JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Structural Analogues of Homoeriodictyol as Flavor Modifiers. Part III: Short Chain Gingerdione Derivatives

JAKOB P. LEY,* SUSANNE PAETZ, MARIA BLINGS, PETRA HOFFMANN-LÜCKE, HEINZ-JÜRGEN BERTRAM, AND GERHARD E. KRAMMER

Symrise GmbH & Co. KG, Flavor & Nutrition Research & Innovation, P.O. Box 1253, 37601 Holzminden, Germany

In order to find new flavor modifiers, various short chain gingerdione derivatives were synthesized as structural analogues of the known bitter masker homoeriodictyol and evaluated by a sensory panel for masking and sweetness enhancing activities. 1-(4-Hydroxy-3-methoxyphenyl)hexa-3,5-dione ([2]-gingerdione) and the homologue 1-(4-hydroxy-3-methoxyphenyl)hepta-3,5-dione ([3]-gingerdione) at concentration ranges 50−500 mg kg^{-1} showed the most promising masking activity of 20−30% against bitterness of a 500 mg kg^{-1} aqueous caffeine solution. Additionally, both compounds were able to reduce the bitterness of a 5 mg kg^{-1} quinine solution by about 20%; however, the bitter tastes of salicine, the model peptide H-Leu-Trp-OH, and KCl solutions were not reduced. Whereas for bitter masking activity a vanillyl moiety seems to be important, some of the tested isovanillyl isomers showed an interesting sweet enhancing effect without exhibiting a significant intrinsic sweetness. The isomer 1-(3-hydroxy-4-methoxyphenyl)hexa-3,5-dione ([2]-isogingerdione) at 100 mg kg^{-1} caused a significant and synergistic increase of 27% of sweet taste of a 5% sucrose solution.

KEYWORDS: Taste modifying; bitter masking; sweet enhancing; gingerdiones

INTRODUCTION

Masking bitter and other negative taste attributes as well as enhancing positive taste qualities such as sweetness is crucial for the food and beverage industry especially in improving the palatability of functional foods enriched with bitter tasting actives such as tea catechins (*1*) or reduced in calories, especially in sucrose or high fructose corn syrup. Several possibilities to counteract bitter taste are known: for example, removal of bitter actives, physical barriers (e.g., (micro)encapsulation, coatings, emulsions, suspensions) (*2*), using lipoproteins (*3*), proteins (*4*), polysaccharides such as pectins (*5*) or cyclodextrins (*6*) for scavenging the tastants, biotransformation of bitter molecules to nonbitter metabolites (e.g., naringin hydrolysis to naringenin (*7*)), using strong flavors or tastants (e.g., salt, sweeteners, acids) or congruent flavors (e.g., chocolate flavor, grapefruit flavor etc.) (*8*), and last but not least, bitter masking molecules, which are somewhat odorless and tasteless (*9*). In recent years some potent new bitter masking molecules were identified: AMP shows a well accepted masking effect against KCl bitterness in sodium reduced formulations (*10*), and a pyridinium glycinyl betaine was able to reduce the bitterness ratings of various concentrations of caffeine (250−2500 mg kg^{-1}) by about 3 units using a scale of 0 (no bitterness) to 5 (very strong) (*11*). Until now, maintaining the sweet taste of calorie reduced applications was mostly achieved by using high impact sweeteners such as saccharin, aspartam, or acesulfam and/or bulk calorie free sweeteners such as sorbitol or erythritol (*12*). But very recently, the use of sweet enahncers such as pyridinium alanyl betaine (*13*) or ethyl butyrate in subthreshold concentrations (*14*) was demonstrated. Both molecules can increase the perceived sweetness of a sugar containing application without exhibiting strong intrinsic sweetness.

Recently, we identified some hydroxybenzoic acid vanillylamides (*15*) and hydroxylated deoxybenzoins (*16*) as new bitter masking compounds related to the active flavanone homoeriodictyol (**1**), which was identified as the active principle of the long known bitter reducing extract from Herba Santa (*17*). Some of the new vanillylamides such as 2,4-dihydroxybenzoic acid *N*-vanillylamide (**2**) showed additionally sweet enhancing properties (*18*) and may be used in flavors to increase preference of low-carbohydrate applications.

Starting with the results from former structure−activity relationships (*17, 15*) we tried to simplify the homoeriodictyol structure and have investigated into the promising class of short chain gingerdiones and related molecules (**Figure 1**) to discover new taste modulators. Longer chain gingerdiones (1-(4-hydroxy-3-methoxyphenyl)-3,5-alkadiones) are minor constituents of the ginger rhizomes in addition to paradols (1-(4-hydroxy-3-methoxyphenyl)-3-alkanones), gingerols (1-(4-hydroxy-3-methoxyphenyl)-5-hydroxy-3-alkanones), and shogaols (1-(4-hydroxy-3-methoxyphenyl)-5*E*-alken-3-ones) (*19*). In dry ginger, [6]-,

* To whom correspondence should be addressed. Phone: + 49 5531 90 1883. Fax: + 49 5531 90 48883. E-mail: jakob.ley@symrise.com.

10.1021/jf8006536 CCC: $40.75
Published on Web 07/04/2008

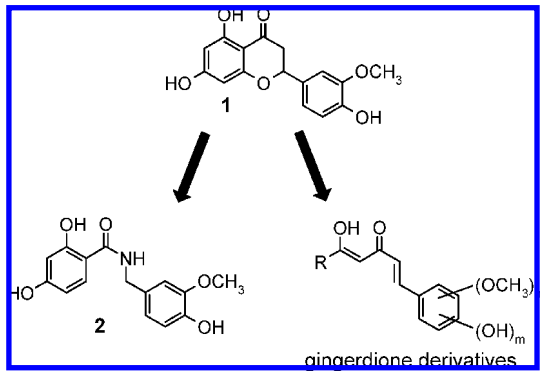

Figure 1. Possible simplifications and modifications of the homoeriodictyol structure.

[8]-, [10]-, and [12]-gingerdiones (*20*) and in fresh ginger [6]-, [8]-, and [10]-dehydrogingerdiones (1-(4-hydroxy-3-methoxyphenyl)-1-alkene-3,5-diones) were described (*21*). Only one short chain derivative, the [3]-dehydrogingerdione (**11**, **Figure 2**), was found in ginger as a trace component (*21*). The antioxidant hispolon 1-(3,4-dihydroxyphenyl)-1*E*-hex-1-en-3,5-dione (**13**, **Figure 2**) was identified in the fungus *Inonotus hispidus* (*22*), but it was never tested for taste effects. In our studies, we have focused on the short chain derivatives due to the intrinsic pungency of most of the longer chain ginger vanilloids. Several new and known relatives were synthesized and screened for their masking effects against caffeine and enhancing effects of sucrose sweetness.

MATERIALS AND METHODS

Dipeptide *N*-L-leucyl-L-tryptophane (H-Leu-Trp-OH) was obtained from Bachem (Weil am Rhein, Germany). Homoeriodictyol (**1**) was isolated as a sodium salt as described in ref (*17*), **3** was from ABCR (Karlsruhe, Germany, order no. TCH0996), **4** was from the Symrise library (prod. no. 113507), curcumin (**19**) was purchased from Sigma-Aldrich (Deisenhofen, Germany), and stevioside from Jenaer Pflanzenprodukte (Jena, Germany). [8]-Gingerol (**15**) and [10]-gingerol (**16**) were isolated in a purity >95% from a commercial ginger oleoresin (Naturex, Avignon, France) using common PHPLC techniques. All other chemicals were from Sigma-Aldrich, Acros (Schwerte, Germany), E. Merck (Darmstadt, Germany), or Lancaster Synthesis (Frankfurt, Germany). NMR spectra were recorded using a Varian VXR400S (^{1}H: 400 MHz) spectrometer (Varian, Darmstadt, Germany) at 25 °C using tetramethyl silane as the internal standard. LC-MS spectra were recorded using the LCQ HPLC system Finnigan MAT HP1100 (Finnigan MAT, Egelsbach, Germany; APCI, atmospheric pressure chemical ionization). Elemental analysis (combustion analysis for C, H, and O and AAS for sodium) was performed by Bayer Technology Services, Germany. Flash chromatography (FC) was performed on a Biotage Flash 40 system (Biotage AB, Uppsala, Sweden) using disposable prep-packed columns. Solvents were dried as needed by using an activated molecular sieve.

(1*E*)-1-(4-Hydroxy-3-methoxyphenyl)hex-1-ene-3,5-dione (5). After stirring boron trioxide (3.76 g) and acetyl acetone (7.58 g) at 100 °C for 1 h, the mixture was cooled to 0 °C. Tributyl borate (23.0 g) was dissolved in dry ethyl acetate (50 mL) and added. At 0 °C, first vanillin (3.76 g) dissolved in dry ethyl acetate (30 mL) and then butylamine (0.5 mL) in dry ethyl acetate (3 mL) were added dropwise. The mixture was slowly warmed to room temperature and stirred for 3 days. Hydrochloric acid (0.4 mol/L, 50 mL, 60 °C) was added, and the phases separated. The organic phase was washed with water, dried with Na_2SO_4, filtered, and evaporated in vacuo. The raw material (6.6 g) was purified by flash chromatography on silica gel 60 using the eluent *n*-hexane/ethyl acetate 1:1 (v/v). Yield: 2.97 g (51% related to vanillin) of yellow oil. HPLC-MS (RP-18-Phase, APCI +): m/z = 235.19 (100%, $[M + H]^+$). ^{1}H NMR (400 MHz, $CDCl_3$, internal standard TMS, *diketo tautomer): δ = 7.53 (1H, d, J = 15.8 Hz, H-1), 7.51* (ca. 5% H, d, J = 16.1 Hz, H-1*), 7,11* (ca. 5% H, ddd, J = 8.2 Hz, J = 2 Hz, J = 0.5 Hz), 7.09 (1H, ddd, J = 8.2 Hz, J = 1.9 Hz, J = 0.5 Hz, H-6′), 7.05* (5% H, d, J = 2 Hz), 7.02 (1H, d, J = 1.9 Hz, H-2′), 6.93* (5% H, d, J = 8.2 Hz), 6.92 (1H, d, J = 8.2 Hz, H-5′), 6.63* (ca. 5% H, d, J = 16.1 Hz), 6.32 (1H, d, J = 15.8 Hz, H-2), 5.91 (1H, bs, OH), 5.62 (1H, s, H-4), 3.94 (3H, s, O-CH_3), 2.16 (3H, s, H-6) ppm. ^{13}C NMR (100 MHz, $CDCl_3$ internal standard TMS): δ = 197.03 (C, C-5), 177.93 (C, C-3), 147.72 (C, C-3′ or C-4′), 146.79 (C, C-4′ or C-3′), 140.06 (CH, C-1), 127.67 (CH, C-1′), 122.65 (CH, C-6′), 120.32 (CH, C-2), 114.82 (CH, C-5′), 109.50 (CH, C-2′), 100.71 (CH, C-4), 55.95 (CH_3, O-CH_3), 26.58 (CH_3, C-6) ppm.

