Synthesis and Sensory Evaluation of Ring-Substituted Dihydrochalcone Sweeteners. 2. Analogues of 3′-Carboxyhesperetin Dihydrochalcone, a High-Potency Dihydrochalcone Sweetener†

Murray L. Whitelaw, Hai-Jung Chung, and James R. Daniel*

Department of Foods and Nutrition, Purdue University, West Lafayette, Indiana 47907

Analogues of 3′-carboxyhesperetin dihydrochalcone were evaluated for taste potency and sweetness quality at both the tip of the tongue and the back of the mouth. High levels of sweetness were seen at the tip of the tongue, while at the back of the mouth higher taste potencies comprised mainly licorice-flavored side-tastes. Alterations to the B-ring isovanillyl portion of 3′-carboxyhesperetin dihydrochalcone reduced both taste potency and sweetness quality, implying that B-ring functionality is crucial for sweet receptor interaction. It is proposed that the 3′-carboxylic acid group enhances sweetness of the dihydrochalcone structure by allowing two conformations to fit into and bind the sweetener receptor, according to the tripartite AH/B/X sweet pharmacophore theory.

We recently reported the synthesis of 3′-carboxyhesperetin dihydrochalcone (**1a**), the most potent dihydrochalcone sweetener currently known (3400 times the potency of 6% sucrose; Whitelaw and Daniel, 1991). The ability of the 3′-carboxy group to enhance taste potency stimulated us to propose that this functionality might promote the A-ring to become more important than the B-ring in sweet receptor interaction. Shallenberger and Acree (1967) hypothesized that sweetness results from reciprocal hydrogen bonding between complimentary AH (hydrogen donating) and B (hydrogen accepting) groups on the sweet molecule and sweetener receptor. According to this theory, two such sweet pharmacophores have been proposed for dihydrochalcones (Crosby et al., 1979). These sweet pharmacophores are displayed in Figure 1, where **1a** is presented in the hypothesized active conformation for dihydrochalcones (DuBois et al., 1977). The mechanism by which the 3′-carboxy group enhances sweetness is unclear. The electron-withdrawing carboxy group may induce the proposed A-ring AH/B unit to become a more effective sweet pharmacophore, or the carboxy group itself, which contains alternative AH/B sites, may act as a receptor-binding sweet pharmacophore. As the 3′-carboxy substituent produces a more polar compound than other ring-substituted hesperetin dihydrochalcones (Whitelaw and Daniel, 1991), it is also plausible that a more favorable hydrophile/lipophile balance is influencing taste potency. Elevated potency of **1a** is accompanied by the sweet, licorice-like aftertaste characteristic for this class of compounds. The presence of a vanillyl substitution pattern as part of many strong flavor compounds (e.g., vanillin, capsaicin, gingerol) suggests that the B-ring isovanillyl (3-hydroxy-4-methoxyphenyl) unit could be responsible for the nonsweet portion of the aftertaste in dihydrochalcones. If the 3′-carboxy group does cause the A-ring to become dominant in sweet receptor interaction, then adjustment of the B-ring functionality might provide a decrease in licorice-flavored side-tastes without sacrificing sweetness. To investigate this idea, we sought to synthesize 3′-carboxyhesperetin dihydrochalcone analogues modified in the B-ring isovanillyl unit.

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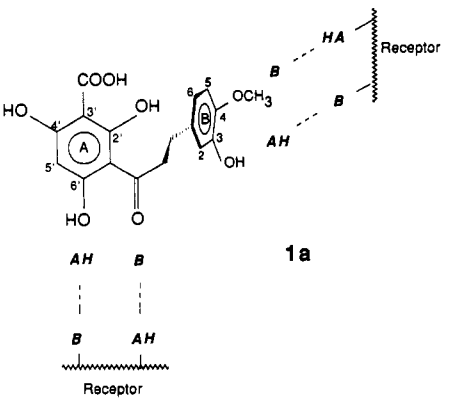

Figure 1. Hypothesized interaction of dihydrochalcones with the sweetener receptor via AH/B sweet pharmacophores.

EXPERIMENTAL PROCEDURES

Synthetic Methods. General Procedures. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by the Purdue microanalytical service. ^{1}H NMR spectra were obtained on a Nicolet NTC-200 spectrometer with tetramethylsilane as an internal reference. High-resolution mass spectra were run on a Kratos MS50 mass spectrometer. Workups that involved extraction into an organic solvent used $MgSO_4$ as a drying agent, which was filtered off before the solvent was evaporated in vacuo. Solvent mixtures are given as a volume/volume ratio. Flash chromatography was carried out on silica gel 60 (EM 9385), 230–400 mesh. All other column chromatography was carried out on silica gel 60–200 mesh. For analytical TLC, aluminum-backed silica gel F-254 plates (Merck) were used. Chalcones were visualized on TLC plates by the appearance of a characteristic red color upon spraying with $KMnO_4/H_2SO_4$ (5 mg/mL), while dihydrochalcones gave intense brown spots when placed in an iodine chamber. Dry organic solvents were obtained by distillation over the appropriate drying agent (Gordon and Ford, 1972) and stored over activated molecular sieves. All starting materials were purchased from either Aldrich or Sigma or prepared as stated in the given reference.

