8.8 Hz, CH), 5.88 (m, 2 × 2H, CH_2); ^{13}C NMR (360 MHz; $CDCl_3$; DEPT; HMQC; HMBC): δ 16.4 [CH_3], 22.7 [CH_2], 30.7 [CH_2], 32.50 [CH_2], 40.1 [CH_2], 49.4 [CH_2], 51.7 [CH_3], 59.6 [CH], 123.9 [C], 144.7 [C], 174.6 [COOMeth], 206.9 [CO]; *diast. B:* HRMS: $C_{12}H_{17}O_3N$; MS (EI): 223 (43; $[M]^+$), 164 (100), 136 (34), 134 (23), 108 (25), 106 (22), 94 (31), 70 (36), 67 (28), 55 (30), 41 (30); ^{1}H NMR (360 MHz; $CDCl_3$, COSY, TOCSY): δ 1.16 (d, 3H, J = 2.2 Hz, CH_3), 1.91 (m, 2 × 2H, CH_2), 1.98−2.25 (m, 4 × 2H, CH_2), 2.39 (m, 2 × 2H, CH_2), 3.24 (m, 2 × 2H, CH_2), 3.69 (s, 3H, CH_3), 4.77 (dd, 1H, J = 3.1, 8.8 Hz, CH), 5.88 (m, 2 × 2H, CH_2); ^{13}C NMR (360 MHz; $CDCl_3$; DEPT; HMQC; HMBC): δ 16.3 [CH_3], 22.6 [CH_2], 30.6 [CH_2], 32.53 [CH_2], 40.0 [CH_2], 49.3 [CH_2], 51.7 [CH_3], 59.1 [CH], 124.1 [C], 144.5 [C], 174.7 [COOMeth], 207.0 [CO].

5-Methyl-4-(1-pyrrolidinyl)-3(2*H*)-furanone (**30**): HRMS: $C_9H_{13}O_2N$; MS (EI): 42 (100), 167 (95), 54 (93), 96 (76), 124 (74); ^{1}H NMR (360 MHz; $CDCl_3$, COSY, TOCSY): 1.82 (m, 2 × 2H, CH_2), 2.23 (s, 3H, CH_3), 3.11 (m, 2 × 2H, CH_2), 4.38 (s, 2H, CH_2); ^{13}C NMR (360 MHz; $CDCl_3$): 14.2 (CH_3), 24.7 (2 × CH_2), 50.6 (2 × CH_2), 72.9 (CH_2), 126.3 (C), 183.0 (C), 198.9 (CO).

4,5-Dimethyl-3-(1-pyrrolidinyl)-2(5*H*)-furanone (**33**): HRMS: $C_{10}H_{15}O_2N$; MS (EI): 181 (82; $[M]^+$), 166 (76), 138 (28), 136 (49), 122 (100), 110 (74), 108 (93), 94 (37), 82 (36), 68 (26), 55 (43), 54 (26), 53 (24), 43 (31), 41 (44);^{1}H NMR (360 MHz; $CDCl_3$, COSY, TOCSY): δ 1.35 (d, 3H, J = 6.6 Hz, CH_3), 1.83 (m, 2 × 2H, CH_2), 2.03 (s, 3H, CH_3), 3.52 (m, 2 × 2H, CH_2), 4.69 (q, 1H, J = 6.6 Hz, CH);^{13}C NMR (360 MHz; $CDCl_3$; DEPT, HMQC, HMBC): δ 11.8 [CH_3], 19.2 [CH_3], 24.9 [2 × CH_2], 49.3 [2 × CH_2], 78.0 [CH], 128.7 [C], 130.5 [C], 170.4 [CO].

*Compounds **20** and **21***. A solution of 3-hydroxy-2-cyclopenten-1-one (100 mmol) or 3-hydroxy-2-methyl-2-cyclopenten-1-one (100 mmol), respectively, was refluxed in the presence of pyrrolidine (400 mmol), acetic acid (400 mmol), and ethanol (600 mL) for 4 h. After cooling the sample to room temperature, the solvent was removed in vacuo, the residue was taken up in water (300 mL), and the pH was adjusted to 14 with concentrated sodium hydroxide solution. The solution was then extracted with diethyl ether (5 × 150 mL) and the combined organic layers were freed from solvent in vacuo. The residual solid was recrystallized from diethyl ether, affording the target compounds as yellow crystals.