1-(4-Hydroxy-3-methoxyphenyl)hexa-3,5-dione (6). (1*E*)-1-(4-Hydroxy-3-methoxyphenyl)hex-1-ene-3,5-dione (**5**) (2.28 g) was mixed with palladium on activated carbon (10%, wet, 0.23 g) in ethanol (100 mL) and was hydrogenated at ambient pressure and room temperature with hydrogen for 2 h (410 mL H_2 uptake). The reaction mixture was filtered, and the filtrate was evaporated in vacuo. The raw material was purified using FC on silica gel 60 using the eluent *n*-hexane/ethyl acetate 2:1 (v/v). Yield: 1.16 g (50%) of colorless oil (slowly crystallizing over months). HPLC-MS (RP-18-Phase, ESI+): m/z = 236.92 (100%, $[M + H]^+$), 219.08 (94%, $[M - HO]^+$). HRMS: calcd. for $C_{13}H_{16}O_4$ 236.1049, found 236.1041. ^{1}H NMR (400 MHz, $CDCl_3$, internal standard TMS, *diketo tautomer): δ = 6.83 (1H, dd, J = 7.2 Hz, J = 0.8 Hz, H-5′), 6.82* (ca. 10% H, m, H-5′), 6.69 (1H, m, H-2′), 6.67 (1H, ddm, J = 7.2 Hz, J = 1.6 Hz, H-6′), 5.53 (1H, s, OH), 5.47 (1H, s, H-4), 3.88* (10% H, s, O−CH_3), 3.86 (3H, s, O−CH_3), 3.54* (10% 2H, s, H-4*), 2.86 (2H, dd, J = 8.4 Hz, J = 7.2 Hz), 2.82* (10% 2H, ddd, J = 6.8 Hz, J = 4.4 Hz, J ca. 1 Hz), 2.559* (10% 2H, m), 2.558 (2H, dd, J = 8.4 Hz, 8.4 Hz), 2.20* (10% 3H, s, H-6*), 2.03 (3H, s, H-6) ppm. ^{13}C NMR (100 MHz, CD_3OD, internal standard TMS): δ = 203.38* (C, C-3*), 193.27 (C, C-3), 191.18 (C, C-5), 146.42 (C, C-3′), 144.00 (C-4′), 132.62 (C-1′), 120.82 (C-6′), 114.38* (CH_2, C-5′*), 114.34 (CH_2, C-5′), 111.01* (CH_2, C-2′*), 110.96 (CH_2, C-2′), 100.13 (CH_2, C-4), 58.13* (CH_3, O-CH_3*), 55.88 (CH_3, O-CH_3), 45.53* (CH_2, C-4*), 40.40 (CH_2, C-2), 31.29 (CH_2, C-1), 29.15* (CH_2), 24.86 (CH_3, C-6) ppm.

(1*E*)-1-(3-Hydroxy-4-methoxyphenyl)hex-1-ene-3,5-dione (7). The dehydrogingerdione **7** was synthesized analogously to **5** using boron trioxide (7.0 g), acetyl acetone (14.3 g), tributyl borate (42.6 g), isovanillin (7.05 g). and butylamine (2 mL). The raw material was recrystallized from ethyl acetate. Yield: 4.95 g of yellow-orange crystals (purity >95%, LC-MS). For analytics, a small sample was purified with FC on silica gel 60 using the eluent *n*-hexane/ethyl acetate 1:1 (v/v) (99.7%, GC). HPLC-MS (RP-18-Phase, ESI+): m/z = 235.0 (100%, $[M + H]^+$), 177.4 (18%). HRMS: calcd. for $C_{13}H_{14}O_4$ 234.0892, found 234.0888. ^{1}H NMR (400 MHz, $CDCl_3$, internal standard TMS): δ = 7.51 (1H, d, J = 15.79 Hz, H-1), 7.14 (1H, d, J = 2.1 Hz, H-2′), 7.02 (1H, ddd, J = 8.3 Hz, J = 2.1 Hz, J = 0,4 Hz, H-6′), 6.85 (1H, d, J = 8.3 Hz, H-5′), 5.69 (1H, s, OH), 6.32 (1H, d, J = 15.8 Hz, H-2), 5.62 (1H, s, H-3), 3.92 (3H, s, O-CH_3), 2.16 (3H, s, H-6) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, internal standard TMS): δ = 197.30 (C, C-5), 177.67 (C, C-3), 148.31 (C, C-4′), 145.91 (C, C-3′), 139.72 (CH, C-1), 128.76 (CH, C-1′), 121.86 (CH, C-6′), 120.92 (CH, C-2), 112.67 (CH, C-5′), 110.89 (CH, C-2′), 100.89 (CH, C-4), 56.02 (CH_3, O-CH_3), 26.91 (CH_3, C-6) ppm.

1-(3-Hydroxy-4-methoxyphenyl)hexa-3,5-dione (8). The hydrogenation of **7** (2.34 g) to **8** was performed as described for [2]-gingerdione (**6**) (g). The crude reaction product (69%, GC) was purified with FC on silica gel 60 using the eluent *n*-hexane/ethyl acetate 2:1 (v/v). Yield: 1.0 g (42%, purity 99.0% GC) of colorless crystals. MS (EI): m/z = 236 ($M^{+\cdot}$, 43%), 150 (14%), 137 (100%), 85 (15%), 43 (17%), 28 (14%). HRMS: calcd. for $C_{13}H_{16}O_4$ 236.1049, found 236.1044. ^{1}H NMR (400 MHz, $CDCl_3$, internal standard TMS, *diketo tautomer): δ = 6.76 (1H, d, J = 8.2 Hz, H-5′), 6.77 (1H, d, J = 2.1 Hz, H-2′), 6.62 (1H, ddm, J = 8.2 Hz, J = 2.1 Hz, H-6′), 5.60 (1H, s, OH), 5.47 (1H, s, H-4), 3.861 (3H, s, O-CH_3), 3.856* (ca. 5% H, s, O-CH_3*), 2.85 (2H, m, H-1), 2.55 (2H, dd, J = 9 Hz, 9 Hz), 2.20* (ca. 5% 3H, H-6*, s), 2.04 (3H, s, H-6) ppm. ^{13}C NMR (100 MHz, CD_3OD, internal standard TMS): δ = 193.30 (C, C-3), 191.09 (C, C-5), 145.56 (C, C-3′), 145.03 (C, C-4′), 134.00 (C, C-1′), 119.68* (CH, C-6′*), 119.65 (CH, C-6′), 114.38* (CH_2, C-5′*), 114.34 (CH_2, C-5′), 110.75*

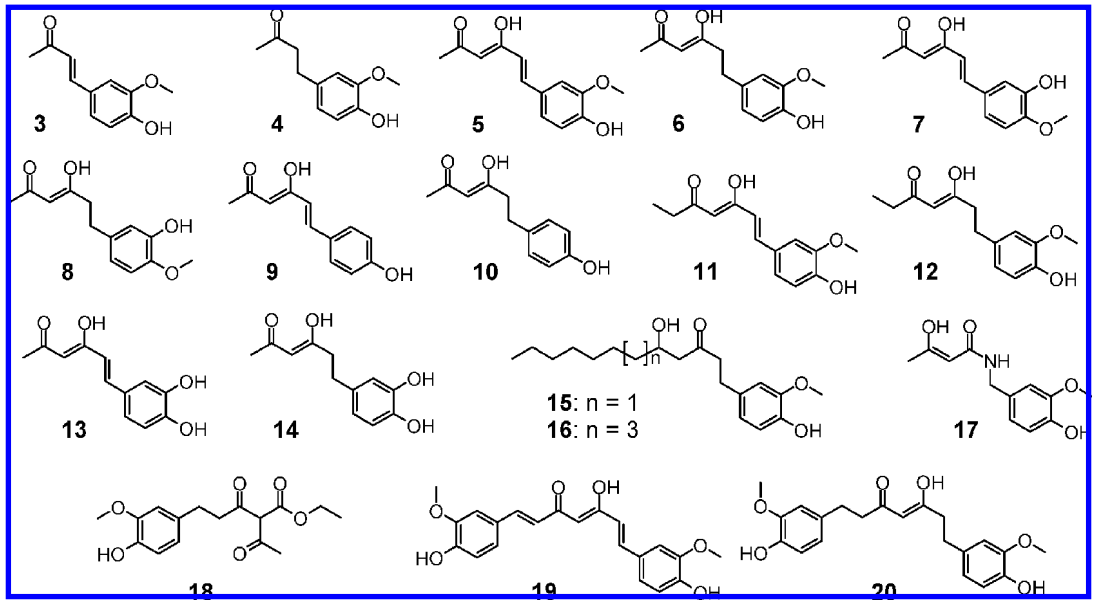

Figure 2. Synthesized and tested compounds related to gingerdiones.

