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Suppression of the Sweetness of 2′,3-Dihydroxy-4-methoxydihydrochalcone by α-Hydroxylation

James G. Sweeny and Guillermo A. Iacobucci*

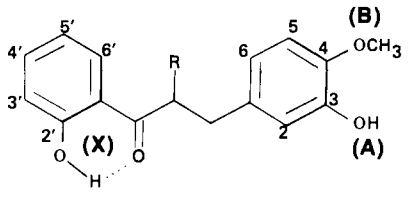
The introduction of an α-hydroxy group to 2′,3-dihydroxy-4-methoxydihydrochalcone (1) has been found to eliminate its sweet taste. This finding has been rationalized following current ideas on the structure–activity relationships of sweet-tasting compounds.

The hypotheses of Shallenberger and Acree (1967) on the structure–activity relationships among sweet-tasting compounds, and the more recent theoretical studies of Kier (1972), have led to the current concept that a sweet molecule interacts with the sweet-taste receptors in a precise fashion involving three binding sites: A, B, and X. Site A is a weak proton-donating group, B is an electronegative atom, and X is a group involved in dispersion interaction (London forces) with the receptor.

The model for glucophore as hypothesized by Kier resulted from the examination of a wide variety of sweet-tasting molecules. It imposes on the three sites a fixed spatial relationship, with distances A–B ~ 2.6 Å, A–X ~ 3.5 Å, and B–X ~ 5.5 Å (Kier, 1972).

Recently, these concepts have been extended by DuBois et al. (1977) to the case of dihydrochalcone sweeteners. It was found that, in order to satisfy Kier's model, the structures should be bent around the flexible $-CH_2CH_2-$ chain to place the 3-OH (site A) and the 4-OCH_3 (site B) in close proximity (about 8 Å) to the H-bonded *o*-hydroxycarbonyl group (site X). The resulting "U-shaped" conformation is characterized by having a pair of hydrophilic legs and a hydrophobic region at the elbow.

The recent disclosure by Yamato et al. (1977) that the simple dihydrochalcone 1 is sweet (100 times the sweetness of sucrose on a weight basis) has prompted us to synthesize the α-hydroxy analogue **2** in an attempt to determine what effect the placing of a hydrophilic substituent at the hydrophobic elbow would have on the sweetness of 1.



(1) R = H

(2) R = OH

Although its sweetness is not as clean as sucrose and hence is of limited applicability, 1 can be readily synthesized and is a good model for sweet dihydrochalcones.

The synthesis of **2** was approached through the metal–ammonia reduction of the properly substituted flavonol, a reaction known to yield primarily α-hydroxydihydrochalcones (Sweeny et al., 1977).

Our first attempt was the Na/NH_3 reduction of the flavonol **3**, readily prepared in 56% yield by the H_2O_2–NaOH oxidation of the chalcone **4** (Yamaguchi, 1960). Instead of the expected α-hydroxydihydrochalcone, the reaction gave the debenzylated flavonol **5** in 46% yield, along with a complex mixture of minor products. Under the Birch conditions, therefore, debenzylation proceeded quite readily and precluded the reduction of the flavonol ring.

To overcome this problem, the methoxymethyl flavonol **7** was synthesized (47%) via H_2O_2–NaOH oxidation of the chalcone **6**.

Birch reduction of **7** for 1 h using an excess of sodium then afforded four major products **8**–**11** in the yields in-

Corporate Research and Development Department, The Coca-Cola Company, Atlanta, Georgia 30301.

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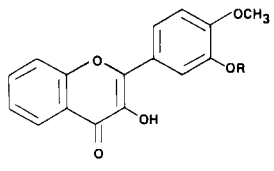
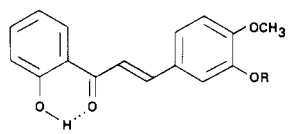
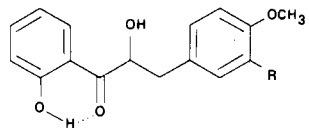


(3) R = $CH_2C_6H_5$
(5) R = H
(7) R = CH_2OCH_3

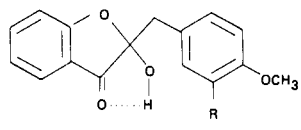


(4) R = $CH_2C_6H_5$
(6) R = CH_2OCH_3

dicated below, and their structures were ascertained from their NMR and MS data (see Experimental Section).