Spectroscopic data: 3-(1-Pyrrolidinyl)-2-cyclopenten-1-one (**20**): HRMS: $C_9H_{13}ON$; MS (EI): 151 (87; $[M]^+$), 122 (100), 108 (31), 95 (45), 94 (29), 67 (20), 53 (26); ^{1}H NMR (360 MHz; DMSO): δ 1.92 (m, 2 × 2H, CH_2), 2.16 (m, 2H, CH_2), 2.60 (m, 2H, CH_2), 3.19 (m, 2H, CH_2), 3.42 (m, 2H, CH_2), 4.70 (s, 1H, CH); ^{13}C NMR (360 MHz; DMSO; HMQC, HMBC): δ 25.9 [2 × CH_2], 28.0 [CH_2], 34.9 [CH_2], 47.9 [CH_2], 49.7 [CH_2], 98.6 [CH], 175.3 [C], 201.8 [CO].

2-Methyl-3-(1-pyrrolidinyl)-2-cyclopenten-1-one (**21**): HRMS: $C_{10}H_{15}ON$; MS (EI): 165 (100; $[M]^+$), 164 (20), 137 (55), 136 (68), 122 (59), 110 (37), 108 (41), 96 (73), 94 (35), 81 (26), 80 (25), 70 (33), 68 (28), 67 (54), 65 (26), 55 (30), 54 (36), 53 (45), 43 (23), 42 (31), 41 (83);^{1}H NMR (360 MHz; DMSO; COSY) δ 1.78 (s, 3H, CH_3), 1.86 (m, 2 × 2H, CH_2), 2.11 (m, 2H, CH_2), 2.52 (m, 2H, CH_2), 3.59 (m, 2 × 2H, CH_2);^{13}C NMR (360 MHz; DMSO; HMQC, HMBC): δ 9.4 [CH_3], 25.5 [2 × CH_2], 27.7 [CH_2], 33.1 [CH_2], 49.4 [2xCH_2], 105.9 [C], 170.8 [C], 201.7 [CO].

*Compound **29***. A mixture of 2-hydroxy-3-methyl-2-cyclopenten-1-one (50 mmol) and L-proline (80 mmol) was refluxed in ethanol (400 mL) for 4 h (Table 1). After cooling the sample to room temperature, the reaction mixture was taken up in water (500 mL), filtered, and the pH was adjusted to 9 by using an aqueous sodium hydroxide solution (1 mol/L). The solution was extracted with dichloromethane (5 × 200 mL), and the aqueous layer was freeze-dried, the residue was taken up in a mixture (15 mL; 5/95, v/v) of methanol and aqueous ammonium formate solution (pH 7.0; 0.1 mol/L) and, then, fractionated by flash chromatography on RP-18 material (15.0 g; Lichroprep 25−40 μm, Merck, Darmstadt, Germany) using the same solvent mixture as the mobile phase. After application of the crude material and chromatography with an eluent flow of 1.5 mL/min, the effluent of a peak detected at λ = 300 nm after 5 h was collected. After evaporation of the solvent and freeze-drying, the material was finally purified by RP-HPLC using the following solvent gradient: after isocratic chromatography with a mixture (10/90, v/v) of methanol and aqueous ammonium formate buffer (pH 7.0; 0.1 mol/L) for 10 min, the methanol content was increased to 100% within 10 min. Monitoring the effluent at 322 nm gave a peak at 9 min, which was collected in several runs. The combined eluates were freeze-dried yielding **29** (3.5 mmol; 7% in yield). LC/MS ($APCI^+$): 210 (100, $[M+1]^+$), 166 (45), 164 (39), 192 (10); ^{1}H NMR (360 MHz; $CDCl_3$, COSY, TOCSY): δ 1.80 (m, 2H, CH_2), 1.95 (s, 3H, CH_3), 2.01 (m, 2H, CH_2), 2.27 (m, 2H, CH_2), 2.34 (m, 2H, CH_2), 3.23 (m, 2H, CH_2), 4.6 (m, 1H, CH).

