Non-Caloric Sweeteners, Sweetness Modulators, and Sweetener Enhancers

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Abstract

For a new sweetness technology to realize strong commercial success, it must be safe, exhibit good taste quality, be sufficiently soluble and stable in food and beverage systems, and be cost effective and patentable. Assessments of the commercial promise of eight synthetic and eight natural non-caloric sweeteners are made relevant to these metrics. High-potency (HP) non-caloric sweeteners, both synthetic and natural, are generally limited in taste quality by (*a*) low maximal sweetness response, (*b*) "off" tastes, (*c*) slow-onset sweet tastes that linger, and (*d*) sweet tastes that adapt or desensitize the gustatory system. Formulation approaches to address these limitations are discussed. Enhancement of the normal sucrose sensory response by action of a sweetener receptor positive allosteric modulator (PAM) has been achieved with very significant calorie reduction and with retention of the taste quality of sucrose. Research on PAM discovery over the past decade is summarized.

INTRODUCTION

High potency (HP): synthetic and natural non-caloric sweeteners are most commonly referred to as HP sweeteners

High-throughput screening (HTS): technology typically employing cell-based assays and robotics enabling assay of tens of thousands of compounds per day

Postive allosteric modulator (PAM): compound that enhances the activity of the sweetener receptor through binding at an allosteric site

Maximal response (R_m): Intensity of sweetness that is the maximal intensity a sweetener will provide in sucrose equivalent units

Dissociation constant (K_d): sweetener/receptor complex equilibrium constant for dissociation in m L^{-1} commonly estimated by least squares regression analysis of C/R data to fit law of mass action function $R = Rm\ C/(K_d + C)$

Response (R): intensity of sweetness in sucrose equivalent units

Sucrose equivalent (SE): measure of sweetness intensity (e.g., 10.0% sucrose w/v = 10 SE sweetness intensity)

Organic chemists have identified a great many synthetic and natural sweet-tasting compounds since the early 1800s. Most of these are much more potent than the common carbohydrate (CHO) sweeteners and are generally referred to as high-potency (HP) sweeteners. A source of fascination for chemists is the diversity of chemical structure among sweeteners, with at least 50 structural classes of organic compounds represented (Marie & Piggott 1991, Shallenberger 1993, DuBois 2000, O'Brien Nabors 2001, Kim & Kinghorn 2002, DuBois et al. 2008b). Most, however, are not commercially viable. To have high commercial potential, a sweetener must rank acceptably on all of the six metrics: safety, taste quality, stability, solubility, cost, and patentability (DuBois 2008a). This is an extreme challenge. Given historical knowledge, the discovery of new and improved sweeteners within existing structural classes is straightforward. However, consumers strongly prefer the taste of sucrose, and HP sweeteners that closely replicate sucrose taste have not been found. HP sweeteners commonly show (*a*) low maximal sweetness intensities, (*b*) "off" tastes (e.g., bitter, metallic, and licorice-like), (*c*) slow-onset sweet tastes that linger, and (*d*) desensitizing sweet taste.

Early in the past decade, work by Charles Zuker (University of California, San Diego), Nicholas Ryba (National Institutes of Health), and their coworkers led to the discovery of the rat sweetener receptor (Nelson et al. 2001). Follow-up work by Xiaodong Li and coworkers (Senomyx) led to the human sweetener receptor (Li et al. 2002). These discoveries enabled development of a cell-based assay and high-throughput screening (HTS) of large synthetic and natural product libraries for the discovery of novel sweeteners as well as sweetness enhancers, more properly referred to as positive allosteric modulators (PAMs).

In this review, we discuss commercially available as well as development-candidate synthetic and natural non-caloric sweeteners. In addition, we discuss progress in the modulation of negative taste attributes of non-caloric sweeteners, and in the development of PAMs, ingredients with breakthrough potential.

NON-CALORIC SWEETENERS

The non-caloric sweetener market has developed over the past three decades, addressing the needs of greater than one billion consumers. Thousands of good-tasting zero- and reduced-calorie food and beverage products are available today. However, many consumers express interest in additional products, especially products with natural non-caloric sweeteners. This section covers synthetic and natural non-calorie sweeteners that have been commercialized or are under development.