(CH, C-2′*), 110.70 (CH, C-2′), 100.13 (CH, C-4), 58.7* (CH_3, O-CH_3*), 56.00 (CH_3, O-CH_3), 45.34* (CH_2, C-4*), 40.10 (CH_2, C-2), 30.89 (CH_2, C-1), 28.84* (CH_2), 24.85 (CH_3, C-6) ppm.

(1*E*)-1-(4-Hydroxyphenyl)hex-1-ene-3,5-dione (9). The dehydrogingerdione **9** was synthesized analogously to **5** using boron trioxide (13.9 g), acetyl acetone (28.4 g), tributyl borate (85.2 g), 4-hydroxybenzaldehyde (12.3 g), and butylamine (2 mL). The raw material was recrystallized from ethyl acetate. Yield: 2.5 g of yellow-orange crystals (purity >95%, LC-MS). MS (EI): m/z = 204 (100%, $M^{+\cdot}$), 186 (40%), 161 (75%), 147 (100%), 119 (32%), 91 (28%, 65 (20%), 43 (43%). HRMS: calcd. for $C_{12}H_{12}O_3$ 248.1049, found 248.1028. ^{1}H NMR (400 MHz, $CDCl_3$, internal standard TMS, *diketo tautomer): δ = 7.53 (2H, m, H-2′, H-6′), 7.49 (1H, d, J = 16 Hz, H-1), 6.81 (2H, m, H-3′, H-5′), 6.56* (13% H, d, J = 16.1 Hz, H-2*), 6.58 (1H, d, J = 16.0 Hz, H-2), 5.85 (1H, s, H-4), 2.18* (13% 3H, s, H-6*), 2.12 (3H, s, H-6) ppm. ^{13}C NMR (100 MHz, CD_3OD, internal standard TMS): δ = 196.53 (C, C-5), 178.19 (C, C-3), 159.56 (C, C-4′), 139.82 (CH, C-1), 130.57* (CH), 130.02 (2 × CH, C-2′, C-6′), 125.71 (C, C-1′), 119.31 (CH, C-2), 115.82* (CH), 115.76 (2 × CH, C-3′, C-5′), 100.39 (CH, C4), 26.29 (CH_3, C-6) ppm.

1-(4-Hydroxyphenyl)hexa-3,5-dione (10). The hydrogenation of **9** (2.00 g) to **10** was performed as described for [2]-gingerdione (**6**). The reaction product was purified with FC on silica gel 60 using the eluent *n*-hexane/ethyl acetate 2:1 (v/v). Yield: 0.5 g (25%) of colorless oil. MS (EI): m/z = 206 ($M^{+\cdot}$, 32%), 120 (23%), 107 (100%), 85 (21%), 77 (12%), 43 (17%). HRMS: calcd. for $C_{12}H_{14}O_3$ 206.0943, found 206.0933. ^{1}H NMR (400 MHz, $CDCl_3$, internal standard TMS, *diketo tautomer): δ = 7.02 (2H, d, J = 8.0 Hz, H-2′, 6′), 7.00* (20% 2H, d, 8.0 Hz, H-2′*, 6′*), 6.76 (2H, d, J = 8.0 Hz, H-3′, 5′), 6.74* (2H, m, H-3′*, 5′*), 5.48 (1H, s, H-4), 3.56* (20% 2H, s, H-4*), 2.85 (2H, m, H-1), 2.80* (2H, m, J = 7.1 Hz, J = 4.5 Hz, H-1*), 2.55 (2H, dd, J = 8.4 Hz, J = 7.2 Hz, H-2), 2.19* (1H, s, H-6*), 2.04 (3H, s, H-6), 1.1* (20% H, 3-OH*) ppm. ^{13}C NMR (100 MHz, CD_3OD, internal standard TMS): δ = 204.15* (C, C-3*), 203.08* (C, C-5*), 193.37 (C, C-3), 191.96 (C, C-5), 154.37* (C, C-4′*), 154.33 (C, C-4′), 132.32 (C, C-1′), 132.08* (C, C-1′*), 129.39* (2 × CH, C-2′*, 6′*), 129.36 (2 × CH, C-2′, 6′), 115.48* (2 × CH, C-3′*, 5′*), 115.41 (2 × CH, C-3′, 5′), 100.21 (CH, C-4), 57.98* (CH_2, C-4*), 45.51* (CH_2, C-2*), 40.20 (CH_2, C-2), 30.94* (CH_3, C-6*), 30.78 (CH_2, C-1), 28.58* (CH_2, C-1*), 24.97 (CH_3, C-6) ppm.

(1*E*)-1-(4-Hydroxy-3-methoxyphenyl)hept-1-ene-3,5-dione (11). Vanillin (6.3 g, 42 mmol), 2,4-hexadione (14.1 g, 124 mmol) prepared according to Ohta et al. (*23*), and boron trioxide (11.5 g, 165 mmol) were mixed with *N,N*-dimethyl formamide (50 mL) and warmed to 90 °C. A solution of isobutylamine (0.041 g, 0.4 mmol) in *N,N*-dimethyl formamide (38 mL) was added dropwise for 2 h. After stirring at 90 °C for 1 h, 55 mL of the solvent was evaporated in vacuo, and water (250 mL) was added. The mixture was stirred at 60 °C for another hour and at room temperature for 12 h. The reaction mixture was extracted with ethyl acetate (200 mL), and the solvents were evaporated. The crude reaction product was suspended in ethyl acetate (50 mL) and stored for several days. The filtered solution (the precipitate contains mainly the curcumin derivative) was purified by FC on silica gel 60 using the eluent ethyl acetate/hexane (25 min 30:70 (v/v), then 10 min isocratic to 50:50). The isolated dehydrogingerdione-[3] (**11**) was recrystallized from ethyl acetate. Yield: 7 g (67%) of yellow crystals. MS (EI): m/z = 248 ($M^{+\cdot}$, 55%), 230 (28%), 219 (49%), 201 (50%), 191 (63%), 177 (100%), 159 (15%), 145 (50%). HRMS: calcd. for $C_{16}H_{14}O_4$ 248.1049, found 248.1028. ^{1}H NMR (400 MHz, $CDCl_3$, internal standard TMS): δ = 7.52 (1H, d, J = 15.7 Hz, H-1), 7.09 (1H, ddd, J = 8.2 Hz, J = 1.9 Hz, J = 0.5 Hz, H-6′), 7.02 (1H, d, J = 1.9 Hz, H-2′), 6.92 (1H, d, J = 8.2 Hz, H-5′), 6.34 (1H, d, J = 15.8 Hz, H-2), 5.86 (1H, bs, 4′-OH), 5.63 (1H, s, 3-OH), 3.94 (3H, s, OCH_3), 2.42 (2H, q, J = 7.5 Hz, H-6), 1.18 (3H, t, J = 7.5 Hz, H-7) ppm. ^{13}C NMR (100 MHz, CD_3OD, internal standard TMS): δ = 201.00 (C, C-5), 177.70 (C, C-3), 147.66 (C, C-4′), 146.78 (C, C-3′), 139.77 (CH, C-1), 127.75 (C, C-1′), 122.61 (CH, C-6′), 120.50 (CH, C-2), 114.81 (CH, C-5′), 109.47 (CH, C-2′), 99.55 (CH, C-2), 55.96 (CH_3, O-CH_3), 33.23 (CH_2, C-6), 9.45 (CH_3, C-7) ppm.

1-(4-Hydroxy-3-methoxyphenyl)hepta-3,5-dione (12). The hydrogenation of **11** (3.5 g) to **12** was performed as described for [2]-gingerdione (**6**). The reaction product was purified with FC on silica gel 60 using the eluent *n*-hexane/ethyl acetate 2:1 (v/v). Yield: 2.9 g (83%) of colorless oil (GC DB1 RI 2002 96.6%). MS (EI): m/z = 250 ($M^{+\cdot}$, 30%), 150 (12%), 137 (100%), 99 (10%), 47 (8%). HRMS: calcd. for $C_{16}H_{14}O_4$ 248.1049, found 248.1028. ^{1}H NMR (400 MHz, $CDCl_3$, internal standard TMS, *diketo tautomer): δ = 6.85−6.81 (1H, m, H-6′), 6.69 (1H, m, H-5′), 6.69−6.64 (1H, m, H-2′), 5.48 (1H, s, H-4), 5.46* (33% 2H, s, H-4*), 3.88* (30% 3H, s, OCH_3*), 3.87 (3H, s, OCH_3), 3.54 (1H, 3-OH), 2.89−2.81 (2H, m, H-1), 2.57 (2H, m, H-2), 2.49* (30% 2H, q, J = 7.2 Hz, H-6*), 2.30 (2H, q, J = 7.6 Hz, H-6), 1.13 (3H, t, J = 7.5 Hz, H-7), 1.04 (30% 3H, t, J = 7.2 Hz, H-7*) ppm. ^{13}C NMR (100 MHz, CD_3OD, internal standard TMS, *diketo tautomer): δ = 204.62* (C, C-5*), 203.55* (C, C-3*), 195.38 (C, C-5), 192.99 (C, C-3), 146.44* (C, C-3′*), 146.39 (C, C-3*), 143.99* (C, C-4′*), 143.97 (C, C-4′), 132,68 (C, C-1′), 132.48* (C, C-1′*), 120.84 (CH, C-6′), 120.82* (CH, C-6′*), 114.34* (CH, C-5′*), 114.31 (CH, C-5′), 111.00* (CH, C-2′*), 110.95 (CH, C-2′), 98.75 (CH, C-4), 57.12* (CH_2, C-4*), 55.90* (CH_3, OCH_3*), 55.88 (CH_3, O-CH_3), 45.52* (CH_2, C-2*), 40.45 (CH_2, C-2), 37.13* (CH_2, C-1*), 31.45 (CH_2, C-6), 31.37 (CH_2, C-1), 29.16* (CH_2, C-1*), 9.65 (CH_3, C-7), 7.46* (CH_3, C-7*) ppm.