*Compound **32**. 3-Hydroxy-4-methyl-2(5H)-furanone.* Following a procedure reported in the literature (*10*), diethyl oxalate (1.03 mol) and ethyl propionate (1.03 mol) were added dropwise to a suspension of sodium ethoxide (1.00 mol) in diethyl ether (300 mL). After refluxing the sample for 3 h, the reaction mixture was cooled to room temperature, the solvent was removed in vacuo (45 mbar) and the residue was taken up in water (160 mL). Formaldehyde (1.0 mol, 35% in solution) was added in small portions while the mixture was cooled in a water bath. The reaction mixture was then heated for 1 h at 50 °C, the ethanol generated was removed in vacuo, the solution was then acidified with concentrated hydrochloric acid (225 mL), and, after addition of water (60 mL) and hydrochinone (100 mg), was refluxed for 4 h. The mixture was then cooled to room temperature, extracted with ethyl acetate (5 × 100 mL), and the combined organic layers were freed from solvent in vacuo. The residue was recrystallized from diethyl ether, affording the 3-hydroxy-4-methyl-2(5*H*)-furanone (38.8 g, 34% in yield) as white crystals. MS (EI): 114 (56; $[M]^+$), 86 (13), 69 (100), 58 (13), 57 (34), 55 (11), 41 (40); ^{1}H NMR (360 MHz; DMSO): δ 1.86 (t, 3H, J = 1.3 Hz, CH_3), 4.63 (q, 2H, J = 1.3 Hz, CH_2); ^{13}C NMR (360 MHz; DMSO): δ 9.5 [CH_3], 69.5 [CH_2], 128.9 [C], 137.0 [C], 169.9 [CO].

*Compound **32***. Following the procedure for the synthesis and purification of **33** as detailed above, **32** was prepared by refluxing 3-hydroxy-4-methyl-2(5*H*)-furanone (100 mmol), acetic acid (100 mmol) and pyrrolidine (100 mmol) in ethanol (225 mL) for 2.5 h. Spectroscopic data of **32**: HRMS: $C_9H_{13}O_2N$; MS (EI): 167 (94; $[M]^+$), 166 (63), 139 (58), 138 (45), 122 (93), 120 (43), 111 (54), 110 (46), 108 (32), 95 (26), 94 (100), 82 (25), 81 (24), 80 (23), 68 (67), 67 (21), 55 (36), 54 (27), 53 (23), 41 (58); ^{1}H NMR (360 MHz; $CDCl_3$, COSY, TOCSY): δ 1.83 (m, 2 × 2H, CH_2), 2.09 (s, 3H, CH_3), 3.52 (m, 2 × 2H, CH_2), 4.53 (s, 2H, CH_2); ^{13}C NMR (360 MHz; $CDCl_3$; DEPT, HMQC, HMBC): δ 12.4 [CH_3], 25.1 [2 × CH_2], 49.5 [2 × CH_2], 71.7 [CH_2], 124.3 [C], 130.7 [C], 171.6 [CO].

Identification of Compound 30 in Dark Malt. Dark malt (100 g, Caraffa special; Weihenstephan, Germany) was frozen in liquid nitrogen, ground in a mortar, and then extracted with dichloromethane (2 × 700 mL). The combined organic layers were concentrated to about 50 mL in vacuo (45 mbar) and the volatile components were isolated by SAFE distillation (*11*) in high-vacuum at 40 °C. The distillate obtained was concentrated to about 1 mL and then fractionated by column chromatography (0.9 × 100 mm) on Al_2O_3 (basic activity III−IV, Merck, Darmstadt, Germany), which was conditioned in pentane. Chromatography was performed using sequentially pentane (100 mL; fraction A), pentane/diethyl ether (8/2, v/v; 100 mL; fraction B), pentane/diethyl ether (5/5, v/v; 100 mL; fraction C), pentane/diethyl ether (2/8, v/v; 100 mL; fraction D), and diethyl ether (100 mL, fraction E). Fraction D was collected and analyzed by GC/MS. By comparison of the retention time as well as mass spectrum with those obtained for the synthetic reference compound, 3(2*H*)-MPF (4.4 μg/kg) could be identified in fraction D.

Sensory Analyses. *Training of the Sensory Panel.* Assessors were recruited from the German Research Center for Food Chemistry and were trained to evaluate odor and taste of aqueous solutions (5 mL each) of the following standard compounds by using a triangle test as described in the literature (*12*): saccharose (50 mmol/L) for sweet taste; lactic acid (20 mmol/L) for sour taste; NaCl (12 mmol/L) for salty taste; caffeine (1 mmol/L) for bitter taste; sodium glutamate (8 mmol/L, pH 5.7) for umami taste; tannin (gallustannic acid; 0.05%) for astringency; (−)-menthol (0.1%) for "cooling" sensation. Sensory analyses were performed in a sensory panel room at 22−25 °C on three different sessions.