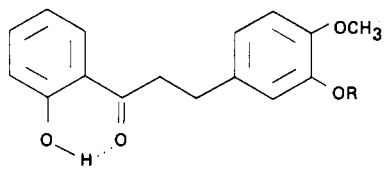
(8) R = H (4%)
(9) R = OCH_2OCH_3 (11%)



(10) R = H (15%)
(11) R = OCH_2OCH_3 (26%)

The formation of both α-hydroxydihydrochalcone **9** and α-hydroxy-2-benzyl-2(3*H*)-benzofuranone **11** is consistent with the results obtained with flavonol itself (Sweeny et al., 1978). The loss of the methoxymethoxy side chain in the formation of **8** and **10** has precedence in the literature (Birch, 1947), where it was found that anisole could be obtained in good yield upon reduction of guaiacol methoxymethyl ether with Na/NH_3.

Refluxing **9** with methanolic HCl for 15 min removed the methoxymethyl group to give α-hydroxydihydrochalcone **2** as a white solid, mp 114–5 °C. For comparison purposes, dihydrochalcone **1** was prepared in two steps by catalytic reduction of **6** to afford **12** (mp 61–2 °C), followed by demethoxymethylation in methanolic HCl.



(12) R = CH_2OCH_3
(1) R = H

Taste test of **1** and **2** by a group of six volunteers, at the 0.1% level in 50% EtOH–H_2O, indicated the former to be sweet as reported (Yamato et al., 1977) and the latter insipid.

These results suggest that the presence of a hydroxy group at the $-CH_2CH_2-$ bridge could prevent sweetness by interfering with the proper folding of **2** suggested by Dubois et al. (1977). Alternatively, the $-CH_2CH_2-$ group may have a direct function in the binding via a lypophilic interaction which is destroyed by introduction of a hydrophilic OH group. A hydrophobic binding site has recently been postulated for the dipeptide sweeteners (Van der Heyden et al., 1978; Ariyoshi, 1976).

EXPERIMENTAL SECTION

Melting points are uncorrected. ^1H NMR spectra were recorded in $CDCl_3$ unless otherwise stated. Chemical shifts are given in parts per million downfield from Me_4Si. Coupling constants (*J*) are in Hz. Abbreviations: s = singlet; b.s. = broad singlet; d = doublet; t = triplet; m = multiplet. Ammonia was supplied by Matheson and was used without further purification. All other reagents were also used as received from the supplier and were reagent grade. Microanalyses were performed by Galbraith Analytical Laboratories, Knoxville, TN.

Reduction of 3-Hydroxy-3′-benzyloxy-4′-methoxyflavone (3). Treatment of 500 mg (1.34 mmol) of **3** with 200 mg of Na in a mixture of 15 mL of THF and 15 mL of NH_3 for 1 h as previously described (Sweeney et al., 1977) gave a light-yellow oil. Trituration with $CHCl_3$ yielded 187 mg (46%) of 3,5-dihydroxy-4-methoxyflavone **5**, mp 203.5–5 °C (MeOH–H_2O), lit. mp 203–3.5 °C (Yamaguchi, 1960).

4-Methoxy-3-methoxymethoxy-2′-hydroxychalcone (6). A mixture of 2.0 g (1.02 mmol) of 4-methoxy-3-methoxymethoxybenzaldehyde (Venturella, 1958), 1.5 g (1.10 mmol) of *o*-hydroxyacetophenone and 15 mL of 95% EtOH was cooled in ice, and a solution of 2 g of KOH in 3 mL of H_2O added. After standing for 24 h at room temperature the mixture was poured into 100 mL of 3% HOAc and extracted with 3 × 25 mL of $CHCl_3$. Drying and evaporating the $CHCl_3$ gave a yellow oil which crystallized from EtOAc–hexane to afford 1.51 g (0.48 mmol, 47%) of **6**: mp 94–4.5 °C; NMR 3.55 (3 H, s OCH_3), 3.90 (3 H, s, OCH_3), 5.30 (2 H, s, OCH_2O), 6.6–8.0 (9 H, m, ArH and –HC=); MS *m/e* (rel intensity): 314 (72), 269 (28), 181 (34), 147 (34) and 121 (100); IR (KBr) cm^{-1} 1635, 1560, 1360, 1140, 1005, and 755. Calcd for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 69.05; H, 5.86.

4′-Methoxy-3′-methoxymethoxy-3-hydroxyflavone (7). To a solution of 5.0 g (15.9 mmol) of chalcone **6** in 240 mL of 50% MeOH–acetone was added 21 mL of 1 N NaOH and 14 mL of 30% H_2O_2 at 5 °C. The mixture was kept in an ice bath for 3 h, then neutralized with 1.8 mL of HOAc and water was added until a faint cloudiness persisted (~100 mL). Storage at 5 °C for 18 h produced 2.45 g (7.46 mmol, 47%) of **7** as a pale-yellow solid: mp 158–9 °C ($CHCl_3$–hexane); MS *m/e* (rel intensity) 328 (48), 298 (9), 296 (8), 284 (9), 283 (9), 266 (7), 238 (9), and 45 (100); IR (KBr) cm^{-1} 3220, 1605, 1510, 1475, 1410, 1265, 1135 and 750. Calcd for $C_{18}H_{16}O_6$: C, 65.85, H, 4.91. Found: C, 65.78; H, 5.00.