General Procedure for the Preparation of Dihydrochalcones. The hydrogenation procedure has been adapted from DuBois et al. (1981). To a 10% solution of chalcone in THF/methanol (2:1), 10% palladium on charcoal was added to provide a 1:5 ratio of catalyst to chalcone. Stirring the reaction mixture under a balloon of hydrogen at 25 °C quickly produced a bright green color, which gradually faded to colorless as hydrogenation became complete. After a specified reaction time, the mixture

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was filtered through Celite and the solvent evaporated to leave crude product.

3-Carboxy-2,4,6-tris(benzyloxy)acetophenone (**2b**). An oxidation procedure similar to that of Bal et al. (1981) was employed. To a solution of 3-formyl-2,4,6-tris(benzyloxy)acetophenone (**2c**, 2.50 g, 5.3 mmol; Whitelaw and Daniel, 1991) in THF (75 mL) and *tert*-butyl alcohol (75 mL) was added 3-methyl-2-butene (27.5 mL). A solution of sodium chlorite (4.38 g, 48.4 mmol) and sodium dihydrogen phosphate (4.38 g, 31.7 mmol) in H_2O (18 mL) was then added dropwise with stirring over 15 min, while the temperature was kept at 25 °C. The solution was vigorously stirred for 45 min, and then H_2O (100 mL) was added and the mixture extracted with ethyl acetate (2 × 100 mL). The combined organic extracts were washed with H_2O (100 mL) and allowed to evaporate in a fume hood overnight. Toluene was added to the residual oil and evaporated in vacuo to leave a yellow oil. Crystallization from benzene/petroleum ether gave a quantitative yield of **2b** as white clusters. Traces of *tert*-butyl alcohol were removed by washing with petroleum ether and filtering: mp 123–125 °C; ^{1}H NMR ($CDCl_3$) δ 2.43 (s, 3 H, CH_3), 5.00 (s, 2 H, OCH_2Ph), 5.04 (s, 2 H, OCH_2Ph), 5.11 (s, 2 H, OCH_2Ph), 6.36 (s, 1 H, Ar′ *H*), 7.35 (s, 15 H, 3 × Ph*H*). Anal. Calcd for $C_{30}H_{26}O_6$: C, 74.68; H, 5.43. Found: C, 74.42; H, 5.34.

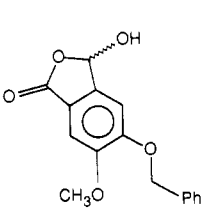
6-Methoxyisovanillin (**4**). A strategy based on that of Rao and Stuber (1983) was employed. A solution of 6-bromoisovanillin (**3**, 1.0 g, 4.3 mmol; Saraf, 1983) and anhydrous copper(II) chloride (140 mg, 1.0 mmol) in DMF (10 mL) was added dropwise to 25 wt % sodium methoxide/methanol (5 mL) at 60 °C. The insoluble brown sludge was stirred and warmed to 100 °C, eventually giving a brown solution. After 1.5 h, the solution was cooled to 25 °C and poured into 2 M HCl (20 mL). The product was extracted into ethyl acetate (2 × 30 mL), and the combined organic layers were washed with water (2 × 30 mL), dried, and evaporated to leave a brown solid. Purification by flash chromatography ($CHCl_3$/EtOAc, 20:1) gave 650 mg (82% yield) of **4** as a yellow solid. Recrystallization from ethyl acetate/hexane gave transparent flakes: mp 144–146 °C; ^{1}H NMR ($CDCl_3$) δ 3.90 (s, 3 H, OCH_3), 3.98 (s, 3 H, OCH_3), 5.43 (s, 1 H, O*H*), 6.47 (s, 1 H, H_5), 7.37 (s, 1 H, H_2), 10.30 (s, 1 H, C*H*O). Anal. Calcd for $C_9H_{10}O_4$: C, 59.34; H, 5.53. Found: C, 59.15; H, 5.86.

3-(Benzyloxy)-4,6-dimethoxybenzaldehyde (**5**). A mixture of **4** (450 mg, 2.5 mmol), benzyl chloride (630 mg, 5.0 mmol), and anhydrous potassium carbonate (760 mg, 5.5 mmol) in dry DMF (20 mL) was stirred at 70 °C for 2.5 h. The mixture was cooled to 25 °C and poured into H_2O (40 mL). The product was extracted into ethyl acetate (2 × 40 mL), and the combined organic layers were washed with water, dried, and evaporated to a yellow oil. Addition of ethanol precipitated a yellow solid, which was filtered and dried. Purification by flash chromatography ($CHCl_3$) gave 540 mg (80% yield) of **5** as a light yellow solid. Recrystallization from ethyl acetate/ethanol gave transparent needles: mp 99–101 °C; ^{1}H NMR ($CDCl_3$) δ 3.92 (s, 3 H, OCH_3), 3.96 (s, 3 H, OCH_3), 5.10 (s, 2 H, OCH_2Ph), 6.50 (s, 1 H, H_5), 7.26 (s, 1 H, H_2), 7.33–7.42 (m, 5 H, Ph*H*), 10.29 (s, 1, H, C*H*O). Anal. Calcd for $C_{16}H_{16}O_4$: C, 70.58; H, 5.92. Found: C, 70.19; H, 6.05.