Oral Cooling Thresholds and Odor Thresholds. Prior to sensory analysis, the purity of the synthetic compounds was proven by GC/MS as well as ^{1}H NMR spectroscopy. Cooling thresholds and retronasal odor thresholds were determined in a triangle test using tap water as the solvent (*8*). The samples (5 mL) were presented in order of increasing concentrations (serial 1:1 dilutions), and the threshold values evaluated in three different sessions were averaged. The values between individuals and separate sessions differed not more than one dilution step.

Topical Testing. An aliquot (0.2 mL) of solutions, containing between 0.0002 and 1.0% of the coolant in water, was applied to a circular area (about 10 cm^2) of the skin surface on the inside of a forearm, midway between the wrist and the elbow, and were rubbed for 30 s. In parallel, an aliquot (0.2 mL) of pure tap water was applied as the blank onto the skin of the other forearm. After 30 s, the skin was dried with a towel. A panel of 10 subjects (male and female) were asked to identify the arm where a "cooling" sensation was detectable, and furthermore, to rank perceived cooling intensity on a scale from 0 (no effect) to 5 (very strong). The values evaluated in three different sessions at 2 days were averaged. The values between individuals and separate sessions differed not more than two scores.

High-Resolution Gas Chromatography/Mass Spectrometry (HRGC/MS). HRGC was performed with a type 5160 gas chromatograph (Fisons Instruments, Mainz, Germany) using an SE-54 (30 m × 0.32 mm i.d. fused silica capillary, DB-5, 0.25 μm; J&W Scientific, Fisons, Mainz, Germany) with on-column injection at 40 °C. After 2 min, the temperature of the oven was raised at 40 °C/min to 50 °C and held for 2 min isothermally, and then raised at 6 °C/min to 230 °C, and held for 5 min. The flow of the carrier gas, helium, was 2.5 mL/min. MS analysis was performed with an MS 95 S (Finnigan, Bremen, Germany) in tandem with the HRGC. Mass chromatography in the electron-impact mode (MS/EI) was performed at 70 eV and in the chemical ionization mode (MS/CI) at 115 eV with isobutane as the reactant gas. High-resolution mass spectrometric analysis (HRMS) of synthetic compounds was performed with the MS 95 S spectrometer running in the electron impact mode by using perfluorokerosine as the reference.

Liquid Chromatography/Mass Spectrometry (LC/MS). An analytical HPLC column (Nucleosil 100-5C18, Macherey and Nagel, Dürren, Germany) was coupled to a LCQ-MS (Finnigan MAT GmbH, Bremen, Germany) using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). After injection of the sample (2−20 μL), analysis was performed using a gradient starting with a solution of aqueous ammonium formate (10 mmol/L; pH 8.2), and increasing the methanol content to 80% within 50 min, and then, to 100% within 5 min.

Nuclear Magnetic Resonance Spectroscopy (NMR). ^{1}H, ^{13}C, DEPT-135, DQF-COSY, HMQC, HMBC spectroscopy were performed on Bruker-AC-200 and Bruker-AM-360 spectrometers (Bruker, Rheinstetten, Germany) using the acquisition parameters previously described (*13*).

RESULTS AND DISCUSSION

The 2-amino-2-cyclopenten-1-ones **8** and **9** (Figure 2), recently identified as the most active cooling compounds in dark malt formed by Maillard reactions from glucose and L-proline, were found to be virtually odor- and tasteless but impart a long-lasting sensation of "cooling" and "freshness" to the oral cavity (*8*). To gain further insights into the molecular requirements of this novel class of cooling compounds for imparting a cooling sensation, a range of derivatives varying either in the amino moiety, the ring size, or the number and position of methyl groups as well as oxygen atoms were synthesized, and their physiological cooling activities were evaluated sensorially. The tongue was found to be very sensitive to the cooling sensation of **8** and **9** (*8*), being well in line with the observation that cooling compounds applied on the tongue appear to penetrate rapidly to the cold nerve receptors (*1*). For that reason, it is believed that the oral activities are a reasonable close approximation to the intrinsic activities of cooling compounds. Determining the oral thresholds of the cooling effect, therefore, appeared to give the best measure of intrinsic cooling activity.