(1*E*)-1-(4-Hydroxy-3-methoxyphenyl)hex-1-ene-3,5-dione (13). In a flame dried apparatus, (1*E*)-1-(4-hydroxy-3-methoxyphenyl)hex-1-en-3,5-dione (**5**) (2.36 g, 10 mmol) was suspended in dichloromethane (20 mL) under nitrogen and cooled to −70 °C. To the solution, BBr_3 in dichloromethane (3.76 g, 15 mmol in 10 mL) was added via a syringe through a rubber septum, and the reaction mixture was maintained at −65 to −70 °C for 4.5 h and then warmed up during the next 16 h. To the solution, ice water (16 g) and ethyl acetate (50 mL) were added, washed with brine, dried over Na_2SO_4, filtered, and evaporated to dryness in vacuo. The raw hispolone (**13**) was purified with FC on silica gel 60 using the eluent *n*-hexane/ethyl acetate 7:3 (v/v). Yield: approximately 10 mg of yellow solid. HRMS: calcd. for $C_{12}H_{12}O_4$ 220.0736, found 220.0732. HPLC-MS (RP-18-Phase, APCI+): *m/z* = 220.97 (100%, $[M + H]^+$), 219.28 (37%). ^{1}H NMR (400 MHz, $CDCl_3$, internal standard TMS): δ = 8.34 (1H, bs, OH), 7.48 (1H, d, J = 15.9 Hz, H-1), 7.16 (1H, d, J = 2 Hz, H-2′), 7.04 (1H, ddd, J = 8.2 Hz, J = 2.1 Hz, J = 0.5 Hz, H-6′), 6.87 (1H, d, J = 8.2 Hz, H-5′), 6.47 (1H, d, J = 15.8 Hz, H-2), 5.78 (1H, s, H-4), 2.11 (3H, s, H-6) ppm. ^{13}C NMR (100 MHz, CD_3OD, internal standard TMS): δ = 197.78 (C, C-5), 179.36 (C, C-3), 148.57 (C, C-3′ or 4′), 146.38 (C, C-4′ or 3′), 140.91 (CH, C-1), 128.35 (C, C-1′), 122.42 (C-6′ or C-2), 120.72 (CH, C-2 or C-6′), 116.41 (CH, C-2′ or C-5′), 115.07 (CH, C-5′ or C-2′), 101.14 (CH, C-4), 26.60 (CH_3, C-6) ppm.

(1*E*)-1-(3,4-Diydroxyphenyl)hexa-3,5-dione (14). 1-(4-Hydroxy-3-methoxyphenyl)hexan-3,5-dione (**6**) (2.39 g, 10 mmol) was treated in the same way as described for hispolon (**13**). The raw dihydrohispolone (**14**) was purified with FC on silica gel 60 using the eluent *n*-hexane/ethyl acetate 7:3 (v/v). Yield: 0.81 g (36%) of off-white solid (GC DB1: >99%). MS (EI): *m/z* = 222 ($M^{+\cdot}$, 32%), 204 (2%), 164 (4%), 136 (29′%), 123 (100%′), 85 (39%), 43 (40%), 28 (79%). HRMS: calcd. for $C_{12}H_{14}O_4$ 222.0892, found 222.0891. ^{1}H NMR (400 MHz, $CDCl_3$, internal standard TMS, *diketo tautomer): δ = 6.77 (1H, d, J = 8.1 Hz, H-5′), 6.76* (20% 1H, d, J = 8.0 Hz, H-5′), 6.69 (1H, d, J = 2.0 Hz, H-2′), 6.67* (20% 1H, d, J = 2.0 Hz, H-2′), 6.59 (1H, dm, J = 8.0 Hz, 2.0 Hz, H-6′), 6.57* (20% 1H, dm, J = 8.1 Hz, J = 2.0 Hz, H-6′), 5.49 (1H, s, H-4), 3.55 (30% 1H, s, H-4), 2.83−2.77 (m, H-1 or H-2), 2.54 (2H, m, H-2 or H-1), 2.20* (20% 3H, m, J = 0.5 Hz, H-6), 2.04 (3H, s, H-6) ppm. ^{13}C NMR (100 MHz, CD_3OD, internal standard TMS, *diketo tautomer): δ = 193.72 (C, C-3), 191.71 (C, C-5), 143.77* (C, C-3′ or 4′), 143.73 (C, C-3′ or 4′), 142.19* (C, C-4′ or 3′), 142.10 (C, C-4′ or 3′), 133.42 (C, C-1′), 120.52 (CH, C-6′), 115.39* (C-2′ or C-5′), 115.34 (2 × CH, C-2′ and C-5′), 100.25 (CH, C-4), 57.97* (CH_2, C-4), 45.37* (CH_2, C-2), 40.16 (CH_2, C-2), 31.00* (CH_3, C-6), 30.94 (CH_2, C-1), 28.79* (CH_2, C-1), 24.89 (CH_3, C-6) ppm.

3-Oxobutyric acid *N*-(4-hydroxy-3-methoxyphenyl)methylamide (17). Vanillylamine hydrochloride (0.99 g, 5.2 mmol) was suspended in 1,4-dioxane (10 mL) and stirred with triethylamine (2 mL) for 2 h. Ethyl acetoacetate (2.01 g, 15.5 mmol) was dissolved in 1,4-dioxane (20 mL) and Chirazyme L2c2 (Boehringer Ingelheim, Germany) was added. Under nitrogen atmosphere, the vanillylamine solution was added portionwise (5 × 2 mL) to the ethyl acetoacetate solution at room temperature during 5 h and stirred for a further 12 h. The precipitate was removed by filtering and washing with dichloromethane, and the combined filtrates were evaporated in vacuo to dryness. The oily residue was recrystallized from ethyl acetate to yield 0.25 g (20%) of pale white crystals. HPLC-MS (RP-18-Phase, APCI+): *m/z* = 474.53 (22%, $[2M]^+$), 237.85 (100%, $[M]^+$). HRMS: calcd. for $C_{12}H_{15}O_4N$ 237.1001, found 237.1004. ^{1}H NMR (400 MHz, $CDCl_3$, internal standard TMS): δ = 7.20 (1H, bs, NH), 6.85 (1H, d, J = 8.0 Hz, H-5′), 6.82 (1H, d, J = 1.9 Hz, H-2′), 6.77 (1H, ddd, J = 8.0 Hz, J = 2.0 Hz, J = 0.6 Hz, H-6), 5.74 (1H, s, OH), 4.37 (2H, d, J = 5.7 Hz, H-7′), 3.88 (3H, s, OCH_3), 3.44 (2H, s, H-2), 2.26 (3H, m, J = 0.4 Hz) ppm. $_{13}$C NMR (100 MHz, CD_3OD, internal standard TMS): δ = 204.53 (C, C-3), 165.34 (C, C-1), 146.74 (C, C-3′), 145.13 (C, C-4′), 129.83 (C, C-1′), 120.76 (CH, C-6′), 114.45 (CH, C-5′), 110.62 (CH, C-2′), 55.96 (CH_3, O−CH_3), 49.63 (CH_2, C-2), 43.50 (CH_2, C-7′), 31.09 (CH_3, C-4) ppm.

Ethyl 2-Acetyl-5-(4-hydroxy-3-methoxyphenyl)-3-oxopentanoate (18). *O*-Benzylvanillin (72.9 g, 302 mmol) (**21**), malonic acid (62.7 g, 603 mmol), and piperidine (3 mL) in dry pyridine (165 mL) were warmed to 90 °C under nitrogen for 3 h and subsequently refluxed for 2 h. The hot reaction mixture was poured on ice/water (600 g) and stirred for 1 h. The *O*-benzylferulic acid (**22**) was filtered off, washed with water, and dried at 40 °C/1 mbar for 12 h. Yield: 83.8 g (294 mmol, 97%); LCMS (RP-18 phase, ESI+): *m/z* = 285 ($[M + H]^+$, 100%). The protected ferulic acid **22** (26.4 g, 93 mmol) was dissolved in toluene (300 mL), and at ambient temperature, thionylchloride (14.3 g, 120 mmol) was added. The mixture was stirred until dissolution for 6 h and subsequently warmed up to 50 °C for 1 h. The solvent and excess thionylchloride were removed by evaporation in vacuo to yield *O*-benzylferulic acid chloride (**23**). In a flame dried apparatus under nitrogen, magnesium turnings (2.67 g) were placed into the flask, and dry ethanol (15 mL), CCl_4 (0.5 mL), and dry diethyl ether (100 mL) were carefully added. The mixture was stirred at 20−23 °C for 4 h. After cooling down to 0 °C, ethyl acetoacetate (13 g, 76 mmol) in dry diethyl ether (30 mL) was added dropwise during 1 h. After cooling to −5 °C, the acid chloride **23** dissolved in dry diethyl ether (100 mL) and dry tetrahydrofurane (150 mL) was added dropwise over a period of 1 h. The mixture was stirred for 12 h at 0−23 °C and was diluted with ice cold 50% sulfuric acid (100 g). The organic phase was washed with water, dried with Na_2SO_4, and filtered, and the filtrate was evaporated to dryness to yield crude **24** (35 g, about 70% purity, 80% yield based on ethyl acetoacetate). A portion was purified with flash chromatography on silica gel 60 using the eluent *n*-hexane/ethyl acetate 3:1 (v/v). Yield: 7.6 g of a >90% fraction. LCMS (RP-18 phase, APCI-): *m/z* = 395, 29 ($[M - H]^-$, 100%). Ethyl 2-acetyl-5-(4-benzyloxy-3-methoxyphenyl)-3-oxopent-2-enoate (1.3 g, 3.3 mmol) (**24**) was dissolved in ethanol (20 mL), Pd−C (5%, wet) was added, and the mixture was hydrogenated at ambient pressure and temperature with hydrogen. After filtration, the filtrate was evaporated to dryness in vacuo, and the crude product was purified with flash chromatography on silica gel 60 using the eluent *n*-hexane/ethyl acetate 2:1 (v/v). Yield: 0.42 g (purity >95%, 1.4 mmol, 42%). LCMS (RP-18 phase, ESI+): *m/z* = 309.6 ($[M + H]^+$, 100%). HRMS: calcd. for $C_{16}H_{20}O_6$ 308.1260, found 308.1272. ^{1}H NMR (400 MHz, $CDCl_3$, internal standard TMS): δ = 6.82 (1H, dd, J = 7.9 Hz, J = 0.3 Hz, H-5′), 6.71 (1H, d, J = 1.9 Hz, H-2′), 6.69 (1H, dd, J = 7.9 Hz, J = 2 Hz, H-6′), 5.59 (1H, s, 4′-OH), 4.25 (2H, q, J = 7.2 Hz, Et−CH_2), 3.86 (3H, d, J = 0.2 Hz, O-CH_3), 3.00−2.95 (2H, m, H-2), 2.91−2.86 (2H, m, H-1), 2.35 (3H, d, J = 0.2 Hz, H-6), 1.32 (3H, t, J = 7.2 Hz, Et−CH_3) ppm. ^{13}C NMR (100 MHz, CD_3OD, internal standard TMS): δ = 198.09 (C, C-3), 195.61 (C, C-5), 167.15 (C, C-1″), 146.45 (C, C-3′), 144.02 (C, C-4′), 132.62 (C, C-1′), 120.89 (CH, C-6′), 114.37 (CH, C-5′), 111.03 (CH, C-2′), 60.84 (CH_2, Et−CH_2), 55.87 (CH_3, O−CH_3), 39.93 (CH_2, C-2), 31.31 (CH_2, C-1), 25.62 (CH_3, C-6), 14.22 (CH_3, Et−CH_3) ppm.