The relationship between sweetness intensity and concentration for HP sweeteners has been demonstrated to be well modeled by the law of mass action, a hyperbolic function of the form $R = R_mC/(K_d + C)$, where R is the response in units of sucrose equivalence (SE) on a percentage (w/v) basis, R_m is the maximal response in SE units, K_d is the apparent sweetener/receptor dissociation constant, and C is the sweetener concentration in mg L^{-1} (DuBois et al. 1991). These equations are commonly referred to as concentration/response (C/R) functions. Sweetener potencies are readily calculated from C/R functions relative to a specific sucrose reference concentration (e.g., 5% sucrose, 10% sucrose, etc.) and are generally calculated on a weight basis. Thus, for example, $P_w(5) = 100$ for an HP sweetener means that, relative to a 5% (w/v) sucrose reference, the sweetener is 100 × more potent. HP sweetener usage in foods and beverages is regulated according to acceptable daily intake (ADI) values assigned by regulatory agencies following review of safety assessment studies. The ADI (mg kg^{-1} body weight day^{-1}) is defined as 1% of the no observable adverse effect level (NOEL) determined in animal and human safety assessment studies.

Synthetic Sweeteners

The first non-caloric sweeteners discovered were synthetic organic compounds identified at a time when taste and smell were key dimensions in their evaluations. Thus, the earliest report of a sweet-tasting synthetic organic compound is that of *m*-nitroaniline (Muspratt & Hofmann 1846). The discoveries of additional sweeteners continued from routine tasting into the twentieth century. As the fields of spectroscopy and chromatography developed in the second half of the twentieth century, routine sensory assessment of compounds was gradually discontinued. Nonetheless, the sweet tastes of many compounds were discovered serendipitously in the years to follow. In this section, we discuss the eight synthetic non-caloric sweeteners illustrated in **Figure 1**. Of these, some were discovered by routine tasting but most by serendipity.

Concentration/response (C/R): mathematical function that describes the relationship between sweetener concentration and response in sucrose equivalent units of sweetness intensity

Acceptable daily intake (ADI): the amount of a food ingredient in mg kg^{-1} body weight day^{-1} that may be safely consumed over a lifetime and that is defined as 1/100 of the no observable adverse effect level

No observable adverse affect level (NOEL): the highest dose of a chemical compound in mg kg^{-1} body weight day^{-1} given in animal or human studies that does not show an adverse effect

Saccharin. The sweet taste of saccharin (SAC) (**Figure 1*a***) was discovered at Johns Hopkins University in 1878 by Constantine Fahlberg, a postdoctoral research associate of Professor Ira Remsen (Fahlberg & Remsen 1879). SAC was the first HP sweetener to be commercialized. At the turn of the twentieth century, it was the first product of the Monsanto Chemical Company. SAC has been reviewed in DuBois 2006.

Physical and chemical properties. SAC and its sodium (SAC-Na) and calcium (SAC-$Ca_{0.5}$) salts are white crystalline solids. The acid form of SAC is sparingly water soluble (0.2% at 20°C), whereas SAC-Na (100% at 20°C) and SAC-$Ca_{0.5}$ (37% at 20°C) are readily soluble. As a solid, SAC is very stable, and in solution it has excellent hydrolytic, thermal, and photo stability. Stability is not affected by temperatures and pHs normally encountered in food/beverage manufacturing. Because of its stability, SAC can be used in cooking, baking, and confectionery (Pearson 2001).

Sensory properties and applications. The C/R function for SAC-Na in water has been determined to be $R = 10.1C/(115 + C)$ (DuBois et al. 1991). From this equation, the R_m for SAC-Na is 10.1 SE. The practical ramifications of this low R_m are that SAC, by itself, cannot be used for products with high sweetness intensities (e.g., ≥7% SE) and that SAC finds its utility in blends with other sweeteners, most commonly where SAC provides ≤5% SE or applications with low sweetness intensities. Thus, in food or beverage systems where SAC-Na is providing a 5% SE, it exhibits a $P_w(5)$ of 450. SAC-Na also exhibits bitter and metallic "off" tastes, particularly at concentrations approaching its R_m. SAC-Na exhibits a rapid onset of sweetness without significant lingering (referred to as sweetness linger) (DuBois & Lee 1983). Blending with other HP sweeteners, such as cyclamate salts (CYC-Na) or aspartame (APM), markedly reduces "off" tastes and sweetness linger. SAC-Na is synergistic with some HP sweeteners, such as CYC-Na and APM, likely because of cooperative binding effects of the different sweeteners at multiple sites on the sweetener receptor (DuBois 2004). Many commercial beverages today are formulated as SAC-Na blends with APM, CYC, and sucralose (SUL). The ADI for SAC is 5 mg kg^{-1} body weight day^{-1}, sufficient for broad food and beverage application.