3′-Carboxy-2′,3,4′,6′-tetrakis(benzyloxy)-4,6-dimethoxychalcone (**7b**). To a solution of **2b** (532 mg, 1.1 mmol) and **5** (300 mg, 1.1 mmol) in dry THF (30 mL) was added 25 wt % sodium methoxide/methanol (5 mL). The clear yellow solution was stirred at 25 °C for 15 h and then poured into 1 M HCl/ice (100 mL). The product was extracted into ethyl acetate (2 × 50 mL), and the combined organic layers were washed with water (2 × 50 mL) and dried. Evaporation of the solvent gave a yellow/green oil, which was purified by flash chromatography ($CHCl_3$/MeOH, 20:1). Crystallization from ethyl acetate/hexane gave pale yellow crystals: mp 155–157 °C; ^{1}H NMR ($CDCl_3$) δ 3.79 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 5.02 (s, 6 H, 3 × OCH_2Ph), 5.09 (s, 2 H, OCH_2Ph), 6.46 (s, 1 H, Ar′, *H*), 6.85 (d, 1 H, *J* = 16 Hz, C*H*Ar), 7.04 (s, 1 H, Ar *H*), 7.19–7.43 (m, 22 H), 7.66 (d, 1 H, *J* = 16 Hz, Ar′,COC*H*). Anal. Calcd for $C_{46}H_{40}O_9$: C, 74.99; H, 5.47. Found: C, 74.71; H, 5.64.

3′-Carboxy-2′,3,4′,6′-tetrahydroxy-4,6-dimethoxydihydrochalcone (**1b**). Chalcone **7b** was hydrogenated for 7 h by the general procedure to give a white solid on workup. Trace impurities were removed by washing with $CHCl_3$ and filtering to yield 180 mg (92%) of **1b**: mp 120 °C (dec); measured mass (FAB) $[M + H]^+$ 379.1032 ($C_{18}H_{18}O_9$ requires $[M + H]^+$ 379.1028); ^{1}H NMR (DMSO-d_6) δ 2.70 (t, 2 H, *J* = 7 Hz, CH_2Ar), 3.20 (t, 2 H, *J* = 7 Hz, Ar′ $COCH_2$), 3.72 (s, 3 H, OCH_3), 3.76 (s, 3 H, OCH_3), 5.54 (s, 1 H, Ar′ *H*), 6.59 (s, 1 H, Ar *H*), 6.62 (s, 1 H, Ar *H*).

3-[2-Oxo-2-[3′-carboxy-2′,4′,6′-tris(benzyloxy)phenyl]ethyl]-5-(benzyloxy)-6-methoxy-1(3H)-isobenzofuranone (**9**). To a solution of **2b** (1.35 g, 2.80 mmol) and 5-(benzyloxy)-6-methoxyphthalaldehydic acid (**8**, 800 mg, 2.80 mmol; Whitelaw and



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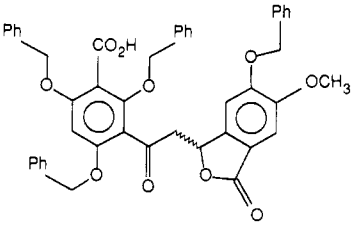

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Daniel, 1991) in anhydrous methanol (45 mL) was added 25 wt % sodium methoxide/methanol (20 mL). The clear yellow solution was refluxed for 23 h. After cooling to 25 °C, the solution was poured into 1 M HCl/ice (100 mL) and the product extracted into ethyl acetate (2 × 50 mL). The combined organic fractions were washed with brine (2 × 50 mL), dried, and evaporated to leave a yellow oil. The oil was dissolved in benzene (200 mL), and *p*-toluenesulfonic acid (100 mg) was added. After stirring for 12 h, TLC ($CHCl_3$/MeOH, 9:1) showed clean conversion of the initial chalcone product **7c** to the higher R_f **9**. Ethyl acetate (20 mL) was added and the organic solution washed with water (2 × 100 mL), dried, and evaporated to leave a yellow oil. Purification by flash chromatography ($CHCl_3$, followed by $CHCl_3$/MeOH, 20:1) gave 1.7 g (81% yield) of **9** as a yellow oil. Crystallization from ethyl acetate/hexane gave small white rosettes: mp 176–178 °C; ^{1}H NMR ($CDCl_3$) δ 3.10 (dd, 1 H, *J* = 18 Hz, 8 Hz, Ar′ COC*H*), 3.36 (dd, 1 H, *J* = 18 Hz, 8 Hz, Ar′ COC*H*), 3.87 (s, 3 H, OCH_3), 4.78 (d, 1 H, *J* = 12 Hz, OC*H*Ph), 4.86 (d, 1 H, *J* = 12 Hz, OC*H*Ph), 4.96 (s, 2 H, OCH_2Ph), 5.06 (s, 2 H, OCH_2Ph), 5.12 (s, 2 H, OCH_2Ph), 5.77–5.80 (m, 1 H, H_3), 6.36 (s, 1 H, $H_{5'}$), 6.87 (s, 1 H, H_4), 7.20–7.34 (m, 21 H). Anal. Calcd for $C_{46}H_{38}O_{10}$: C, 73.59; H, 5.10. Found: C, 73.20; H, 4.91.