1,7-Bis(4-hydroxy-3-methoxyphenyl)-hepta-3,5-dione (20). Tetrahydrocurcumin (**20**) was obtained by hydrogenation of curcumin (1.5 g, 4.1 mmol) analogous to the described procedure for **6** and subsequently purified with flash chromatography on silica gel using the eluent *n*-hexane/ethyl acetate 1:1. Yield: 0.60 g (39%). LC-MS (APCI+): *m/z* = 389.8 (100%, $[M + H_2O]^+$). ^{1}H NMR (400 MHz, $CDCl_3$, internal standard TMS): δ = 6.83 (2H, m), 6.68 (4H, m), 5.48 (2H, OH), 5.42 (1H, s, H-4), 3.86 (3H, s, O−CH_3), 2.85 (4H, m), 2.55 (4H, m) ppm. ^{13}C NMR (100 MHz, CD_3OD, internal standard TMS, *diketo tautomer): δ = 203.44* (C, C-3*, C-5*), 193.24 (C, C-3, C-5), 146.43 (C, C-3′), 143.96 (C, C-4′), 132.56 (C, C-1′), 132.34* (C, C-1′*), 120.81 (CH, C-6′), 114.35 (CH, C-5′), 111.02* (CH), 110.95 (CH, C-2′), 99.82 (CH, C-4), 57.65* (OCH_3*), 55.87 (CH_3, C-2), 45.52*, 40.38 (CH_2), 31.31 (CH_2, C-1) ppm.

Sensory Studies. All raw sensory data were analyzed using the standard functions of Microsoft Excel 97. For the calculation of statistical significance, Student's matched pair test was used.

Dose Response Plots for Sucrose and Caffeine. Series of dilutions of sucrose (0.29−83% by matter) and caffeine (100−28 340 mg kg^{-1}) were presented to the panelists (trained, 25 and 20, respectively) in the order of increasing concentration (unknown to the panelist) with the advice to rate directly without backward tasting again. The rating was given on an unstructured 15 cm scale from left (nothing) to right (strongest taste effect). The test was performed twice, and the values were averaged and recalculated to a scale from 1 to 10.

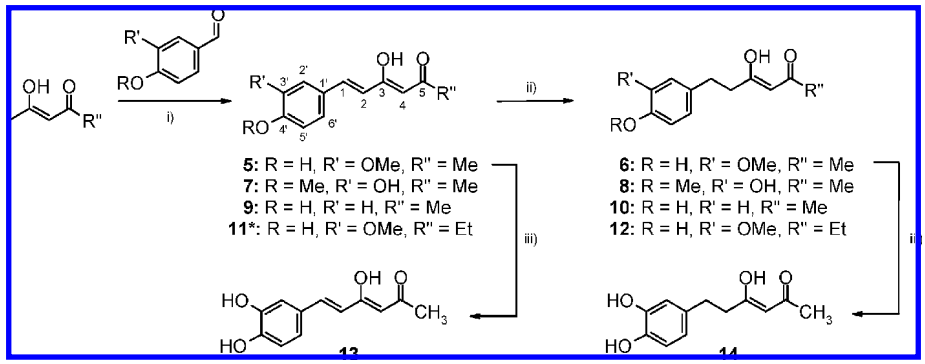

Figure 3. Synthesis of gingerdione derivatives **5**−**14**: (**i**) (a) B_2O_3, acetyl acetone, (b) Bu_3B, EtOAc, (c) aldehyde, EtOAc, and (d) *n*-$BuNH_2$/EtOAc; (**ii**) Pd−C, H_2, EtOH; (**iii**) BBr_3, CH_2Cl_2, −70 °C, room temperature. * alternative method: (**i**) (a) B_2O_3, 3,5-heptadienone, aldehyde, isobutylamine, DMF, 90 °C.

Bitter Masking and Sweet Enhancing Activity. For screening and dose response studies of taste modulating effects, the test compounds were added directly to an aqueous solution of the appropriate bitter or sweet compound. Occasionally, the mixture was treated for several minutes in an ultrasonic bath to improve the dissolution process. Panelists (healthy adults, no tasting problems known) were trained on caffeine as a bitter standard and sucrose as sweet standard. Studies were performed in the morning hours 1 to 2 h after breakfast, during which time they were not allowed to drink black or green tea or coffee due to adaptation to caffeine; only one test per day was performed. A minimum of 10 testers were used in the descriptive test. For all experiments, the test solutions were coded, and in the case of color or cloudiness, the cups were covered using an aluminum foil. Panelists were advised to test randomly mixed samples in the given order by the sip and spit method. For bitter masking activity, the bitter compound was tested only in one concentration (given in the tables, e.g., 500 mg kg^{-1} for caffeine) showing sufficient bitterness that was determined in preliminary tests. For screening of sweet enhancing activity, 5% sucrose solution was used. For screening purposes, the taste quality was absolutely rated on a scale of 1 (no taste) to 10 (very strong taste). Relative flavor modification activity (in %) was calculated by averaging the intensity ratings for each the test and blank solution and then using the following formula: averaged intensity $rating_{test}$/averaged intensity $rating_{blank} - 1$.

Determination of Intrinsic Sweetness. For determination of intrinsic sweetness, an aqueous solution of the test compound in the appropriate concentration was compared to a series of dilutions of sucrose (0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, and 10%). The values of all panelists for each concentration of the test compound were averaged. The plots were given as percent sucrose equivalents.

RESULTS AND DISCUSSION

Synthesis. The dehydrogingerdiones **5**, **7**, **9**, and **11** were synthesized using the known procedure starting from an excess of an alka-3,5-dione-boron complex, which is reacted with the appropriate benzaldehyde with the aid of triethylborate under basic catalysis (*24*). When the aldehyde was used in more than an equimolar ratio, the main product is the appropriate curcumin derivative. The remaining amounts of the bis-coupling products were easily separated by crystallization and if necessary by flash chromatography. Syntheses of [2]- and [3]-dehydrogingerdione (**5**, **11**) as well as for [2]-isogingerdione (**7**) were described earlier (*25*) as well as for the *p*-hydroxy derivative **9** (*26*). In the case of **11**, we have chosen an improved variation of the method using DMF as the solvent without the use of triethyl borate as described for an improved synthesis of curcumin (*27*). The reaction was much more straightforward and much easier to clean up. Fortunately, the reaction yielded as main component the intended regioisomer, and the alternative condensation product (1*E*)-2-methyl-1-(4-hydroxy-3-methoxyphenyl)hex-1-en-3,5-dione was found only in amounts <5% and was separated by flash chromatography.

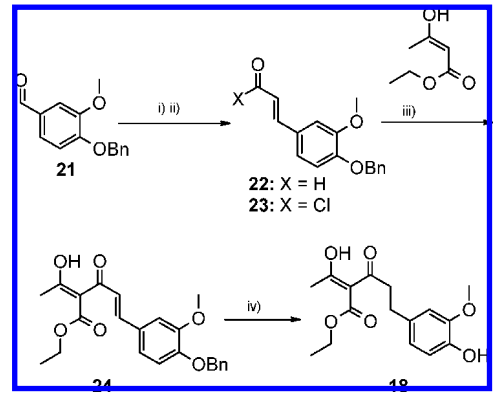

Figure 4. Synthesis of gingerdione derivative **18**: (**i**) malonic acid, pyridine, piperidine (97%); (**ii**) $SOCl_2$, toluene (quant.); (**iii**) Mg/EtOH/THF/DEE (80%); (**iv**) H_2, Pd−C, EtOH (42%).

The gingerdiones **6**, **8**, **10**, and **12** were easily obtained by Pd catalyzed hydrogenation at ambient pressure. The reaction mixtures contained as main components the final products and as side products the appropriate gingerols in amounts <5%. Hispolon (**13**) and dihydrohispolon (**14**) were synthesized using the well-established cleavage of the methyl ether moiety of [2]-dehydrogingerdione (**5**) and [2]-gingerdione (**6**), respectively, with BBr_3 in dichloromethane (**Figure 3**). The reaction yielded only very small amounts of **5** due to interference of the conditions with the unsaturated skeleton and due to losses during the workup. Tetrahydrocurcumin (**20**) was prepared starting from commercial curcumin (**19**) using the same hydrogenation conditions; the analytical data corresponded to known data from the literature (*28*).