Cyclamate. The sweet taste of cyclohexylsulfamic acid, or cyclamate (CYC) (**Figure 1*b***), also known as cyclamic acid, in salt form (CYC-Na) was discovered in 1937 by Michael Sveda working in the laboratory of Professor Audrieth at the University of Illinois (Audrieth & Sveda 1944). CYC discovery and commercialization enabled the first high quality reduced- and zero-cal foods and beverages in the 1950s and 1960s. Prior to that, only SAC was available, and SAC-sweetened foods and beverages exhibited significant "off" tastes to most people. CYC has been reviewed by DuBois (2006).

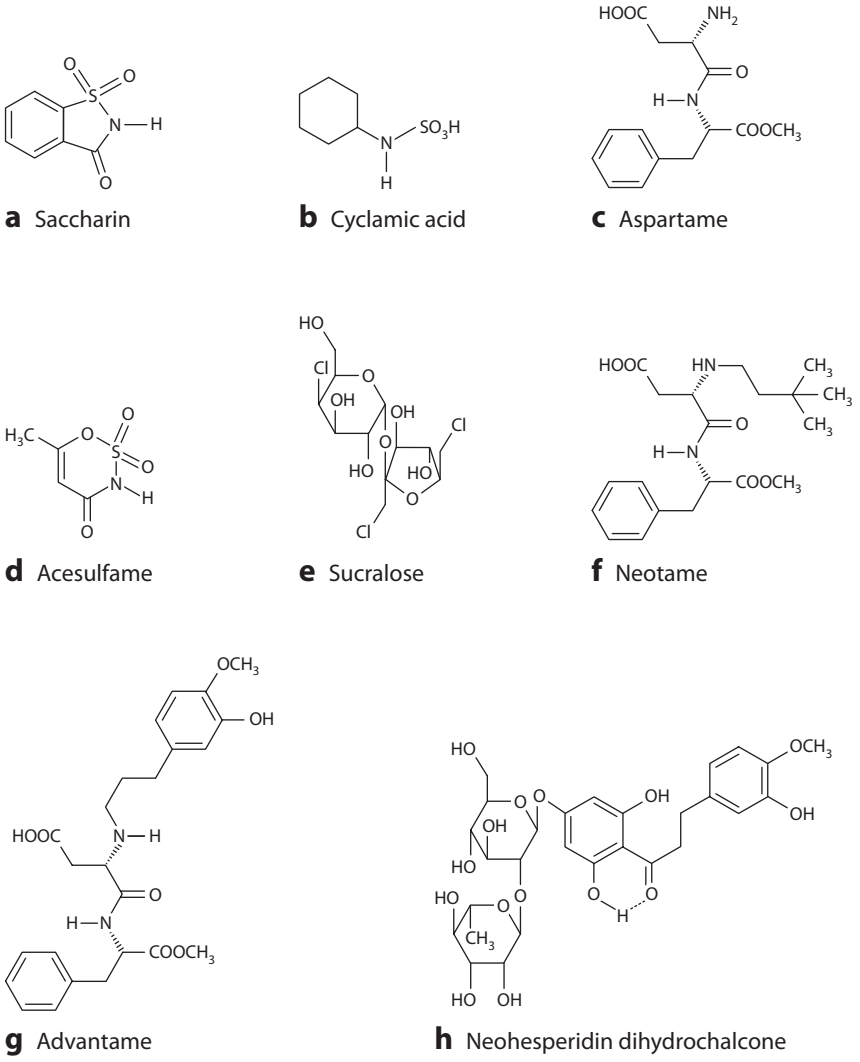


Figure 1

Synthetic compounds used today in foods and beverages as sweeteners or flavors: (*a*) saccharin, (*b*) cyclamic acid, (*c*) aspartame, (*d*) acesulfame, (*e*) sucralose, (*f*) neotame, (*g*) advantame, and (*h*) neohesperidin dihydrochalcone.

Physical and chemical properties. CYC and its sodium (CYC-Na) and calcium (CYC-$Ca_{0.5}$) salts are crystalline solids. In its acid form (CYC-H), it is a strong acid with pK_a of 1.71 (Spillane & Thomson 1977, Benson & Spillane 1980). Although CYC-H has good water solubility (~13.3% at 20°C), its high acidity results in preference for the very soluble CYC-Na (~20% at 20°C) or CYC-$Ca_{0.5}$ (~25% at 20°C) salts (Beck 1980). As a solid, it is very stable, and in solution, slow hydrolysis yields cyclohexylamine (CHA) and inorganic sulfate. Given safety concerns about CHA, FDA scientists determined CHA levels in a selection of food products, finding CHA in the majority of them, albeit at low levels (Fazio et al. 1970). Interestingly, CHA levels, even in

the most acidic products (e.g., cola beverages), were not significantly changed after four months at ambient temperature. In summary, CYC sweeteners are quite stable and find application in a broad spectrum of products, including beverages, baked goods, and confectionary products (Bopp & Price 2001).