1-(2-Hydroxyphenyl)-3-(4-methoxy-3-methoxymethoxyphenyl)propanone (12). To a solution of 500 mg (1.60 mmol) of chalcone **6** in 30 mL of EtOAc was added 25 mg of 5% Pd/C and the mixture hydrogenated for 1 h under 1 atm H_2. Uptake stopped at 40 mL (1.8 mmol). Filtration followed by evaporation of the filtrate gave a thick oil which was crystallized from $CHCl_3$–hexane at −10 °C to yield 342 mg (68%) of dihydrochalcone (12), mp 61–2 °C; NMR 3.1 (4 H, m, CH_2), 3.50 (3 H, s, OCH_3), 3.80 (3 H, s, OCH_3), 5.20 (2 H, s, OCH_2O), 6.7–7.8 (7 H, m, ArH); IR (KBr) cm^{-1} 2965, 2830, 1645, 1510, 1490, 1445, 1250, 1200, 1150, 1130, 1075, 1000, 755; MS *m/e* (rel intensity) 316 (35), 256 (9), 178 (17), 151 (38), 137 (22), 121 (100), 45 (96). Calcd for $C_{18}H_{20}O_5$: C, 68.34; H, 6.37. Found: C, 68.15; H, 6.42).

1-(2-Hydroxyphenyl)-3-(4-methoxy-3-hydroxyphenyl)propanone (1). A solution of 100 mg of dihydrochalcone (12) (0.32 mmol) in 4 mL of MeOH containing 1 drop 6 N HCl was heated at reflux for 15 min. Upon cooling it was poured into 20 mL of 0.1% $NaHCO_3$ and extracted with 2 × 20 mL of $CHCl_3$. Drying and evaporating the $CHCl_3$ afforded a light-yellow oil. Crystallization from EtOH–H_2O gave 74 mg (0.27 mmol, 85%) of **1** mp 98–9 °C; NMR 2.7–3.3 (4 H, m, CH_2), 3.65 (3H, s, OCH_3), 6.5–7.6 (7 H, m, ArH); IR (KBr) cm^{-1} 3450, 3380, 1640, 1585, 1510, 1490, 1445, 1365, 1295, 1255, and 750; MS *m/e* (rel intensity) 272 (89), 254 (15), 239 (9), 151 (30), 137 (100), 121 (67), and 93 (13). Calcd for $C_{16}H_{16}O_4$:

C, 70.57; H, 5.92. Found: C, 70.62; H, 6.01.

Birch Reduction of 4′-Methoxy-3′-methoxymethoxy-3-hydroxyflavone (7). One gram (3.05 mmol) of **7** was reduced with 250 mg (11 mmol) of Na in 50 mL of liquid NH_3 as described above. The combined products from three reactions were chromatographed on a 35 × 2.5 cm silica gel column using 15 and 20% EtOAc in hexane as eluant. The four products in order of elution were as follows.

(*a*) *1-(2-Hydroxyphenyl)-2-hydroxy-3-(4-methoxyphenyl)propanone* (**8**). 106 mg (4%) as an oil. NMR 3.1 (2 H, m, CH_2), 3.80 (3 H, s, OCH_3), 5.30 (1 H, m, C_2H), and 6.6–7.8 (8 H, m, ArH); IR (neat) cm^{-1} 3470, 2965, 1640, 1610, 1510, 1300, 1245, and 750; MS *m/e* (rel intensity) 272 (2), 254 (2), 151 (3), 134 (2), 123 (4), 122 (11) and 121 (100); *Oxime*, mp 138–9.5 °C; IR (KBr) cm^{-1} 3320, 3260, 1610, 1515, and 1250; MS *m/e* (rel intensity) 287 (2), 121 (100), 91 (34). Calcd for $C_{16}H_{17}NO_4$: C, 66.88; H, 5.96; N, 4.88. Found: C, 66.74; H, 6.27; N, 4.88.

(*b*) *2-Hydroxy-2-(4-methoxybenzyl)-2(3H)-benzofuranone* (**10**). 376 mg (1.39 mmol, 15%); mp 117–8.5 °C, lit. mp 120 °C (Chopin et al., 1964); IR (KBr) cm^{-1} 1720; MS *m/e* (rel intensity) 270 (8), 122 (13), 121 (100), 91 (7), 78 (12), and 77 (12).