3′,6-Dicarboxy-2′,3,4′,6′-tetrahydroxy-4-methoxydihydrochalcone (**1c**). To a solution of **9** (600 mg, 0.80 mmol) in THF (30 mL) and methanol (30 mL) was added 20% aqueous NaOH (0.5 mL). After 5 min of stirring, 20% palladium on charcoal (120 mg) was added and the mixture stirred under a balloon of hydrogen for 7 h. Neutralization with 6 N HCl (0.5 mL) preceded filtration through Celite and evaporation of solvent. The cream-colored solid was washed successively with $CHCl_3$ and H_2O to give 260 mg (83% yield) of **1c** as a white solid: mp 180 °C (dec); measured mass (FAB) $[M + H]^+$ 393.0816 ($C_{18}H_{16}O_{10}$ requires $[M + H]^+$ 393.0821); ^{1}H NMR (DMSO-d_6) δ 3.11–3.26 (m, 4 H, CH_2CH_2), 3.77 (s, 3 H, OCH_3), 5.57 (s, 1 H, Ar′ *H*), 6.74 (s, 1 H, H_5), 7.40 (s, 1 H, H_2).

Methyl 5-Formyl-2-methoxybenzoate (**6**). A mixture of 5-formyl-2-hydroxybenzoic acid (1.30 g, 7.8 mmol), dimethyl sulfate (1.85 mL, 19.5 mmol), and anhydrous potassium carbonate (2.90 g, 21.0 mmol) in acetone (60 mL) was stirred at 55 °C for 4 h. The mixture was then poured into 0.1 M HCl (50 mL) and extracted with ethyl acetate (2 × 75 mL). The combined organic layers were washed with water (100 mL) and dried, and the solvent was evaporated to leave a yellow oil which solidified on standing. The solid was washed with petroleum ether and filtered to yield 1.27 g (83%) of **6**. Recrystallization from methanol/water gave powdery white clusters: mp 81–83 °C; ^{1}H NMR δ 3.92 (s, 3 H, CO_2CH_3), 4.01 (s, 3 H, OCH_3), 7.12 (d, 1 H, *J* = 9 Hz, H_5), 8.03 (dd, 1 H, *J* = 9 Hz, 2 Hz, H_6), 8.33 (d, 1 H, *J* = 2 Hz, H_2), 9.92 (s, 1 H, C*H*O). Anal. Calcd for $C_{10}H_{10}O_4$: C, 61.85; H, 5.19. Found: C, 61.67; H, 5.05.

3-Carboxy-2′,4′,6′-tris(benzyloxy)-4-methoxychalcone (**7d**). To a solution of 2,4,6-tris(benzyloxy)acetophenone (**2a**, 2.26 g, 5.2 mmol; Whitelaw and Daniel, 1991) and **6** (1.00 g, 5.2 mmol) in benzene (100 mL) and THF (40 mL) was added 25 wt % sodium methoxide in methanol (15 mL). After 14 h of stirring at 25 °C, the solution was evaporated to half volume and poured into 1 M

HCl/ice (100 mL). The product was extracted into ethyl acetate (2 × 75 mL), and the combined organic layers were washed with brine (2 × 75 mL). After drying and evaporation of solvent, a yellow oil was obtained. Purification by flash chromatography ($CHCl_3$) gave 2.90 g (94% yield) of **7d** as a yellow oil. Crystallization from ethyl acetate/hexane gave fine yellow needles: mp 154–156 °C; ^1H NMR ($CDCl_3$) δ 4.09 (s, 3 H, OCH_3), 5.01 (s, 2 H, OCH_2Ph), 5.03 (s, 4 H, 2 × OCH_2Ph), 6.27 (s, 2 H, 2 × Ar′ *H*), 6.95 (d, 1 H, *J* = 16 Hz, *CH*Ar), 7.05 (d, 1 H, *J* = 9 Hz, H_5), 7.23–7.39 (m, 16 H), 7.71 (dd, 1 H, *J* = 9 Hz, 2 Hz, H_6), 8.23 (d, 1 H, *J* = 2 Hz, H_2). Anal. Calcd for $C_{38}H_{32}O_7$: C, 75.99; H, 5.37. Found: C, 75.76; H, 5.47.

3-Carbomethoxy-2′,4′,6′-tris(benzyloxy)-4-methoxychalcone (**7e**). A solution of **7d** (2.00 g, 3.3 mmol) and *p*-toluenesulfonic acid (100 mg) in methanol (100 mL) and dichloromethane (40 mL) was refluxed for 48 h. The clear yellow solution was then cooled to 25 °C and the solvent evaporated. The residue was dissolved in chloroform (50 mL), washed with 0.01 M HCl (50 mL), and then dried and evaporated to a yellow oil. Crystallization from ethyl acetate/methanol gave 1.50 g (74% yield) of **7e** as a white solid: mp 142–144 °C; ^1H NMR (DMSO-d_6) δ 3.80 (s, 3 H, CO_2CH_3), 3.88 (s, 3 H, $ArOCH_3$), 5.11 (s, 6 H, 3 × OCH_2Ph), 6.53 (s, 2 H, 2 × Ar′ *H*), 6.97 (d, 1 H, *J* = 16 Hz, *CH*Ar), 7.26–7.45 (m, 18 H), 7.88–7.91 (m, 2 H, 2 × Ar*H*). Anal. Calcd for $C_{39}H_{34}O_7$: C, 76.21; H, 5.58. Found: C, 76.19; H, 5.58.