Sensory properties and applications. The C/R function in water determined for CYC-Na is $R = 15.2C/(3140 + C)$ (DuBois et al. 1991). Given the high R_m of 15.2% SE, it may be possible to use CYC-Na in single-sweetener systems as well as in blends. However, because of its low potency and regulatory limitations, CYC-Na is only used in blends. From the C/R function, CYC-Na, $P_w(5) = 32$. CYC-Na exhibits weak bitter and salty tastes at 10% SE and higher concentrations, but no significant "off" tastes in blends with SAC-Na and other HP sweeteners (Carr et al. 1993). CYC-Na has a sucrose-like temporal profile with no significant sweetness linger and with rapid sweetness onset (DuBois & Lee 1983). Finally, CYC-Na is synergistic with some (e.g., SAC-Na) but not all HP sweeteners, thereby enabling use at lower than predicted concentrations and enabling cost savings and taste quality improvements better than otherwise anticipated (Schiffman et al. 1995). CYC is not permitted for use in the United States, but is allowed in many other countries, with an ADI of 11 mg kg^{-1} body weight day^{-1}.

Aspartame. The sweet taste of APM (**Figure 1*c***) was discovered by James Schlatter at G. D. Searle Pharmaceutical Company in 1965 (Mazur et al. 1969). Today, it is the most widely used HP sweetener. APM has been reviewed in Butchko et al. 2001.

Physical and chemical properties. APM is a crystalline solid with water solubility of approximately 1% at 25°C. In crystalline form, it is very stable, and in solution, its stability is pH dependent with maximal stability observed at pH ~4.3. At low pH, APM, a dipeptide ester, hydrolyzes to the dipeptide aspartylphenylalanine and methanol, as well as undergoes slow conversion to its β-linked dipeptide ester isomer, which further hydrolyzes to β-aspartylphenylalanine. At higher pHs, APM cyclizes to form a diketopiperazine and methanol. Ultimately, APM degradation proceeds through all of these pathways to its constituent amino acids, aspartic acid and phenylalanine (Homler 1984). APM is generally regarded as a sweetener suitable for low pH applications (e.g., beverages), but at the low extreme of acceptable stability. APM is insufficiently stable for neutral pH applications (e.g., baked goods).

Sensory properties and applications. The C/R function in water determined for APM is $R = 16.0C/(560 + C)$ (DuBois et al. 1991). Given the high R_m of 16.0% SE, APM can be used both in single and blended sweetener applications. From the C/R function, $P_w(5) = 200$ and $P_w(10) = 110$. It exhibits clean sweet taste without "off" tastes as observed for SAC-Na. It has slightly delayed onset with moderate sweetness linger (DuBois & Lee 1983). APM is claimed to enhance flavors, particularly citrus flavors. APM has been used in single sweetener systems but is more commonly employed in blends with other HP sweeteners (e.g., SAC-Na, acesulfame-K and CYC-Na). APM has an ADI in the United States of 50 mg kg^{-1} body weight day^{-1}, the highest for any HP sweetener.

Acesulfame. The sweet taste of oxathiazinone dioxide salts was discovered by Clauss & Jensen in 1970 at Hoechst AG (Clauss & Jensen 1973). Subsequently, the specific oxathiazinone dioxide known as acesulfame (ACE) (**Figure 1*d***) was chosen for development as its potassium salt (ACE-K). ACE-K has been reviewed in Lipinski & Hanger 2001.

Flavor extract manufacturing association (FEMA): organization responsible for regulation of flavors used in the United States

Physical and chemical properties. ACE-K is a crystalline solid with good water solubility (27% at 20°C). ACE-K is very stable in solid form and adequately stable to hydrolysis and light exposure for use in beverage applications (Federal Register 1988). Under conditions designed to simulate cola beverages, 15% is lost in one year at 25°C and 25% in three months at 40°C (Federal Register 1988), with degradation leading to acetoacetamide-N-sulfonic acid, acetoacetamide, acetoacetic acid, and acetone. However, no sweetness loss problems have been reported for ACE-K in food or beverage applications.

