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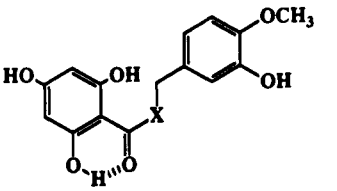
Isovanillyl Sweeteners. Amide Analogues of Dihydrochalcones

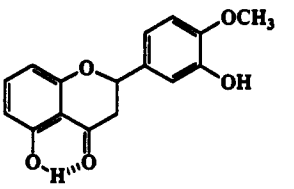
Leland J. Chinn,*[1] D. Eric Walters, Kenneth Williams, and Robert H. Mazur

Conformational energy calculations of hesperetin dihydrochalcone and its corresponding amide in which an NH group has been substituted for the ketone α-methylene group exhibit global energy minima in the extended conformation. In contrast, the dihydrochalcone amide analogue in which an N-CH_3 group has replaced the ketone α-methylene group exhibits global energy minimum when the molecule adopts a folded conformation as observed with the sweet flavanones. Although hesperetin dihydrochalcone is sweet, neither of the amides was found to be sweet. The N-CH_3 analogue was found to be bitter. Either increased hydrophobicity about the carbonyl region or the added bulk of the methyl group may be the cause for the bitterness. The steric effect of the methyl group may interfere with binding to the sweetness receptor.

In the course of studying the temporal properties of the dihydrochalcone sweeteners **1a**, DuBois et al. (1977) concluded that the active conformer of the sweet dihydrochalcones is folded as in a flavanone. Some flavanones such as **2** are potently sweet (Yamato et al., 1977). This report describes an amide analogue of the dihydrochalcone sweeteners that was expected, on the basis of conformational energy calculations, to be able to adopt a folded conformation similar to that of the flavanones.



1a X = CH_2
1b X = NH
1c X = N-CH_3



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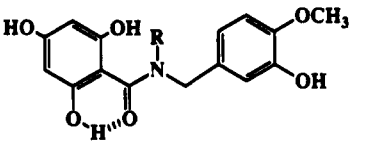

3a R = H
3b R = Me

EXPERIMENTAL SECTION

Conversion of Phloroglucinolcarboxylic Acid Tris(methylcarbonate) to the Acid Chloride 5b. An 800-mg sample of acid **5a** was dissolved in 3.5 mL of $CHCl_3$. To this solution was added 600 mg of PCl_5. The reaction mixture was stirred at room temperature for 1.5 h. The residual solid was removed by filtration. The filtrate was evaporated to dryness at room temperature under reduced pressure to afford **5b** as a viscous oil. The oil was dried at room temperature in vacuo for an additional 5.5 h before further use.

Preparation of Isovanillin Oxime. A mixture of 72.4 g of isovanillin, 65.2 g of hydroxylamine hydrochloride, 80 mL of pyridine, and 400 mL of MeOH was heated under reflux for 16 h. The MeOH was removed by distillation under reduced pressure. The residue was extracted with EtOAc. The EtOAc extract was washed successively with H_2O, 3 N HCl, and H_2O again, dried (Na_2SO_4), and concentrated until crystals appeared. The crystalline oxime was collected: yield 73 g; mp 142–145 °C. Anal. Calcd for $C_8H_9NO_3$: C, 57.48; H, 5.43; N, 8.38. Found: C, 57.11; H, 5.31; N, 8.38.

Preparation of Isovanillylamine (4a). A 29.3-g sample of isovanillin oxime was reduced in 300 mL of EtOH over 8.8 g of Raney Ni. After the calculated amount of hydrogen had been adsorbed, the catalyst was removed by filtration. The filtrate was concentrated until crystals appeared. The crystalline product **4a** was collected; mp 150–158 °C. Anal. Calcd for $C_8H_{11}NO_2$: C, 62.79; H, 7.24; N, 9.14. Found: C, 62.16; H, 7.04; N, 8.50. The low nitrogen analysis is due to the presence of isovanillyl alcohol formed as a byproduct in the reduction. It can be removed either by chromatography or by sublimation at 100 °C (0.1

New Sweetener Research Group, NutraSweet Research and Development, Mt. Prospect, Illinois 60056.
[1]Present address: Chemistry Department, Biola University, La Mirada, CA 90639.