Acetoacetic acid vanillylamide (**17**) was prepared via amidation of ethyl acetoacetate with vanillylamine in 1,4-dioxane/triethylamine using *Candida antarctica* lipase (Chirazym c2 L2) as catalyst and subsequent recrystallization. Amidation reactions using lipases are a known conversion (*29*), but to our knowledge, the reaction with ethyl acetoacetate and benzyl amines was never described.

The branched gingerdione ethyl-5-(4-hydroxy-3-methoxyphenyl)-2-(1-oxoethyl)-3-oxopentanoate (**18**) was synthesized as shown in **Figure 4** analogously to ethyl-5-(4-hydroxyphenyl)-2-(1-oxoethyl)-3-oxopentanoate, as described by Doherty (*30*). *O*-Benzyl vanillin (**21**) was converted to *O*-benzyl ferulic (**22**) acid via malonic ester condensation and reacted with thionyl-chloride to yield the appropriate acid chloride **23**. The latter was coupled to ethyl acetoacetate using magnesium in diethyl

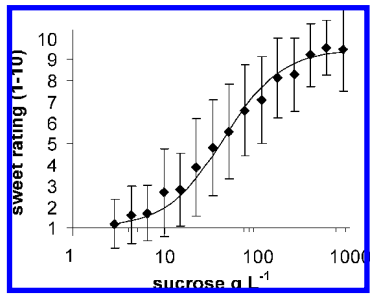

Figure 5. Sweetness of 0.29−83% aqueous sucrose solutions (25 panelists, free choice on a 15 cm unstructured scale, recalculation to scale 1−10).

ether/THF/ethanol to give **24** and subsequently hydrogenated to yield the final product **18**.

All compounds were purified to at least 95% and characterized using (if applicable) GC-MS, LC-MS, and HRMS in the case of new or poorly described products and NMR techniques prior to taste tests. In all cases, the beta-keto system showed tautomerization, and in the solvent used for recording NMR spectra, the main tautomers (>80%) were the enol-isomers.

Screening for Taste Modulating Effects. In our efforts to screen for taste modulating compounds, we used double blinded duo testing. The methods are generally state of the art and used for the screening of taste influencing substances since their introduction by Jellinek in 1966 (*31*). Important criteria for reliable results is a trained panel with sufficient participants, randomized and blinded sampling, and preferably only one tasting session per day, which is best performed in the morning. The panelists record their bitterness impression on a hedonic scale. Some other working groups have developed the half-site tongue test, which may be preferred for very small sample volumes (*32*). We did not use the latter method because in preliminary tasting sessions the masking and enhancing effects of known taste modulators could not be fully reproduced due to the very low volumes. An alternative test, the half-mouth method (*33*) using higher amounts of test solutions was not used due to the sophisticated panelist training necessary for reliable results.

Prior to the screening for sweetness enhancing effects, we tested the sweetness/concentration relationship of neat sucrose solution. The panelists were asked to rate the sweetness of different concentrations of sucrose on an unstructured scale of 15 cm, which was recalculated to a rating from 1 (weak) to 10 (very strong). Despite the fact that most sweet applications (mainly beverages, ice cream etc.) contain between 8 and 15% sucrose, we chose for screening a lower concentration of 5% sucrose solution as test medium because then changes in sweetness could most easily be detected (**Figure 5**). A 5% sucrose solution is about 25% less sweet compared to a 6% sucrose solution when compared directly in the duo test. Therefore, for real applications, a sweetness improving flavor compound should exhibit an enhancing effect of at least 25%. Similar to sweetness rating, a dose rating plot was determined using a series of dilutions of caffeine in water (cf. **Figure 6**). In contrast to sugar as test medium, the acceptance of a 1000−2000 mg kg^{-1}caffeine solution is very low. Therefore, we decided to perform the tests using 500 mg kg^{-1} caffeine. The ratings during a single masking trial at 500 mg kg^{-1} caffeine showed generally higher values when they were compared to the ratings of the same caffeine concentration in the series of dilutions. The differences may be caused by the strong lingering and adaptation effects of caffeine during the dose−response plot tastings.

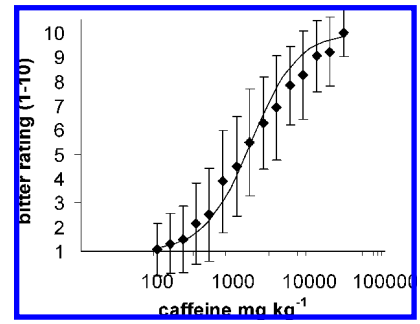

Figure 6. Bitterness of 100−28 340 mg kg^{-1} aqueous caffeine solution (20 panelists, free choice on a 15 cm unstructured scale, recalculation to scale 1−10).

Taste modulating effects lower than 15% were considered only as weak from a practical point of view and are in nearly all cases statistically not significant using the low number of available panelists (10−20). Effects lower than 10% were not considered as valid. In most cases, masking or enhancing effects larger than 25 or 30% are statistically significant and such molecules were evaluated as potential taste modulators.

The most simplified structural elements of homoeriodictyol, dehydrozingerone (**3**), and zingerone (**4**) showed only moderate taste modulation effects (cf. **Table 1**). In the latter case, the concentration used was much lower than for most other test compounds due to the strong intrinsic flavor of **4**. [2]-Dehydrogingerdione (**5**) and to a much higher extend [2]-gingerdione (**6**) showed a pronounced, and in the case of the latter, highly significant masking effect against caffeine bitterness. The sweet enhancing effect on a 5% sucrose solution of both molecules was somewhat weak. Both molecules showed no detectable intrinsic sweetness, and therefore, we conclude that they act as real bitter masking compounds. Exchange of the vanillyl against an isovanillyl aromatic moiety leads to a change in the activity pattern; whereas the masking activity was lower compared to **5**, the sweetness enhancing activity of the [2]-isogingerdione was much higher and highly significant in contrast to the regioisomer. The substitution pattern on the aromatic ring seems to be crucial for activity: the simple *p*-hydroxyphenyl derivatives **9** and **10** showed much lower taste modulation effects as compared to their higher substituted counterparts. Elongation of the alky chain leads to lower efficacy for masking activity: the naturally occurring [3]-dehydrogingerdione (**11**) showed only weak masking and no sweetness enhancing effect. The corresponding [3]-gingerdione (**12**) was remarkably effective against caffeine but weaker than the lower homologous **6**. The sweetness enhancing effect of **12** was higher than for **6** but much lower compared to the [2]-isogingerdione (**8**). Because of the low amounts available, hispolon (**13**) was only tested by qualitative judgment in sucrose and caffeine solutions; at 50 mg kg^{-1}, it exhibited only a weak bitter enhancing effect and was not able to increase sweetness. Dihydrohispolon (**14**) demonstrated at 100 mg kg^{-1} a pronounced dull taste/flavor and was therefore not tested for bitter masking activity. It showed only a weak sweet enhancing effect. The long chain relatives [8]- and [10]-gingerol (**15**, **16**) showed no activity at all. The test concentration of the latter two compounds was 10 times lower as compared to most of the other molecules due to the intrinsic flavor, which was yet remarkably pungent at 10 mg kg^{-1}. *N*-Vanillyl acetoacetamide **17**, the aza-analogue of **6**, as well as the branched variation of **6**, the 2-ethylcarboxy [2]-gingerdione (**18**), showed only moderate and nonsignificant taste modulation

Table 1. Evaluation of Taste Modulation Effects of Compounds Compared to Homoeriodictyol **1** against 500 mg kg^{-1} Caffeine and 5% Sucrose, Respectively[a]

	bitter masking (500 mg kg^{-1} caffeine)		sweet enhancing (5% sucrose)		
compd	panelists (all/masking)[c]	reduction of bitter rating	panelists (all/enhancing)[d]	enhancing of sweet rating	profile (100 mg kg^{-1} in 5% sucrose)
1	10/10	43%* ($p < 0.05$)	15/9	18%	weak, sweet, vanillic, phenolic
3	16/8	10%	16/1	−15%	sweet, balsamic, numbing, neutral
4	15/8	0.7% (1 mg kg^{-1})		n.d.	spicy, clove, smoky (1 mg kg^{-1})
5	12/9	21% ($p < 0.07$)	16/10	15%	neutral, drying
6	17/15	35%* ($p < 0.005$)	15/9	11%	weak, cream, sweet, vanillic
7	16/12	23%* ($p < 0.05$)	16/7	−4%	weak, dry dusty, balsamic, tingling
8	15/11	17%	16/13	27%* ($p < 0.005$)	weak, phenolic, smoky, medicinal
9	16/9	6%	16/5	2%	weak, soapy, sweet, dry-dusty, balsamic
10	16/8	17%	16/9	10%	weak, raspberry, sweet, mouth feel
11	16/9	18%	14/6	0%	weak, sweet, vanillic, ginger
12	16/13	27%* ($p < 0.05$)	16/11	19% ($p < 0.07$)	weak, vanillic, smoke, phenolic
13		more bitter (50 mg kg^{-1})[b]		no difference (50 mg kg^{-1})[b]	mouth feel, astringent
14		n.d.	16/7	8%	dull, anthranilate
15	16/9	−4% (10 mg kg^{-1})	16/9	16% (5 mg kg^{-1})	roasty, coffee (10 mg kg^{-1})
16	16/6	−3% (10 mg kg^{-1})		n.d.	burning, numbing (10 mg kg^{-1})
17	15/10	12%	16/8	10%	neutral
18	16/6	−5%	16/10	11%	woody, card board
19	16/7	−6% (1 mg kg^{-1})	16/3	−2% (1 mg kg^{-1})	sweet, bitter, caramelic (1 mg kg^{-1})
20	11/6	9%	15/6	−1%	fatty, sweet almond, fruity, mouth feel, long lasting

[a] Test concentration 100 mg kg^{-1}; * significant ($p < 0.05$); n.d. = not determined. [b] Only qualitative judgment due to low amounts available. [c] Ratio of number of all panelists against number of panelists who rated the bitterness of test solution lower than standard solution. [d] Ratio of number of all panelists against number of panelists who rated the sweetness of test solution higher than standard solution.

activities. The related diarylheptanoids curcumin (**19**, only 1 mg kg^{-1} tested due to the strong flavor profile) and tetrahydrocurcumin (**20**) showed no taste modulation effects at all.