Sensory properties and applications. The C/R function in water determined for ACE-K is $R = 11.6C/(470 + C)$ (DuBois et al. 1991). Given the low R_m of 11.6% SE, ACE-K, like SAC-Na, applications are limited to blends in which contribution is limited to ≤5% SE. From the C/R function, $P_w(5) = 140$. At higher concentrations approaching its R_m, it exhibits bitter and metallic "off" tastes. However, "off" taste is not a problem for ACE-K in blends with sweetness contribution ≤5% SE. ACE-K exhibits quick onset sweetness and low sweetness linger. Blends of ACE-K and APM are in common use in the food and beverage industry. APM/ACE-K blends are advantaged by sweetness synergy (approximately 30%), enabling reduction in sweetener levels, thereby providing cost reduction and taste quality improvement. The ADI for ACE-K established in the United States is 15 mg kg^{-1} body weight day^{-1}.

Sucralose. The sweet taste of SUL (**Figure 1*e***) was discovered in a collaborative program between Professor Leslie Hough's laboratory at Queen Elizabeth College (University of London) and scientists at Tate & Lyle. SUL was subsequently developed by Tate & Lyle in collaboration with McNeil Specialty Products, a division of Johnson & Johnson (Hough & Phadnis 1976, Hough 1984). Of the commercially developed synthetic HP sweeteners, SUL is the most expensive on a cost/SE basis. Use of SUL has been reviewed in Goldsmith & Merkel 2001.

Physical and chemical properties. SUL is a crystalline solid that is freely soluble in water (28.3% at 20°C). As a dry powder, SUL is stable and as the microparticulated commercial product, it has adequate shelf life. However, at elevated temperatures, degradation with discoloration occurs. In solution, SUL has good hydrolytic stability at all food and beverage system pHs and good light stability, and consequently finds broad utility in food and beverage categories.

Sensory properties and applications. The C/R function in water determined for SUL is $R = 14.7C/(142 + C)$ (DuBois et al. 1991). Given the high R_m of 14.7% SE, SUL can be used both in single and blended sweetener applications. From the C/R function, $P_w(5) = 680$ and $P_w(10) = 330$. SUL exhibits sweetness with a slight delay in onset and with moderate linger, similar to that of APM. SUL is most commonly employed in blends with other HP sweeteners (e.g., ACE-K). In the United States, SUL has an ADI of 5 mg kg^{-1} body weight day^{-1}. Recently, Senomyx scientists reported on the first PAM of the sweetener receptor, a compound specific to SUL (Servant et al. 2010). It has been developed by Senomyx and is approved in the United States by the Flavor Extract Manufacturers Association (FEMA) as an artificial flavor.

Neotame. The sweet taste of neotame (NTM) (**Figure 1*f***) was discovered in 1992 by Claude Nofre and Jean-Marie Tinti at Universitė Claude Bernard in Lyon, France (Nofre & Tinti 1996). NTM was developed and commercialized by The NutraSweet Company. NTM has been reviewed in Prakash et al. 2002.

Physical and chemical properties. NTM is a crystalline solid with solubility in water of approximately 1% at 25°C. In solid form, NTM is very stable, and in solution, it shows highest stability at pH 4.5. At low pH, NTM, a dipeptide methyl ester, hydrolyzes to the dipeptide carboxylic acid, the nonsweet major metabolite of NTM in humans. NTM does not cyclize to a diketopiperazine, as is the case for APM.

Sensory properties and applications. The C/R function determined for NTM in water is $R = 15.1/(9.18 + C)$ (K. M. Gibes, unpublished results). Given the high R_m of 15.1% SE, it appears that NTM could be used in both single and blended sweetener applications. From the C/R function, $P_w(5) = 11{,}000$ and $P_w(10) = 5{,}600$. NTM exhibits clean sweetness with no "off" tastes. However, its sweetness shows a pronounced delay and linger, significantly longer than APM. NTM can be blended with CHO or HP sweeteners. NTM has been determined to be synergistic with SAC (Pajor & Gibbs 2000). However, because of NTM's strong sweetness linger, its principal utility is in reduced-calorie products, for which up to 25% calorie reduction can be achieved without significant decrement in taste quality. In the United States, NTM has an ADI of 0.3 mg kg^{-1} body weight day^{-1}.

Advantame. The sweet taste of advantame (AVM) (**Figure 1*g***) was discovered at the Ajinomoto Company (Amino et al. 2003), where it was subsequently developed and commercialized. AVM has been reviewed in Bishay & Bursey 2011.