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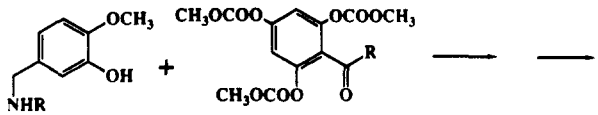
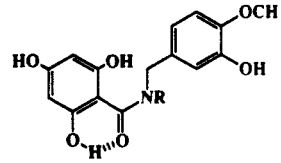
mm). A sample of **4a** after sublimation gave the following results. Anal. Found: C, 62.60; H, 7.41; N, 8.92.

Preparation of *N*-Isovanillylphloroglucinolcarboxamide (1b). To a solution of approximately 800 mg of the acid chloride of phloroglucinolcarboxylic acid tris(methyl carbonate) (**5b**) in 10 mL of CH_2Cl_2 were added 415 mg of isovanillylamine (**4a**) and 0.5 mL of pyridine.



4a R = H
4b R = CH_3

5a R = OH
5b R = Cl



1b R = H
1c R = CH_3

The reaction mixture was stirred at room temperature for 2 h after which it was diluted with H_2O. The CH_2Cl_2 phase was separated and washed successively with 5% HCl, H_2O, 5% $NaHCO_3$, and H_2O again, dried (Na_2SO_4), and evaporated to dryness to afford a yellow viscous oil. The oil was treated with 20 mL of 10% KOH. The reaction mixture was stirred at room temperature for 4 h. It was then extracted with EtOAc. The EtOAc extract was discarded. The aqueous solution was acidified with 3 N HCl. The acidified mixture was extracted with EtOAc. The EtOAc extract was washed successively with H_2O, 5% $NaHCO_3$, and H_2O again, dried (Na_2SO_4), and evaporated to dryness to afford 290 mg of a pink viscous oil. Crystallization from MeOH–H_2O furnished 121 mg of the amide **1b** as a salmon-colored crystalline product. The product melted, resolidified between 120 and 140 °C, and then remelted at 210–211 °C. The NMR and IR spectra are in complete accord with those reported by DuBois et al. (1981b). Anal. Calcd for $C_{15}H_{15}NO_6 \cdot 0.25H_2O$: C, 58.15; H, 5.04; N, 4.52. Found: C, 58.21; H, 5.05; N, 4.34.

Preparation of Isovanillylmethylamine (4b). A 10-g sample of isovanillin and 30 g of methylamine were hydrogenated in 150 mL of EtOH over 5% Pd/C. After the calculated amount of hydrogen was adsorbed, the catalyst was removed by filtration. The filtrate was evaporated to dryness. The solid residue was crystallized from EtOAc to furnish **4b**: mp 141–143 °C; IR (KBr) 3680 (OH), 3540 (NH), 1590 (aromatic C=C) cm^{-1}; NMR (Me_2SO-d_6, 80 MHz) δ 525–547 (m, aromatic H), 298 (OCH_3), 277 (ArC-H_2N), 178 (CH_3N) Hz. Anal. Calcd for $C_9H_{13}NO_2$: C, 64.65; H, 7.83; N, 8.38. Found: C, 64.74; H, 7.46; N, 8.35.

Preparation of *N*-Isovanillyl-*N*-methylphloroglucinolcarboxamide (1c). To a solution of 8.0 g of the acid chloride **5b** of phloroglucinolcarboxylic acid tris(methyl carbonate) in 100 mL of CH_2Cl_2, cooled in an ice bath, were added 3.2 g of isovanillylmethylamine (**4b**) and 15 mL of $(C_2H_5)_3N$. The reaction mixture was stirred at room temperature for 69 h and then acidified with 1.2 N HCl. The CH_2Cl_2 phase was separated, washed with H_2O, dried (Na_2SO_4), and distilled to dryness under reduced pressure to afford a lightly colored foam. TLC of the foam indicated it was essentially one substance.

The foam was dissolved in 150 mL of H_2O containing 15 g of KOH. The reaction mixture was stirred at room temperature for 6 h after which it was extracted with EtOAc. The EtOAc extract was discarded. The aqueous solution was acidified with 6 N HCl. A gum separated out and was removed. The acidified solution was extracted with *n*-BuOH. The *n*-BuOH extract was washed with H_2O, dried (Na_2SO_4), and distilled to dryness under reduced pressure. The residual oil was dissolved in dioxane, and the dioxane solution, after filtration, was evaporated to dryness. The residue was crystallized from EtOAc to afford 2.7 g of **1c** as a yellow crystalline product, mp 146–147 °C; **1c** appeared to be solvated: IR (KBr) 1620 (aromatic C=C) cm^{-1}; NMR (Me_2SO-d_6, 80 MHz) δ 738 (OH), 728 (OH), 697 (OH), 527–550 (m, aromatic H), 484 ($ArCH_2$), 285 (CH_3O), 215 (CH_3N). Anal. Calcd for $C_{16}H_{17}NO_6 \cdot 1.5C_4H_8O_2$: C, 58.53; H, 6.47; N, 3.10. Found: C, 58.10; H, 5.89; N, 3.43.