3-Carbomethoxy-2′,4′,6′-trihydroxy-4-methoxydihydrochalcone (**1e**). Chalcone **7e** (998 mg, 1.63 mmol) was hydrogenated for 5 h under the usual conditions. A light brown solid was obtained on workup. Washing and filtering with $CHCl_3$/petroleum ether removed a trace nonpolar impurity. Purification by flash chromatography ($CHCl_3$/acetone, 10:1) gave 450 mg (80% yield) of **1e** as a white powder: mp 186–189 °C; measured mass (CI) $[M + H]^+$ 347.1123 ($C_{18}H_{18}O_7$ requires $[M + H]^+$ 347.1130); ^1H NMR (DMSO-d_6) δ 2.89 (m, 2 H, CH_2Ar), 3.28 (m, 2 H, Ar′ $COCH_2$), 3.78 (s, 6 H, OCH_3, CO_2CH_3), 5.81 (s, 2 H, 2 × Ar′ *H*), 7.06 (d, 1 H, *J* = 8 Hz, Ar *H*), 7.41 (d, 1 H, *J* = 8 Hz, Ar *H*), 7.52 (s, 1 H, Ar *H*).

3-Carboxy-2′,4′,6′-trihydroxy-4-methoxydihydrochalcone (**1d**). A solution of **1e** (100 mg, 0.29 mmol) in 5% aqueous KOH (3 mL) was stirred at 25 °C for 1 h. Cooling to 5 °C and acidifying to pH 1 with 10% HCl precipitated a white solid. The solid was filtered, washed with water, and air dried. Trace impurities were removed by washing with $CHCl_3$ to give 92 mg (96% yield) of **1d** as a white powder: mp 230 °C (dec); measured mass (CI) $[M + H]^+$ 333.0964 ($C_{17}H_{16}O_7$ requires $[M + H]^+$ 333.0974); ^1H NMR (DMSO-d_6) δ 2.85 (m, 2 H, CH_2Ar), 3.26 (m, 2 H, Ar′ $COCH_2$), 3.78 (s, 3 H, OCH_3), 5.81 (s, 2 H, 2 × Ar′ *H*), 7.03 (d, 1 H, *J* = 8 Hz, Ar *H*), 7.37 (d, 1 H, *J* = 8 Hz, Ar *H*), 7.52 (s, 1 H, Ar *H*).

3,3′-Dicarboxy-2′,4′,6′-tris(benzyloxy)-4-methoxychalcone (**7f**). To a solution of **6** (240 mg, 1.24 mmol) and **2b** (600 mg, 1.24 mmol) in methanol (10 mL) was added 25 wt % sodium methoxide/methanol (2.8 mL), and the solution was stirred at 25 °C for 24 h. The solution was evaporated to half volume and poured into 1 M HCl/ice (20 mL). The product was extracted into ethyl acetate (2 × 20 mL), and the combined organic layers were washed with brine, dried, and evaporated. The yellow oil was purified by flash chromatography (EtOAc/MeOH, 7:1) and crystallized from chloroform/petroleum ether to give 590 mg (74% yield) of **7f** as fine yellow crystals: mp 170–173 °C; ^1H NMR (DMSO-d_6) δ 3.88 (s, 3 H, OCH_3), 4.93 (s, 2 H, OCH_2Ph), 5.23 (s, 2 H, OCH_2-Ph), 5.27 (s, 2 H, OCH_2Ph), 6.93 (s, 1 H, Ar′ *H*), 7.05 (d, 1 H, *J* = 16 Hz, *CH*Ar), 7.16–7.47 (m, 17 H), 7.86–7.91 (m, 2 H), 12.98 (br s, 2 H, 2 × COO*H*). Anal. Calcd for $C_{39}H_{32}O_9$: C, 72.66; H, 5.00. Found: C, 72.35; H, 5.34.

3,3′-Dicarboxy-2′,4′,6′-trihydroxy-4-methoxydihydrochalcone (**1f**). Chalcone **7f** (500 mg, 0.78 mmol) was hydrogenated in the usual manner for 5 h. Upon workup and purification by flash chromatography ($CHCl_3$/MeOH, 1:1) a brown solid was obtained. Washing with chloroform and filtering gave 229 mg (78% yield) of **1f** as a light brown solid: mp 171–174 °C; measured mass (FAB) $[M + H]^+$ 377.0878 ($C_{18}H_{16}O_9$ requires $[M + H]^+$ 377.0873); ^1H NMR (DMSO-d_6) δ 2.85 (t, 2 H, *J* = 8 Hz, CH_2Ar), 3.31 (t, 2 H, *J* = 8 Hz, Ar′ $COCH_2$), 3.79 (s, 3 H, OCH_3), 5.56 (s, 1 H, Ar′ *H*), 7.04 (d, 1 H, *J* = 9 Hz, H_5), 7.39 (dd, 1 H, *J* = 9 Hz, 2 Hz, H_6), 7.52 (d, 1 Hz, *J* = 2 Hz, H_2).