(*c*) *1-(2-Hydroxyphenyl)-2-hydroxy-3-(4-methoxy-3-methoxymethoxyphenyl)propanone* (*9*). 336 mg (1.01 mmol, 11%) oil. Further purified by preparative TLC on silica gel. NMR 3.05 (2 H, m, CH_2), 3.45 (3 H, s, OCH_3), 3.85 (3 H, s, OCH_3), 5.15 (2 H, s, OCH_2O), 5.30 (1 H, m, C_2H), and 6.8–7.8 (7 H, m, ArH); IR (neat) cm^{-1} 3480, 2960, 1645, 1510, 1445, 1265, 1155, 1135, 1080, 1000, and 755; MS *m/e* (rel intensity) 332 (13), 182 (14), 181 (100), 151 (12), 137 (15), and 121 (33).

(*d*) *2-Hydroxy-2-(4-methoxy-3-methoxymethoxybenzyl)-2(3H)-benzofuranone* (**11**). 787 mg (2.38 mmol, 26%) oil. NMR 3.15 (2 H, b.s., CH_2), 3.45 (3 H, s, OCH_3), 3.75 (3 H, s, OCH_3), 5.15 (2 H, s, OCH_2O), and 6.7–7.7 (7 H, m, ArH); IR (neat) cm^{-1} 3380, 2960, 1715, 1610, 1460, 1255, 1140, and 1000; MS *m/e* (rel intensity) 330 (4), 314 (3), 182 (23), 181 (100), 151 (16), 137 (38), 121 (31), 88 (22), and 70 (44). *Dioxime*, mp 188–9.5 °C; IR (KBr) cm^{-1} 3260, 1515 and 1365; MS *m/e* (rel intensity) 360 (1), 342 (9), 207 (14), 181 (13), 177 (13), 163 (15), 151 (12), 148 (14), 137 (16), 121 (18), 120 (27), 119 (58), 92 (38), and 91 (100). Calcd for $C_{18}H_{20}N_2O_6$: C, 59.99; H, 5.59; N, 7.77. Found: C, 59.29; H, 5.51; N, 7.64.

1-(2-Hydroxyphenyl)-2-hydroxy-3-(4-methoxy-3-hydroxyphenyl)propanone (2). A solution of 250 mg (0.75 mmol) of **9** in 10 mL of MeOH containing 2 drops of 6 N HCl was heated at reflux for 15 min and then added to 100 mL of 0.5% $NaHCO_3$. Extraction with 3 × 25 mL of $CHCl_3$ and drying and evaporation of the $CHCl_3$ gave a yellow oil. Crystallization from EtOAc–hexane afforded 196 mg (0.68 mmol, 91%) of **2**: mp 114–5 °C; NMR 3.0 (2 H, m, CH_2), 3.80 (3 H, s, OCH_3), 5.30 (1 H, m, C_2H), and 6.7–7.8 (7 H, m, ArH); IR (KBr) cm^{-1} 3430, 3260, 1650, 1520, 1450, 1240, 1130, 1085, and 975. MS *m/e* (rel intensity) 288 (5), 256 (5), 270 (10), 138 (16), 137 (100), 122 (13), and 121 (33). Calcd for $C_{16}H_{16}O_5$: C, 66.66; H, 5.59. Found: C, 66.53, H, 5.56.

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Formation of (*E*)-Hex-2-enal and (*Z*)-Hex-3-en-1-ol by Fresh Leaves of *Brassica oleracea*

Alexander J. MacLeod* and Harris E. Pikk

The production of (*E*)-hex-2-enal and (*Z*)-hex-3-en-1-ol by fresh leaves of cabbage has been shown to be due to a series of reactions, starting with the action of lipoxygenase on linolenic acid. The 13-hydroperoxide of linolenic acid thus formed decomposes due to the action of inactivated hemoprotein enzyme catalyst to give (*Z*)-hex-3-enal. This can then both isomerize to give (*E*)-hex-2-enal and it can also be reduced by alcohol dehydrogenase/NADH to (*Z*)-hex-3-en-1-ol. No obvious explanation could be found for these reactions occurring almost exclusively in the outer leaves of the plant.

(*E*)-Hex-2-enal (*trans*-hex-2-enal) and (*Z*)-hex-3-en-1-ol (*cis*-hex-3-en-1-ol) are extremely common aroma products of green leaves, so much so that they have been termed "leaf aldehyde" and "leaf alcohol". Many examples can be quoted of their production from fresh foliage (e.g., Walbaum, 1918; Bedoukian, 1963; Hatanaka and Ohno, 1971; Major et al., 1972; Major and Thomas, 1972), vegetables (e.g., Schormuller and Grosch, 1962; Forss et al., 1962; Eriksson 1967; Fleming et al., 1968; MacLeod and MacLeod, 1968, 1970a; Kazeniac and Hall, 1970; Buttery et al., 1971), and fruits (e.g., Winter and Sundt, 1962; Anderson and von Sydow, 1964; Drawert et al., 1966; Anjou

Department of Chemistry, Queen Elizabeth College, London University, London W.8. 7AH, United Kingdom.

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