Computational Procedures. Conformational energy calculations were carried out with Chemlab II (Molecular Design Ltd., San Leandro, CA). Structures were generated from standard bond lengths and angles and then fully optimized with a modified version of Allinger's MM2 program (Allinger, 1977; Allinger and Yuh, 1980), with additional parameters generated by the method of Hopfinger and Pearlstein (1984). Conformational energies as a function of torsional rotation were calculated with the same program, rotating the torsional angle indicated in increments of 15° or 30°, fixing the torsion angle, and allowing the remainder of the molecule to fully optimize.

RESULTS AND DISCUSSION

Replacement of the ketone α-methylene group in hesperetin dihydrochalcone **1a** with an NH group affords **1b**, which DuBois et al. (1981b) found to be tasteless. The tasteless nature of **1b** was attributed to its being exclusively in the extended conformation (**3a**) as a result of amide resonance. In order to confirm the observation of DuBois and co-workers, we prepared **1b** for tasting. However, our synthetic approach was different from that utilized by DuBois et al. In their approach, the synthesis was initiated with an electrophilic attack of chlorosulfonyl isocyanate on 1,3,5-tris(benzyloxy)benzene. We chose a more direct route, which we felt would have more general applicability. Isovanillylamine (**4a**), prepared by hydrogenating isovanillin oxime, was acylated with acid chloride **5b** of phloroglucinolcarboxylic acid tris(methyl carbonate) (Fischer and Strauss, 1914). The resultant product was hydrolyzed with aqueous potassium hydroxide to furnish **1b**. Our product was identical with that of DuBois et al. Confirming their report, we found **1b** to be tasteless.

We carried out conformational energy calculations on the dihydrochalcone **1a** and its amide analogue **1b**. As expected, the hydrogen-bonded hydroxyaryl ketone system has a strong tendency to remain planar. Figure 1 shows the energy as a function of rotation about the CO–X bond for the two molecules. These calculations were carried out by fixing the Ar–CO–X–CH_2 torsion angle in 15° or 30° increments and optimizing the bond lengths, bond angles, and torsion angles of the rest of the molecule. Both molecules exhibit a global energy minimum when the torsion angle Ar–CO–X–CH_2 is 180° (extended conformation). The dihydrochalcone **1a** also has low-energy minima (approximately 1.9 kcal/M above the global minimum) when the torsion angle is 90° or 270° (folded conformations). For the amide **1b**, there are no low-energy conformers other than the extended one; the folded conformers are expected to be at least 7 kcal/M above the global minimum.

Since both **1a** and **1b** exhibit a global energy minimum when the torsion angle Ar–CO–X–CH_2 is 180°, the sweetness of **1a** is likely due to the existence of the low-energy folded conformers (Ar–CO–X–CH_2 = 90° or 270°). This stimulated us to introduce an *N*-methyl group into

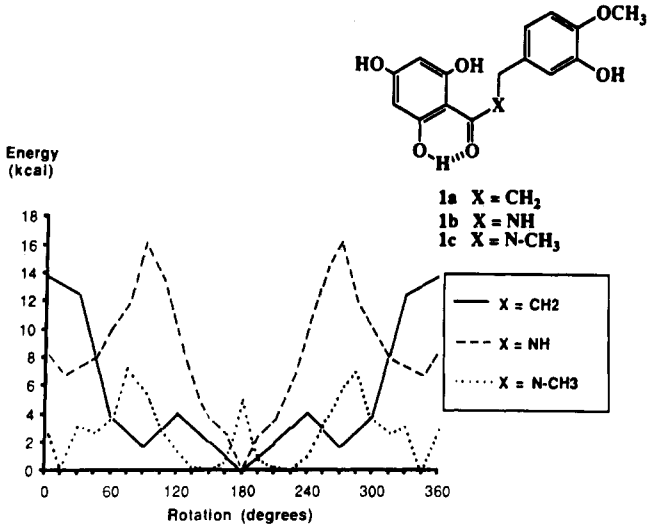


Figure 1. Conformational energy as a function of rotation about the CO–X bond for compounds **1a** (X = CH_2), **1b** (X = NH), and **1c** (X = *N*-CH_3).