3′-Carboxy-2′,3,4′,6′-tetrakis(benzyloxy)chalcone (**7g**). To a solution of 3-(benzyloxy)benzaldehyde (300 mg, 1.41 mmol) and **2b** (682 mg, 1.41 mmol) in dry THF (45 mL) and methanol (10 mL) was added 25 wt % sodium methoxide/methanol (2 mL). The solution was stirred at 25 °C for 20 h and then evaporated to half volume and poured into 1 M HCl/ice (25 mL). The product was extracted into ethyl acetate (2 × 20 mL), and the combined organic layers were washed with brine, dried, and evaporated. The yellow oil product was purified by flash chromatography ($CHCl_3$/MeOH, 8:1). Crystallization from benzene/petroleum ether gave 750 mg (79% yield) of **7g**: mp 130–133 °C; ^1H NMR ($CDCl_3$) δ 5.01 (s, 2 H, OCH_2Ph), 5.05 (s, 4 H, 2 × OCH_2Ph), 5.14 (s, 2 H, OCH_2Ph), 6.41 (s, 1 H, Ar′ *H*), 6.95 (d, 1 H, *J* = 16 Hz, *CH*Ar), 7.07–7.09 (m, 2 H, 2 × Ar *H*), 7.21–7.41 (m, 23 H). Anal. Calcd for $C_{44}H_{36}O_7$: C, 78.09; H, 5.36. Found: C, 77.70; H, 5.40.

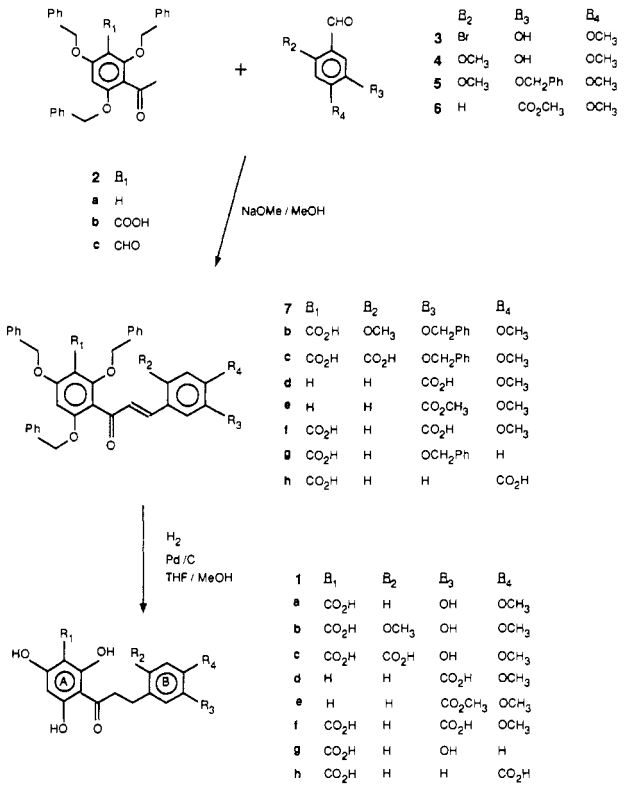
3′-Carboxy-2′,3,4′,6′-tetrahydroxydihydrochalcone (**1g**). Chalcone **7g** (500 mg, 0.74 mmol) was hydrogenated for 5 h by the usual procedure to give a tan solid on workup. The solid was washed with $CHCl_3$ and then purified by flash chromatography (EtOAc/MeOH, 7:1) to give 184 mg (78% yield) of **1g** as a light yellow solid: mp 152–155 °C (dec); measured mass (FAB) $[M + H]^+$ 319.0822 ($C_{16}H_{14}O_7$ requires $[M + H]^+$ 319.0818); ^1H NMR (DMSO-d_6) δ 2.80 (t, 2 H, *J* = 8 Hz, CH_2Ar), 3.30 (t, 2 H, *J* = 8 Hz, Ar′ $COCH_2$), 5.51 (s, 1 H, Ar′ *H*), 6.55–6.66 (m, 3 × Ar *H*), 7.03–7.10 (m, 1 H, Ar *H*).

3′,4-Dicarboxy-2′,4′,6′-tris(benzyloxy)chalcone (**7h**). To a solution of 4-carboxybenzaldehyde (300 mg, 2.0 mmol) and **2b** (965 mg, 2.0 mmol) in dry THF (30 mL) and dry methanol (10 mL) was added 25 wt % sodium methoxide/methanol (3 mL). After stirring for 18 h at 25 °C, the solution was evaporated to half volume and poured into 1 M HCl/ice (30 mL). The product was extracted into ethyl acetate (2 × 30 mL), and the combined organic layers were washed with brine, dried, and evaporated. The yellow oil product was purified by flash chromatography ($CHCl_3$/MeOH, 3:1). Crystallization from ethyl acetate/hexane gave 896 mg (73% yield) of **7h** as a white solid: mp 139–143 °C (dec); ^1H NMR (DMSO-d_6) δ 4.94 (s, 2 H, OCH_2Ph), 5.24 (s, 2 H, OCH_2Ph), 5.29 (s, 2 H, OCH_2Ph), 6.96 (s, 1 H, Ar′ *H*), 7.19–7.48 (m, 17 H), 7.80 (d, 2 H, *J* = 8 Hz, $H_2 + H_6$), 7.96 (d, 2 H, *J* = 8 Hz, $H_3 + H_5$), 13.14 (s, 2 H, 2 × COO*H*). Anal. Calcd for $C_{38}H_{30}O_8$: C, 74.26; H, 4.92. Found: C, 74.17; H, 5.23.