Evaluation of [2]-Gingerdione (6) and [3]-Gingerdione (12) as Bitter Masking Compounds. Prior to further evaluation for gingerdione **6**, an Ames test (*Salmonella typhimorum* reversed mutation assay) with/without metabolic activation was performed according to OECD guidelines (*34*). Gingerdione **6** showed no mutagenic effect in the *Salmonella typhimorum* strains TA98, TA100, TA102, TA1535, and TA1537 each carried out with/without metabolic activation. Because of the structural similarity, the isogingerdione **8** and gingerdione **12** were not tested using the Ames protocol.

In **Figure 7**, gingerdiones **6** and **12** exhibit clear dose−response behavior against the bitterness of caffeine. Analogously to homoeriodictyol (*17*), the masking effect reaches a maximum activity at a 100 mg kg^{-1} level. As shown in **Table 2**, the activity of the gingerdiones against the bitterness of caffeine and quinine is quite similar, whereas both compounds in contrast to homoeriodictyol, do not reduce salicin bitterness at the tested concentration of 100 mg kg^{-1}. Gingerdione **6** was not active against bitter peptides, whereas gingerdione **12** exhibited a weak, nonsignificant masking effect against bitterness of KCl.

In general, the masking effect of the gingerdiones against caffeine and quinine are quite comparable to homoeriodictyol and 2,4-dihydrobenzoic acid *N*-vanillylamide (**2**) (*15*), whereas the tested activity patterns of **6** and **12** show some remarkable differences in respect to salicin. This is of interest for elucidation of the hitherto unknown masking mechanism of homoeriodictyol derivatives. In our opinion, these structural classes may act as antagonists for some but not the whole set of bitter taste receptors.

Evaluation of [2]-Isogingerdione (8) as a Sweetness Enhancer. The isovanillyl moiety is known as a typical structural element occurring in high potency sweeteners (*35, 36*). Therefore, we evaluated the intrinsic sweetness of a series of dilutions of isogingerdione **8** to exclude a simple additive sweetening effect. For comparison, the sweetness of sucralose was calculated according to DuBois et al. (*37*) (**Figure 8**) and

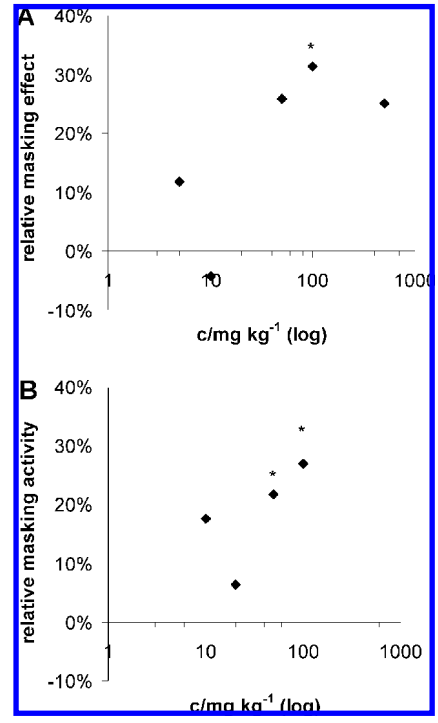

Figure 7. (**A**) [2]-Gingerdione (**6**) and (**B**) [3]-gingerdione (**12**) dose/activity plot for bitterness masking of a 500 mg kg^{-1} caffeine solution determined by paired duo test (trained panel, $n = 16$). * significant, $p < 0.05$.

plotted along with the measured sweetness of solutions containing **8**. The intrinsic sweetness of **8**, even at 250 mg kg^{-1}, was at about 1% sucrose equivalent, and it was 0.58% sucrose equivalent at 100 mg kg^{-1}. Most panelists did not perceive a 0.58% sucrose solution as sweet (averaged rating on the 1−10 scale at 1.5).

To determine synergistic activity of **8**, we have compared calculated and experimental sweetness ratings of 5% sucrose solutions containing various amounts of isogingerdione **8** (**Figure 9**). For calculation reasons, we have normalized the absolute sweet ratings (1−10) to sucrose equivalents (SE) using

Table 2. Bitter Masking Effect of 100 pp mg kg^{-1} [2]-Gingerdione (**6**) and [3]-Gingerdione (**12**) against Various Bitter Compounds[a]

	[2]-gingerdione (**6**)		[3]-gingerdione (**12**)	
bitter compd	panelists (all/masking)[b]	reduction of bitter rating	panelists (all/masking)[b]	reduction of bitter rating
caffeine, 500 mg kg^{-1}	17/15	35%*	16/13	27%*
quinine, 5 mg kg^{-1}	16/10	21%*	15/10	22%
salicine, 250 mg kg^{-1}	16/6	3%	16/7	2%
H-Leu-Trp-OH, 2000 mg kg^{-1}	16/6	1%		n.d.
KCl, 0.5%		n.d.	15/8	5%

[a] Parameters cf. **Table 1**. * significant, $p < 0.05$. n.d. = not determined. [b] Ratio of number of all panelists against number of panelists who rated the bitterness of test solution lower than standard solution.

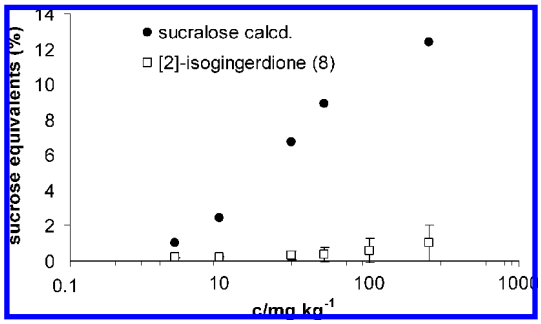

Figure 8. Intrinsic sweetness of [2]-Isogingerdione (**8**) determined by direct comparison of a series of dilutions of test compounds against standard sucrose solutions (0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, and 10%) (trained panel, $n = 23$). Sucralose sweetness was calculated according to DuBois et al. (*37*), $R = 13c(\text{sucralose})^{1.4}/(110^{1.4} + c(\text{sucralose})^{1.4})$. The units of R are % sucrose equivalents; the units of c are mg kg^{-1}.

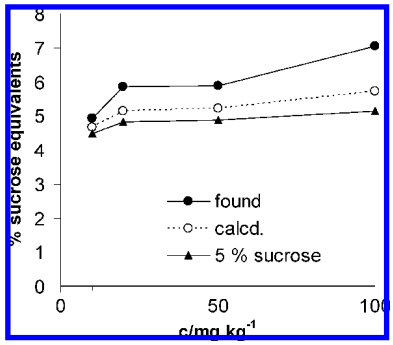

Figure 9. Dose−response plot of sweetness enhancing activity of isogingerdione **8** in 5% sucrose solution compared to the calculated sum of sucrose equivalents of the ratings for a 5% sucrose solution (calculated using the plot in **Figure 5**) and the intrinsic sweetness of **8** (trained panel, $n = 15$).

the plot in **Figure 5**. For each concentration, the SE of the 5% sucrose solutions (**Figure 9**, solid triangles), the sum of the SE of the 5% sucrose solution, the appropriate intrinsic SE of **8** (**Figure 9**, open cirles), and the measured SE of the test solution containing 5% sucrose and **8** (**Figure 9**, solid circle) were plotted. At 10, 20, 50, and 100 mg kg^{-1}, respectively, the calculated synergistic effect was 5, 14, 13, and 23%. The effect at 100 mg kg^{-1} is evident. As a result, we conclude that an amplification of sweetness response to sucrose can be induced not only by low amounts of high intensity sweeteners but also by very weak sweet molecules.

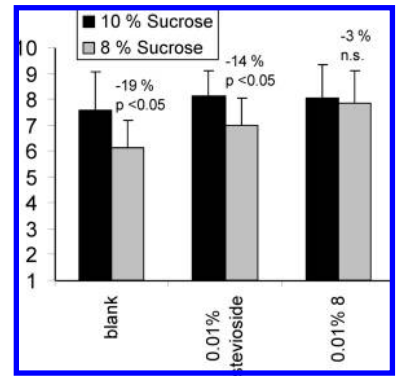

Figure 10. Change in sweetness ratings of a 10% sucrose solution against an 8% sucrose solution (blank), an 8% sucrose solution containing 0.01% stevioside, and an 8% sucrose solution containing gingerdione **8** determined via a paired duo test (trained panel, $n = 15$); significance was determined using Student's t test.

More important for application is the comparison of full sugar applications with sugar reduced variants treated with a sweetness enhancer. Therefore, we performed a paired duo test using a 10% sucrose solution against an 8% sucrose solution with/without test compound **8** (**Figure 10**). For comparison, stevioside as a typical high intensity sweetener was used. Surprisingly, only **8** was able to increase the perceived sweetness of the 8% sucrose solution to a level of 10%.

By starting from the structure of the bitter masking compound homoeriodictyol, it is possible to reduce the complexity of the flavanone skeleton without reduction of the masking effect against caffeine and quinine, as shown for the short chain gingerdiones **6** and **12**. In contrast, the ability to reduce salicin bitterness is not evident. Further modifications of the structural motifs (chain elongation, different substitution pattern, reducing flexibility) cause activity loss. Interestingly, the isogingerdione **8** is able to induce sweetness enhancing effects in model solutions without exhibiting an intrinsic sweet taste.