Physical and chemical properties. AVM is a crystalline solid with solubility in water of approximately 0.10% at 25°C. In solid form, AVM is very stable, and in solution, it shows pH-dependent stability similar to that of APM. At low pH, AVM, a dipeptide methyl ester, hydrolyzes to the dipeptide carboxylic acid, the nonsweet major human metabolite. AVM does not cyclize to a diketopiperazine as is the case for APM.

Sensory properties and applications. The C/R function determined for AVM in water is reported to be $R = 15.8/(2.6 + C)$, where $R_m = 15.8$. Given the R_m of 15.8% SE, it would appear that AVM could be used both in single and blended sweetener applications. From the C/R function, $P_w(5) = 42{,}000$ and $P_w(10) = 22{,}000$. AVM is reported to exhibit clean sweetness without "off" tastes. However, sweetness onset for AVM is slow to develop and sweetness linger is very significant, much greater than that of APM. Although AVM does not yet have regulatory approval as a sweetener, its principal application is likely to be in reduced-calorie, rather than zero-cal, products. AVM, however, is approved by FEMA as an artificial flavor.

Neohesperidin dihydrochalcone. The sweet taste of neohesperidin dihydrochalcone (NDC) (**Figure 1*b***) was discovered by Robert Horowitz and Bruno Gentili in 1950 at the United States Department of Agriculture laboratory in Pasadena, California while studying the bitter tastes of citrus flavonoid glycosides (Horowitz & Gentili 1969). Neohesperidin, the flavanone glycoside precursor of NDC, occurs naturally in the Seville orange. NDC has been reviewed in Borrego & Montijano 2001.

Physical and chemical properties. NDC is a crystalline solid with solubility in water of approximately 0.05% at 25°C. In solid form, NDC is very stable and in solution it shows good stability at pHs relevant to foods and beverages (Borrego & Montijano 2001).

Sensory properties and applications. The C/R function in water determined for NDC (DuBois et al. 1991) is $R = 9.8C/(53 + C)$. Given the low R_m of 9.8% SE, NDC applications are expected to be limited to blends. From the C/R function, $P_w(5) = 910$. NDC exhibits bitter, cooling, and licorice-like "off" tastes (DuBois et al. 1981). And NDC's temporal profile is quite different from that of sucrose, with sweetness developing noticeably slower and lingering much longer than both sucrose and APM (DuBois & Lee 1983). NDC is not approved in the United States as a sweetener. It is, however, approved for use at $\leq$10 mg L^{-1} by FEMA as an artificial flavor (Smith et al. 1996).

Natural Sweeteners

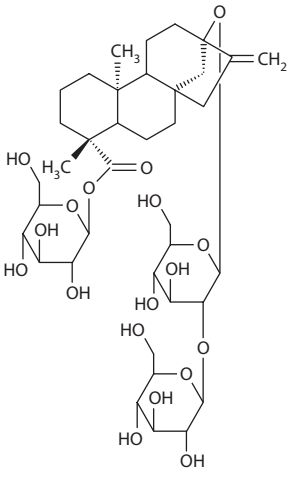
Natural non-caloric sweeteners are gaining popularity with the commercialization of rebaudioside A, the first natural HP non-caloric sweetener to be allowed for use in the United States as a general purpose sweetener. It is clear from the patent literature that commercialization of other natural HP sweetener options is under consideration. Exemplary are the efforts of Cargill (Abraham et al. 2009) and Ajinomoto (Amino & Hirasawa 2004) on monatin, Tate & Lyle in collaboration with BioVittoria (Scott-Thomas 2011) on Luo Han Guo fruit concentrate, and Tate & Lyle (Carlson et al. 2010) and Novozyme (Vind et al. 2011) on brazzein. As has been found for some synthetic HP sweeteners, some natural HP sweeteners (e.g., thaumatin and glycyrrhizic acid salts) have flavor modulatory properties and have been commercialized as flavors. In this section, the eight natural non-caloric sweeteners illustrated in **Figure 2** are discussed. These sweeteners have either been commercialized as sweeteners or flavors, or are under development.