3′,4-Dicarboxy-2′,4′,6′-trihydroxydihydrochalcone (**1h**). Chalcone **7h** (300 mg, 0.49 mmol) was hydrogenated for 5 h under the standard conditions to give a light yellow solid on workup. Purification by flash chromatography ($CHCl_3$/MeOH, 2:1) gave 124 mg (73% yield) of **1h** as a yellow powder: mp 93–95 °C; measured mass (FAB) $[M + H]^+$ 347.0770 ($C_{17}H_{14}O_8$ requires $[M + H]^+$ 347.0767); ^1H NMR (DMSO-d_6) δ 2.96 (t, 2 H, *J* = 8 Hz, CH_2Ar), 3.37 (t, 2 H, *J* = 8 Hz, $ArCOCH_2$), 5.53 (s, 1 H, Ar′ *H*), 7.38 (d, 2 H, *J* = 8 Hz, $H_2 + H_6$), 7.86 (d, 2 H, *J* = 8 Hz, $H_3 + H_5$).

Sensory Evaluation. Novel dihydrochalcones were assessed as nonmutagenic to three strains (TA 98, TA 100, TA 102) of *Salmonella typhimurium* according to the methods of Marion and Ames (1983) and showed no single-dose acute toxicity in mice at 200 and 400 mg/kg. Taste evaluations were carried out by the most consistent 8 panelists from a group of 20 volunteers undertaking training sessions [methods detailed in Whitelaw and Daniel (1991)]. Testing was performed with a standard "sip and spit" procedure using dilute sweetener concentrations (between 20 and 100 ppm, 5-mL volumes) of similar taste intensity to the 6% sucrose reference. In the evaluation of **1a** it was noted the potent, clean sweetness was detected at the tip of the tongue, while side-tastes and aftertaste were dominant only when the solution was taken toward the back of the tongue and mouth. The test solutions were thus evaluated for peak taste potency and percentage sweet/nonsweet components at both the tip of the tongue and the back of the mouth. Panelists were instructed to hold the solution at the tip of the tongue until a maximum response was noted. Perceived taste intensity and percentage sweetness were then rated against the 6% sucrose reference, for which the panelists preassigned a number. After tilting the head back and rolling the solution over the back of the tongue, the panelists repeated these evaluations to give a second set of data. Perceived intensities given by the panelists were "normalized" to bring parity to individual scaling systems. A normalized intensity is the ratio of a rated intensity to the geometric mean

Scheme I. Synthesis of Dihydrochalcones



of all intensities, scored at either the front or the back of the mouth, by the panelist for the session, e.g., normalized intensity of compound A given by

$$I_{N(A)} = I_A/(I_A I_B I_C ... I_n)^{1/n} \quad (1)$$

Normalized intensities (I_N) were calculated for each solution, as determined by each individual panelist, for both sites in the mouth. Normalized intensities for each compound were then averaged, corrected to the scale of reference (1), and multiplied by the dilution factor (concentration of sucrose reference/concentration of sweetener) to give the reported taste potencies on a weight basis.

$$\text{taste potency} = \frac{I_N \text{ sweetener}}{I_N \text{ reference}} \times \frac{60000 \text{ ppm}}{\text{sweetener ppm}} \quad (2)$$

Data widely outside the 95% confidence limits were rejected by the t-value method (Gordon and Ford, 1972).

RESULTS AND DISCUSSION

Synthesis. Target molecules were formed by aldol condensation of 2,4,6-tris(benzyloxy)acetophenones (**2a** or **2b**) and appropriately functionalized benzaldehydes to give intermediate chalcones (**7b–h**). Hydrogenation of chalcones led to the desired dihydrochalcones (**1b–h**) in high yield (Scheme I). Throughout the course of these syntheses we discovered that the aldol condensation could be vastly improved by using sodium methoxide/methanol as a base rather than the previously used aqueous potassium hydroxide/ethanol. We also found the hydrogenations proceed smoothly, without need of pressure, by stirring the reactions at 25 °C under a balloon of hydrogen. Chalcone **7c** readily cyclized to intermediate **9** upon reaction workup. Alkaline hydrogenation conditions reverted **9** to the open chain **7c**, thus enabling smooth conversion to **1c**.