ACKNOWLEDGMENT

We would like to thank Katharina Reichelt and Franziska Benedix for compilation of the dose−response plots for sucrose and caffeine and Martina Herrmann and Holger Joppe for isolation of the gingerols. Special thanks to Debbie Kennison for proof reading of the manuscript.

LITERATURE CITED

(1) Drewnowski, A.; Gomez-Carneros, C. Bitter taste, phytonutrients, and the consumer: a review. *Am. J. Clin. Nutr.* **2000**, *72*, 1424–1435.

(2) Sohi, H.; Sultana, Y.; Khar, R. K. Taste masking technologies in oral pharmaceuticals: recent developments and approaches. *Drug Dev. Ind. Pharm.* **2004**, *30*, 429–448.

(3) Katsugari, Y.; Sugiura, Y.; Lee, C.; Otsuji, K.; Kurihara, K. Selective inhibition of bitter taste of various drugs by lipoprotein. *Pharm. Res.* **1995**, *12*, 658–662.

(4) Sonoda, C.; Sakai, K.; Murase, K. Bitternis relieving agent. WO 00 21,390, 2000.

(5) Hayashi, N.; Ujihara, T.; Kohata, K. Reduction of catechin astringency by the complexation of gallate-type catechins with pectin. *Biosci., Biotechnol., Biochem.* **2005**, *69*, 1306–1310.

(6) Binello, A.; Cravotto, G.; Nano, G. M.; Spagliardi, P. Synthesis of chitosan-cyclodextrin adducts and evaluation of their bitter-masking properties. *Flavour Fragrance J.* **2004**, *19*, 394–400.

(7) Busto, M. D.; Mezaa, V.; Ortega, N.; Perez-Mateos, M. Immobilization of naringinase from *Aspergillus niger* CECT 2088

in poly(vinyl alcohol) cryogels for the debittering of juices. *Food Chem.* **2007**, *104*, 1177–1182.
(8) Psczola, D. Ingredients—flavor enhancement: taking the mask off. *Food Technol.* **2004**, *58*, 56–69.
(9) Ley, J. P. Bitter masking bitter Ttaste by molecules. *Chem. Percept.* **2008**, *1*, 58–77.
(10) Salemme, F. R.; Barndt, R. Compositions and methods for producing a salty taste in foods or beverages. U.S. Patent 2006 286,276, 2006.
(11) Soldo, T.; Hofmann, T. Application of hydrophilic interaction liquid chromatography/comparative taste dilution analysis for identification of a bitter inhibitor by a combinatorial approach based on Maillard reaction chemistry. *J. Agric. Food Chem.* **2005**, *53*, 9165–9171.
(12) Duffy, V. B.; Sigman-Grant, M.; Powers, M. A.; Elmore, D.; Myers, E. F.; Quagliani, D.; Spano, M.; Stitzel, K. F.; Taylor, S.; Earl, R.; Connor, S. Position of the American dietetic association: use of nutritive and nonnutritive sweeteners. *J. Am. Diet. Assoc.* **2004**, *104*, 255–275.
(13) Ottinger, H.; Soldo, T.; Hoffmann, T. Discovery and structure determination of a novel Maillard-derived sweetness enhancer by application of the comparative taste dilution analysis (cTDA). *J. Agric. Food Chem.* **2003**, *51*, 1035–1041.
(14) Labbe, D.; Rytz, A.; Morgenegg, C.; Ali, S.; Martin, N. Subthreshold olfactory stimulation can enhance Ssweetness. *Chem. Sens.* **2007**, *32*, 205–214.
(15) Ley, J. P.; Blings, M.; Paetz, S.; Krammer, G.; Bertram, H.-J. New bitter masking compounds: hydroxylated benzoic acid amides of aromatic amines as structural analogues of homoeriodictyol. *J. Agric. Food Chem.* **2006**, *54*, 8574–8579.
(16) Ley, J. P.; Kindel, G.; Paetz, S.; Krammer, G. Hydroxydeoxybenzoins and the use thereof to mask a bitter taste. WO 2006 106,023, 2006.
(17) Ley, J. P.; Krammer, G.; Reinders, G.; Gatfield, I.-L.; Bertram, H.-J. Evaluation of bitter masking flavanones from Herba Santa (*Eriodictyon californicum* (H. & A.) Torr., Hydrophyllaceae). *J. Agric. Food Chem.* **2005**, *53*, 6061–6066.
(18) Ley, J. P.; Paetz, S.; Kindel, G.; Freiherr, K.; Krammer, G. E. Enhancers for sweet taste from the world of non-volatiles. In *Sweetness and Sweeteners—Biology, Chemistry and Psychophysics*; Weerasinghe, D. K., DuBois, G., Eds.; Oxford University Press: New York, 2008; pp 400−409.
(19) The number in the square brackets denotes the chain length of the residue added to the parent compound zingerone (**4**). Kikuzaki, H. Ginger for drug and spice purposes. *Herbs, Bot. Teas* **2000**, 75–105.
(20) Jolad, S. D.; Lantz, R. C.; Solyo, A. M.; Chen, G. J.; Bates, R. B.; Timmermann, B. N. Fresh organically grown ginger (*Zingiber officinale*): composition and effects on LPS-induced PGE_2 production. *Phytochemistry* **2004**, *65*, 1937–1954.
(21) Jolad, S. D.; Lantz, R. C.; Chen, G. J.; Bates, R. B.; Timmermann, B. N. Commercially processed dry ginger (*Zingiber officinale*): composition and effects on LPS-stimulated PGE_2 production. *Phytochemistry* **2005**, *66*, 1614–1635.
(22) Ali, N. A. A.; Jansen, R.; Pilgrim, H.; Liberra, K.; Lindequist, U. Hispolon, a yellow pigment from *Inonotus hispidus*. *Phytochemistry* **1996**, *41*, 927–929.
(23) Ohta, H.; Ozaki, K.; Tsuchihashi, G.-I. Regio- and enantioselective reduction of α,γ-diketones by fermenting baker's yeast. *Agric. Biol. Chem.* **1986**, *50*, 2499–2502.
(24) Masuda, T.; Matsumura, H.; Oyama, A.; Takeda, Y.; Jitoe, A.; Kida, A.; Hidaka, K. Synthesis of (±)-cassumins A and B, new curcuminoid antioxidants having protective activity of the living cell against oxidative damage. *J. Nat. Prod.* **1998**, *61*, 609–613.
(25) Belliotti, T. R.; Connor, D. T.; Flynn, D. L.; Kostlan, C. R.; Nies, D. E. Preparation of novel styrylpyrazoles, styrylisoxazols, and analogs as 5-lipoxygenase inhibitors. EP 245,825, 1987.
(26) Mann, G.; Beyer, L.; Arrieta, A. Preparation of unsymmetrical curcuminoids. *Z. Chem.* **1987**, *27*, 172–173.
(27) Wegner, C. Method for production of curcumin. WO 2006 089,894, 2006.
(28) Osawa, T.; Sugiyama, Y.; Inayoshi, M.; Kawakishi, S. Antioxidative activity of tetrahydrocurcuminoids. *Biosci., Biotech., Biochem.* **1995**, *59*, 1609–1612.
(29) de Zoete, M. C.; Kock-van Dalen, A. C.; van Rantwijk, F.; Sheldon, R. A. Lipase-catalyzed ammoniolysis of lipids. A facile synthesis of fatty acid amides. *J. Mol. Catal. B: Enzym.* **1996**, *2*, 141–145.
(30) Doherty, D. G. The hydrolysis of carbon-carbon bonds by α-chymotrypsin. *J. Am. Chem. Soc.* **1955**, *77*, 4887–4892.
(31) Jellinek, G. Moderne Verfahren der sensorischen analyse. *Planta Med.* **1966**, *14*, 21–33.
(32) Shikata, H.; McMahon, D. B. T.; Breslin, P. A. Psychophysics of taste lateralization on anterior tongue. *Percept. Psychophys.* **2000**, *62*, 684–694.
(33) Galopin C.; Tigani, L.; Furrer, S. Structure property relationship of chemosensates. In *Structure Property Relationship of Chemosensates*; Hofmann, T., Rothe, M., Schieberle, P., Eds.; Deutsche Forschungsanstalt für Lebensmittelchemie: Garching, Germany, 2005; pp 61−67.
(34) Final Report; No. 18432/18/04; Laboratory of Pharmacology and Toxicology GmbH & Co. KG: Hamburg, Germany, 2007.
(35) Arnoldi, A.; Bassoli, A.; Merlini, L. Progress in isovanillyl sweet compounds. *Food Chem.* **1996**, *56*, 247–253.
(36) Bassoli, A.; Drew, M. G. B.; Hattotuwagama, C. K.; Merlini, L.; Morini, G.; Widen, G. R. W. Quantitative structure-activty relationships of sweet isovanillyl derivatives. *Quant. Struct.−Act. Relat.* **2001**, *20*, 3–16.
(37) DuBois, G. E.; Walters, D. E.; Schiffman, S. S.; Warwick, Z. S.; Booth, B. J.; Pecore, S. D.; Gibes, K.; Carr, B. T.; Brands, L. M. A systematic study of cxoncentration-response relationships of sweeteners. In *Sweeteners: Discovery, Molecular Design, and Chemoreception*; Walters, D. E., Orthoefer, F. T., DuBois, G. E., Eds.; American Chemical Society: Washington, DC, 1991; pp 261−276.

Received for review March 3, 2008. Revised manuscript received May 5, 2008. Accepted May 10, 2008. Parts of the study were presented on the ACS National meeting, Atlanta, March 2006, and the eighth Wartburg Symposium on Flavour Chemistry & Biology, February 28 to March 2, 2007, Eisenach, Germany.

JF8006536