Influence of Amino Moiety on Cooling Activity. In a first set of experiments, the pyrrolidine ring in compounds **8** and **9** was substituted with either piperidine, diethylamine, dibutylamine, or dihexylamine, respectively. To achieve this, a homologous series of six 2-amino-2-cyclopenten-1-ones was synthesized by a hydroxy/amine exchange from 2-hydroxy-3-methyl-2-cyclopenten-1-one and the corresponding secondary amine in the presence of acetic acid in ethanol. The yields of the reaction products, namely, 3-methyl-2-(1-piperidinyl)-2-cyclopenten-1-one (**10**), 5-methyl-2-(1-piperidinyl)-2-cyclopenten-1-one (**12**), 3-methyl-2-diethylamino-2-cyclopenten-1-one (**11**), 5-methyl-2-diethylamino-2-cyclopenten-1-one (**13**), 5-methyl-2-dibutylamino-2-cyclopenten-1-one (**14**), and 5-methyl-2-dihexylamino-2-cyclopenten-1-one (**15**), are given in Table 1; the corresponding structures are displayed in Figure 2. The compounds were purified by column chromatography and characterized by mass spectrometry as well as by 1D- and 2D-NMR experiments. Comparison of the NMR data of these derivatives with those reported recently for **8** and **9** (*8*), as expected, showed similar chemical shifts in the proton as well as in the carbon spectra for the cyclopentenone moiety but reflected the variations in the amino moiety. After the purity of each synthetic compound was proven by GC/MS as well as ^{1}H NMR spectroscopy, the cooling as well as the retronasal odor thresholds were determined by a trained sensory panel using triangle tests and tap water as the solvent. The results of the sensory evaluation demonstrated that enlargement of the pyrrolidine ring by one methylene

Table 2. Comparison of Cooling and Odor Thresholds of α-Keto Enamines[a]

compound	cooling threshold	odor threshold	odor quality	ratio (cool/ odor)
15				
20		234.0−390.0	odorless, bitter	
21		82.0−138.0	odorless, bitter	
26	2213.0−3688.0	553.0−922.0	mint-like	4.0
24	1605.0−2675.0	50.2−83.6	amine-like	32.0
22	495.0−830.0	62.0−106.0	rubber-like	8.0
29	490.0−735.0		odorless	<1
27	467.0−700.8	935.0−1558.0	rubber-like	0.5
28	112.0−188.0		odorless	<1
31	100.0−140.0	30.0−60.0	nutty, roasty	2.7
23	90.5−150.8	45.2−75.4	faintly mint-like	2.0
17	68.0−113.3	136.0−226.6	faintly mint-like	0.5
10	60.0−100.0	80.0−120.0	faintly amine-like	0.8
19	51.3−85.5	25.7−42.8	rubber-like	2.0
14	48.4−67.8	2.4−4.8	mint-like	16.0
18	32.5−53.5	16.0−27.0	rubber-like	2.0
11	30.0−50.0	2.0−3.0	carvone-like	16.0
8	29.0−43.5	43.5−72.5	faintly amine-like	0.8
25	26.9−44.8	3.4−5.6	rubber-like	8.0
16	26.7−42.5	13.4−22.4	faintly mint-like	2.0
12	16.0−24.0	12.0−20.0	faintly mint-like	2.7
13	12.0−20.0	6.0−9.0	curcuma-like	2.1
9	4.5−9.0	2.6−5.2	faintly mint-like	1.7
33	2.0−4.0	32.0−64.0	faintly mint-like	≪0.1
30	1.5−3.0		odorless	≪0.01
32	0.02−0.06		odorless	≪0.01
(−)-menthol	0.9−1.9	0.1−0.2	mint-like	9.5

[a] Cooling and retronasal odor threshold concentrations [mg/kg] were determined in tap water by using a triangle test (*8*).

group led to a significant increase of the cooling threshold, e.g., the cooling activity of **12** was lower by a factor of 3 than that found for **9** (Table 2). Opening of the pyrrolidine ring showed no drastic influence on the cooling threshold, e.g., **13** showed only a 2-fold higher threshold when compared to **9**. However, further increase of the aliphatic amino moiety by two methylene groups led to a drastic decrease of cooling activity, e.g., a 9 or 4 times lower cooling activity was determined for **14** when compared to **9** or **13**, respectively. Increasing the chain length of the amino moiety to six carbon atoms completely diminished the "cooling" perception, e.g., **15** did not impart any cooling effect but produced a burning sensation on the tongue and a tingle in the mouth (Table 2).