Sensory Evaluation. Taste potencies and percentage sweetness values for the prepared dihydrochalcones are given in Table I. The strong dependence of perceived taste on the position in the mouth where the tasting takes

Table I. Taste Potencies and Sweetness Character of 3′-Carboxyhesperetin Dihydrochalcone Analogues[a]

	front of mouth		back of mouth		
compd[b]	potency[c]	% sweetness	potency[c]	% sweetness	n^d
1a	2650 (600)	85 (9)	4180 (1080)	31 (11)	15
1b	1260 (240)	50 (20)	2860 (170)	15 (9)	13
1c	190 (70)	45 (22)	390 (110)	23 (14)	15
1d	tasteless		tasteless		
1e	tasteless		tasteless		
1f	tasteless		tasteless		
1g	330 (110)	56 (15)	1040 (80)	26 (12)	13
1h	tasteless		tasteless		

[a] Values compared to 6% aqueous sucrose (±2 SEM). [b] Aqueous solutions of 20 ppm for **1b**, 50 ppm for **1c**, and 100 ppm for **1d** and **1e**. [c] Weight basis. [d] Number of evaluations.

place is well illustrated by compound **1a**. Extreme potency and almost pure sweetness is detected at the tip of the tongue, where sweetness is known to be most intense. Toward the back of the tongue and mouth, however, sidetastes overwhelm sweetness as overall potency increases. Our results support the proposal of interaction with other taste receptors complicating the sweetness component contribution of dihydrochalcones and producing strong aftertaste (DuBois et al., 1981). The trend of diminishing sweetness accompanying increasing taste potency as solutions were rolled toward the back of the mouth was general for all dihydrochalcones tested. The ability of a carboxylic acid at the 3′-position to enhance sweetness stimulated preparation of **1d**, where the B-ring hydroxyl AH of hesperetin dihydrochalcone is replaced by a carboxylic acid. A carboxylic acid at this position has potential to act as the proposed hydrogen-donating AH, while also enhancing water solubility. Both this compound and its methyl ester, **1e**, are tasteless. Even with the taste-enhancing 3′-carboxylic acid present, this B-ring substitution pattern (**1f**) remains tasteless. Compound **1h**, containing a 4-carboxy group on the B-ring, is also tasteless. These results are consistent with our previous observations that any variation in the B-ring isovanillyl unit dramatically reduces sweetness. A lone 3-hydroxy group on the B-ring (**1g**) yields reduced taste potency and percentage sweetness compared to the parent **1a**, in similar fashion to 4-demethoxyneohesperidin dihydrochalcone (100 × 5% sucrose; Krbecheck et al., 1968) being of lower sweetness than neohesperidin dihydrochalcone (340 × 8.6% sucrose; DuBois et al., 1981). Compounds **1b** and **1c** were prepared to investigate the influence of electron-withdrawing and -donating groups para to the proposed B-ring phenolic AH. It was thought that altering the electronic constitution of the B-ring AH might influence and perhaps lessen interaction with nonsweet taste receptors. These compounds, however, suffer both reduced potencies and taste qualities. All sweet-tasting dihydrochalcones tested contain the characteristic lingering aftertaste, which we qualitatively assessed as increasing proportionately with the taste potency increases seen in 3′-carboxyhesperetin dihydrochalcone analogues.

Evaluation of these analogues suggests that the B-ring isovanillin unit remains of prime importance for inducing sweetness, even though the 3′-carboxylic acid of the A-ring greatly potentiates this sweetness. An explanation for this elevation could lie in the hypothetical assignment of an AH/B/X tripartite sweet pharmacophore to dihydrochalcones by DuBois et al. (1977). In this assignment a Kier X site (proposed site of lipophilic interaction with the sweetener receptor; Kier, 1972) lies in the center of the A-ring o-hydroxypropionyl unit. For compound **1a**, two conformations that map AH, B, and X sites into

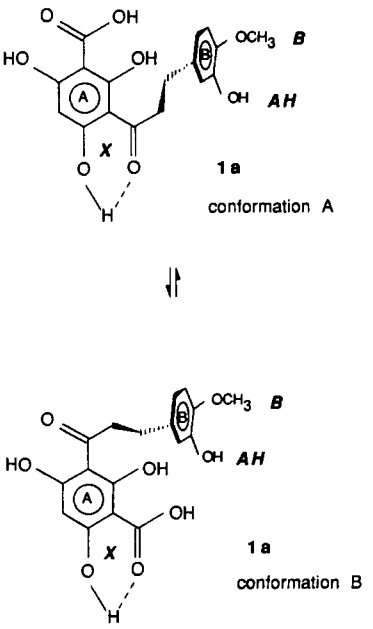


Figure 2. Conformations of **1a** containing similar AH/B/X tripartite sweet pharmacophores.

identical position are possible (Figure 2). Flipping conformation A over (180° through the plane of the page, followed by anticlockwise rotation by 60°) allows the 3′-carboxy-4′-hydroxy unit to form the X site of conformation B. This leaves the aliphatic chain and B-ring projecting out from the plane of the page. Rotating the aliphatic chain to project the B-ring back into the page allows the 3-hydroxy and 4-methoxy groups of conformation B to occupy AH and B positions identical with those of conformation A. These contortions are easily seen if models are constructed. The existence of more than one conformational form of **1a** being able to fit, bind, and activate the sweetener receptor might therefore be the reason that a 3′-carboxylic acid produces enhanced sweetness potency.

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