Steviol glycosides, stevioside, and rebaudioside A. The leaves of *Stevia rebaudiana* (Bertoni), a perennial shrub native to a border region of Paraguay and Brazil, have been known to taste sweet for hundreds of years. Identification of the sweet components of stevia leaves began early in the twentieth century but was not concluded until 1955 with the report of the complete structure of stevioside (STV) (Mosettig & Nes 1955) (**Figure 2*a***). Further work in the 1970s by Professor Osamu Tanaka and coworkers (Hiroshima University) showed that stevia leaf extracts contain eight additional steviol glycosides (SGs), with rebaudioside A (REBA) (**Figure 2*b***) being the second most abundant (Kohda et al. 1976). As a result of recent work, stevia is now known to contain at least 10 SGs (Prakash et al. 2008). All of these compounds have a common aglycone known as steviol (*ent*-13-hydroxykaur-16-en-18-oic acid), and they differ in the number and the types of sugars attached (Bakal & O'Brien Nabors 1986, Kinghorn & Soejarto 1991, Kinghorn et al. 2001). Recently, REBA, in purified form ($\geq$97%), also known as rebiana, and SG mixtures ($\geq$95% STV, rebaudiosides A, B, and C, steviolbioside, dulcoside A, and rubusoside) have become commercially available. SGs have been reviewed in Carakostas et al. 2008 and Carakostas et al. 2011.

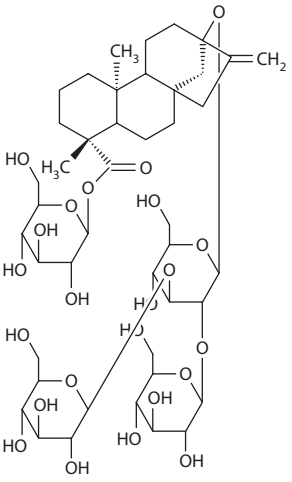
Physical and chemical properties. SGs are solids and exist in various crystalline and amorphous forms. Amorphous, anhydrous, and alcohol solvate forms of REBA and STV readily provide supersaturated solutions in water (>20% w/v at 25°C). The equilibrium solubilities of REBA and STV, however, are only 0.8% and 0.13% (w/v) at 25°C, respectively (Prakash et al. 2008).

Figure 2

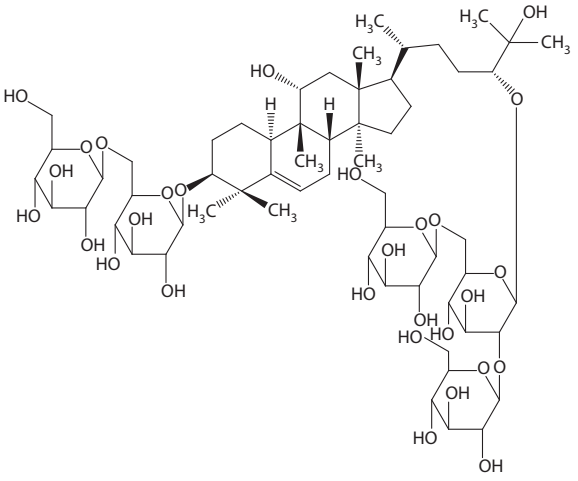
Natural organic compounds used, or under consideration for use, in foods and beverages as sweeteners or flavors: (*a*) stevioside, (*b*) rebaudioside A, (*c*) mogroside V, (*d*) erythritol, (*e*) glycyrrhizic acid, (*f*) thaumatin, (*g*) brazzein, and (*h*) monatin.



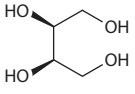
a Stevioside



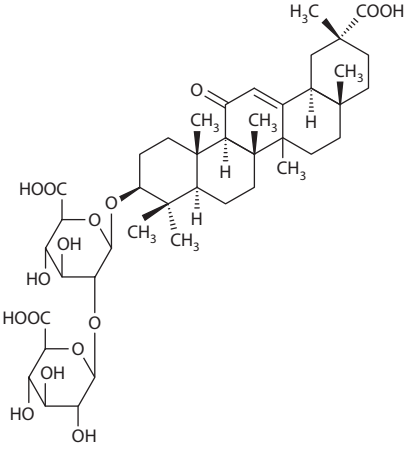
b Rebaudioside A



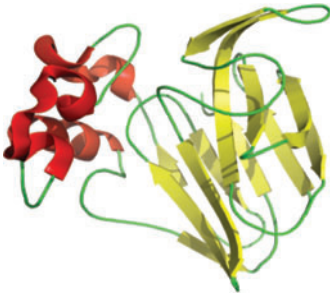
c Mogroside V



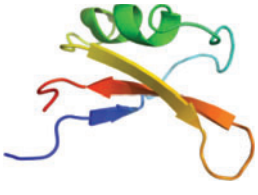
d Erythritol



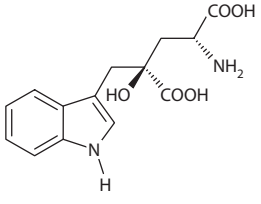
e Glycyrrhizic acid



f Thaumatin



g Brazzein



h Monatin

High fructose starch syrup (HFSS): sweetener manufactured from corn or other sources of starch and most commonly 55% fructose or 42% fructose with the balance made up predominantly of glucose