Influence of Number and Position of Alkyl Groups on Cooling Activity. To study how additional methyl groups or the substitution of a methyl group by an ethyl group are influencing the cooling activity of **8** and **9**, 5-ethyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one (**16**), (*E*)-4,5-dimethyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one (**17**), 3,5-dimethyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one (**18**), 3,4-dimethyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one (**19**), 3-(1-pyrrolidinyl)-2-cyclopent-1-one (**20**), and 2-methyl-3-(1-pyrrolidinyl)-2-cyclopent-1-one (**21**) were synthesized (Table 2; Figure 3). After structure confirmation (MS, NMR) and final purification, the target compounds were used for sensory analysis. Although the structures of the compounds **16** (*14*), **17** (*14*), **19** (*14*), **20** (*15*), and **21** (*16*) have been reported earlier, neither their ^{13}C NMR data are available in the literature, nor their cooling properties have been studied as yet. The

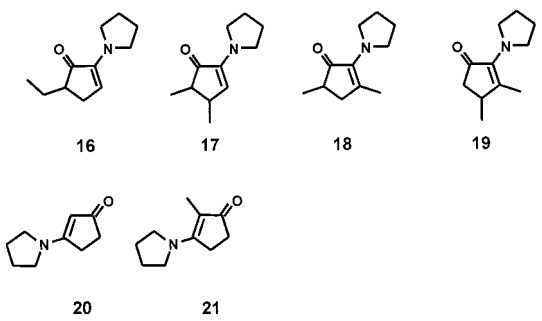

Figure 3. Synthetic α-keto enamines varying in the number and position of alkyl groups.

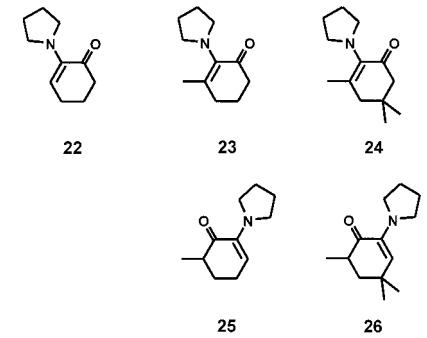

Figure 4. Synthetic α-keto enamines with six-membered carbocycle.

results showed that both the insertion of an additional methyl group, as well as the substitution of the methyl group by an ethyl group, led to a significant increase of their cooling activity, e.g., **17** or **16** were evaluated with 13- or 5-fold higher cooling thresholds when compared to **9** (Table 2). The position of the methyl groups influenced the cooling activities to a somewhat lower extent, e.g., **18** showed only by a 1.5 and 2 times lower cooling threshold than **19** and **17**, respectively. Changing the positions of the methyl group and the amino moiety in the molecule resulted in a total loss of cooling activity, e.g., the *β*-keto enamines **20** and **21** did not show any cooling effect (Table 2).

Influence of Ring Size on Cooling Activity. A third set of experiments were aimed at investigating the influence of the ring size of the carbocycle on the sensory attributes of the α-keto enamines. To achieve this, 2-(1-pyrrolidinyl)-2-cyclohexen-1-one (**22**), 3-methyl-2-(1-pyrrolidinyl)-2-cyclohexen-1-one (**23**), 3,5,5-trimethyl-2-(1-pyrrolidinyl)-2-cyclohexen-1-one (**24**), 6-methyl-2-(1-pyrrolidinyl)-2-cyclohexen-1-one (**25**), and 4,4,6-trimethyl-2-(1-pyrrolidinyl)-2-cyclohexen-1-one (**26**) were prepared and, after purification, the sensory data were compared with those found for the 2-amino-2-cyclopenten-1-ones (Table 2; Figure 4). Although the structure of compounds **22** has been reported earlier in the literature (*15*), neither its ^{13}C NMR data are as yet available nor its cooling property has been studied. The results showed that ring enlargement of the 2-cyclopenten-1-one led to a significant decrease in cooling activity, e.g., **25** and **23** were evaluated with 5- and 3-fold higher cooling thresholds as the corresponding cyclopentenones **9** and **8**, respectively (Table 2). Both subtraction of the methyl group as well as as insertion of additional methyl groups nearly quenched the cooling sensation, e.g., **22** and **26** showed 19- and 82-fold higher cooling thresholds when compared to **25**, respectively.