1b to produce **1c**. Conformational energy calculations on **1c** (Figure 1) indicate that it exhibits global energy minima when the torsion angle Ar–CO–X–CH_2 is 150° or 210° (approximately extended conformation). However, folded conformers approximating the conformation of structure **2** should also be relatively stable, for there are two local energy minima at torsion angles of 15° and 345°, less than 1 kcal/M above the global minima. The likelihood that a significant percentage of the molecules of **1c** will exist in folded conformations led us to predict that **1c** would be sweet.

Synthesis of **1c** was accomplished in the manner described for the synthesis of **1b**. Isovanillylmethylamine (**4b**), prepared by reductive amination of isovanillin with methylamine, was acylated with the acid chloride **5b** (Fischer and Strauss, 1914). The product was hydrolyzed to afford **1c**. Prior to sensory analysis, our sample of **1c** was established to have a purity of greater than 95% by HPLC. It was negative in the Ames *Salmonella*/microsome assay (Ames et al., 1975) using five tester strains of *Salmonella typhimurium* in both the presence and absence of an Arochlor-induced rat liver homogenate metabolic activation system. An acute oral toxicity test of **1c** was conducted in fasted mice at a dosage of 50 mg/kg. There were no deaths or other signs of treatment in the animals over a period of 7 days. Sensory analysis indicated that **1c** produces only a bitter taste.

It is interesting to speculate as to why **1c** is bitter and not sweet. The presence of the *N*-methyl group may increase the hydrophobicity about the carbonyl region, which could result in the molecule being bitter rather than sweet (DuBois and Stephenson, 1985; DuBois et al., 1981a). An alternative possibility is that in the postulated sweet conformer of **1a**, the CH_2–Ar′ group is essentially orthogonal to the plane of the rest of the molecule (Figures 1 and 2a). In contrast, **1c** and **1b** prefer to have the CO–N–CH_2

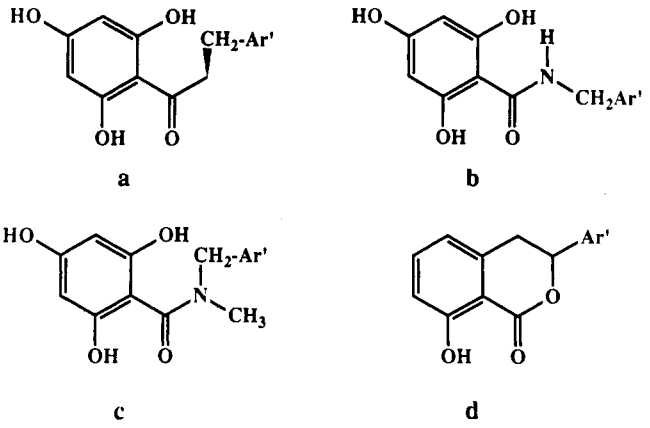
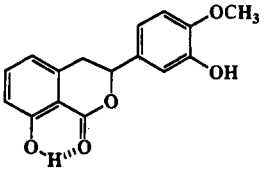


Figure 2. Some accessible conformations of dihydrochalcones and other compounds in the present study: (a) dihydrochalcone **1a**; (b) amide analogue **1b**; (c) *N*-methylamide analogue **1c**; (d) phyllodulcin (**6**).

system nearly coplanar with the trihydroxyphenyl system (Figures 1 and 2b,c). Although neither **1b** nor **1c** is sweet, phyllodulcin (**6**) is potently sweet despite the fact that the



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phenyl–CO–X–CH_2 system can also be regarded as lying essentially in one plane (Figure 2d). Thus, it is possible that either increased hydrophobicity about the carbonyl region or the added steric bulk of the methyl group causes **1c** to be bitter rather than sweet. The bulkiness of the methyl group could sterically interfere with binding to the sweetness receptor.

Registry No. **1a**, 35400-60-3; **1b**, 76820-15-0; **1c**, 107408-11-7; **4a**, 89702-89-6; **4b**, 54542-57-3; **5a**, 107408-12-8; **5b**, 107408-13-9; $HONH_2$·HCl, 5470-11-1; $MeNH_2$, 74-89-5; isovanillin, 621-59-0; isovanillin oxime, 51673-94-0.

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