Influence of Number and Position of Oxygen Atoms on Cooling Activity. To study the role of

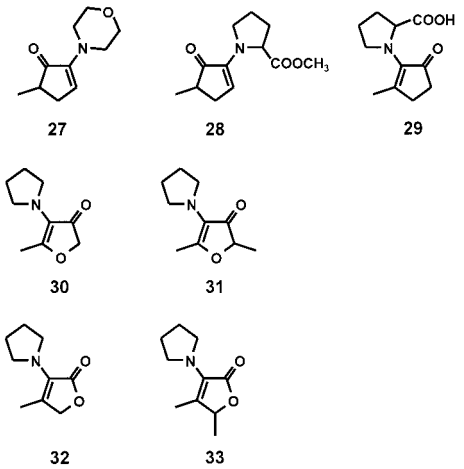

Figure 5. Synthetic α-keto enamines with additional oxygen in the amino moiety or the carbocycle, respectively.

additional oxygen atoms in the amino moiety, 3-methyl-2-(1-morpholino)-2-cyclopenten-1-one (**27**), 3-methyl-2-(2-carboxy-1-pyrrolidinyl)-2-cyclopenten-1-one (**29**), and 5-methyl-2-(2-methoxycarbonyl-1-pyrrolidinyl)-2-cyclopenten-1-one (**28**) were synthesized, purified, and sensorially evaluated (Table 2; Figure 5). Although the structure of compound **27** has been reported earlier, neither its ^{13}C NMR data, nor its cooling property has been studied so far. Only low cooling activities were determined for **29** and **28**, e.g., higher thresholds by factors of 17 or 90 were found when compared to **8** and **9**, respectively (Table 2). Also incorporation of the oxygen atom into a piperidine moiety nearly suppressed any cooling effect, e.g., **27** was evaluated with 29-fold higher threshold concentrations as the corresponding **12** (Table 2).

To evaluate how the cooling activity is influenced upon insertion of an oxygen atom into the 2-cyclopenten-1-one ring, in addition, 5-methyl-4-(1-pyrrolidinyl)-3(2*H*)-furanone (**30**) and 2,5-dimethyl-4-(1-pyrrolidinyl)-3(2*H*)-furanone (**31**) were synthesized from pyrrolidinium acetate and 5-methyl-4-hydroxy-3(2*H*)-furanone or 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, respectively (Table 1; Figure 5). Substitution of the methylene group at C(4) in **8** resulted in a strong increase in cooling activity, e.g., the threshold of **30** (1.5−3.0 ppm) is 16-fold below the threshold concentration determined for **8** and reaches nearly the cooling activity found for (−)-menthol (Table 2). But in contrast to minty smelling (−)-menthol, compound **30** did not show any odor activity. In addition, the identification of **30** (4.4 μg/kg) in dark malt verified its natural occurrence in thermally processed foods and demonstrated **30** as the most active, odorless cooling agent reported so far in nature. Adding a second methyl group to **30** led, however, to a significant decrease of the cooling activity, e.g., a 3- or 53-times higher cooling threshold was found for **31** when compared to **8** and **30**, respectively (Table 2).

Finally, the oxygen atom was shifted from the 4- into the 5-position of the cyclopentenone ring resulting in the γ-lactones **32** and **33** (Table 1; Figure 5). This change in the molecule led to the most drastic increase in cooling activity, e.g. compound **32** exhibited a strong cooling effect at the very low threshold concentration of 0.01−0.06 mmol/L (Table 2). On the basis of these systematic studies, the lactone **32** was found as the most potent cooling compound among these α-keto enamines, e.g. the threshold concentration of **32** is lower by a factor of 35 or 906 than that found for (−)-menthol or **8**, respectively.

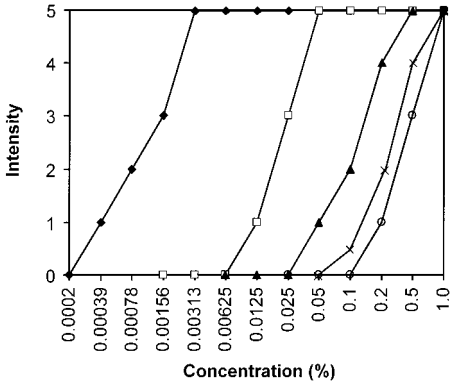

Figure 6. Topical testing of compounds **32** (−◆−), **33** (−□−), **30** (−▲−), **9** (−○−), and (−)-menthol (−×−) on the inside of the forearm. Cooling effect on skin was determined in tap water by using a topical test scoring the cooling intensity on a scale from 0 (no effect) to 5 (very strong).

Comparison of "Cooling" and Odor Thresholds. In comparison to (−)-menthol, most of these "cooling" agents were evaluated as odorless or showed only faint odors (Table 2). Calculating the ratio of cooling threshold to odor threshold revealed values below 0.1 for compounds **30**, **32**, and **33**, clearly demonstrating that these compounds might be used as cooling agents without exhibiting any odor. In comparison, for (−)-menthol the odor theshold is lower by a factor of 9.5 than the cooling threshold, thus indicating that it is not possible to evoke a "cooling" effect in a food product without having a significant mint odor. Other keto enamines also showed faint odors besides the cooling effect, e.g., the thresholds of the amine-like odor of **24**, the mint-like note of **14**, and the carvone-like aroma of **13** were found to be 32- or 16-fold below the threshold concentration required for "cooling" (Table 2). On the basis of these data, it might be concluded that, apart from the "cooling" effect, there is no common property of cooling compounds; for instance, there is no association between minty smell and "cooling".

Topical Cooling Activity. To answer the question whether these novel α-keto enamines also impart a cooling sensation to the skin, topical tests were performed using compounds **9**, **30**, **32**, and **33** as examples and (−)-menthol as the control. To achieve this, aliquots of aqueous solutions containing the cooling agent in increasing concentrations between 0.0002 and 1.0% were applied to a circular area of the skin surface on the inside of the forearm, midway between the wrist and the elbow, and were rubbed for 30 s. In parallel, an aliquot of pure tap water was applied as the blank onto the skin of the other forearm. The trained sensory panel was asked to rank the cooling intensity on a scale from 0 (no effect) to 5 (very strong). As given in Figure 6, all the compounds tested showed an significant cooling effect on the skin; however, they strongly differed in their cooling activities. Compound **32** showed by far the highest "cooling" activity and was already topically detectable in a solution containing 0.00039% of the lactone (Figure 6). Increasing the concentration of **32** intensified the "cooling" sensation on the skin reaching the maximum score of 5 at a concentration of 0.00156%. In comparison, all the other compounds tested in that concentration did not impart any "cooling" effect. Further increase of the concentration revealed compound **33** as the second effective coolant, which was topically

detected when applied in concentrations of ≥0.0125% (Figure 3). In comparison to these lactones, the 3(2*H*)-furanone **30** showed a significantly lower topical cooling activity, e.g., a significant cooling effect could be perceived in concentrations above 0.05% in water. Performing these topical experiments with (−)-menthol showed significantly lower effectivities for the terpene when compared to **30**, **32**, or **30**, e.g., the (−)-menthol imparted a cooling sensation in concentrations of ≥0.2%, which is above the topical detection threshold found for compound **32** by a factor of 512. Taking all these data into account, it might be concluded that, the lactones **32** and **33** in particular, showed, besides their strong oral cooling activities, also significantly higher topical cooling activities as well-known cooling agents such as, e.g., (−)-menthol.

The data obtained for these cyclic α-keto enamine derivatives clearly show that the "cooling" activity as well as the odor quality change with variations in the amino moiety as well as in the carbocyclic ring, thereby illustrating that cooling and odor thresholds cannot be predicted from chemical structures but have to be investigated based on systematic sensory studies with pure reference compounds. These investigations indicated that candidates of these cyclic α-keto enamine derivatives, in particular, **32**, **30**, **8**, and **9**, might be used to evoke certain cooling effects during consumption of nonmint food compositions such as drinking water, confectionary products, malted and citrus beverages, and fruity or browned flavors. In additon, these compounds might be used as active cosmetic ingredients in hair shampoos, shower gels, roll-on deodorants, refreshing body lotions, as well as in oral and dental care products, providing a pleasant "freshness" and a long-lasting "cooling" effect on the skin, without the need for employing alcohol.

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