In contrast to the rapid dissolution rates of amorphous, anhydrous, and alcohol solvate forms of REBA, REBA as a hydrate is very slow to dissolve in water (<0.2% w/v at 25°C for 5 min). As a dry powder at ambient temperature and with controlled humidity, STV is stable for at least two years, and REBA is stable for at least three years. In solution, REBA is most stable in the 4–8 pH range and noticeably less at pH < 2. In aqueous solutions (pH 2–8), the major degradation products of REBA are rebaudioside B, the REBA Δ^{15-16} isomer, and the REBA Δ^{16-17} hydration product.

Sensory properties and applications. The C/R functions in water reported for STV and REBA are R = 9.9C/(410 + C) and R = 10.0C/(200 + C), respectively (DuBois et al. 1991). Given the low R_ms of 9.9 and 10.0% SE, STV and REBA applications are expected to be limited to blends. From its C/R functions, $P_w(5) = 120$ for STV and $P_w(5) = 250$ for REBA. The sweet tastes of STV and REBA are accompanied by bitter and licorice-like "off" tastes, more notable for STV than REBA (Prakash et al. 2008). REBA sweetness is slightly delayed in onset and lingers significantly longer than APM. Owing to low R_m, as well as "off" tastes and significant sweetness linger, REBA's principal use will be in blends with caloric [e.g., sucrose, high fructose starch syrup (HFSS), fructose, glucose, etc.] and non-caloric sweeteners (e.g., erythritol, mogrol glycosides, etc.). In 2008, REBA was affirmed as GRAS (generally recognized as safe) for all applications in foods and beverages with an ADI of 4 mg kg^{-1} body weight day^{-1} on a steviol aglycone basis (12.2 mg kg^{-1} body weight day^{-1} on a REBA basis). In the United States, on notification of these studies, FDA responded with a letter of no objection to market REBA at ≥97% purity. SG sweeteners are approved in 31 other countries.

Mogrol glycosides, Luo Han Guo sweetener, and mogroside V. The fruit of *Siraitia grosvenorii* (Swingle), also known as *Momordica grosvenorii* (Cucurbitaceae), and more commonly known as Luo Han Guo (LHG), have been consumed for centuries in traditional Chinese medicine for the treatment of dry cough, sore throat, dire thirst, and constipation. Also, LHG fruit has been known to be intensely sweet for centuries. Identification of the major sweet component of LHG fruit was achieved in the 1980s with the report of the structure of mogroside V (MOGV) (Takemoto et al. 1983) (**Figure 2*c***). Further work led to the identification of seven additional sweet-tasting mogrol glycosides (MGs), including mogrosides II, III, IV, and VI, isomogroside V, 11-oxomogroside, and siamenoside I. These MGs have the common mogrol triterpenoid aglycone and differ in the sugars attached. Recently, semipurified MG sweeteners have become available as LHG fruit juice concentrates (LHGFCs). MGs have been reviewed in BioVittoria 2009.

Physical and chemical properties. The commercially available LHGFC sweeteners are light yellow powders with limited water solubility (approximately 0.1% at 25°C) (I. Prakash & R. I. San Miguel, unpublished results). As a dry powder, LHGFC is claimed to be stable for at least one year under conditions of controlled humidity at ambient temperature. Hydrolytic and light stability data are not available.

Sensory properties and applications. A sweetness potency of 95 is reported for BioVittoria's LHGFC (PureLo® brand) containing approximately 33% MOGV, without specification of the sucrose reference concentration (BioVittoria 2009). Also, sweetness potencies reported for Guilin Layn Natural Ingredients Corporation's LHGFC (Go-Luo® brand) are as follows: 25% MOGV LHGFC, $P_w(5) = 160$; 45% MOGV LHGFC, $P_w(5) = 210$; and 55% MOGV LHGFC, $P_w(5) = 250$ (Guilin Layn Natural Ingredients Corporation 2010). In addition, MOGV, as a pure compound, is claimed to exhibit $P_w(5) = 256$ (Kinghorn & Soejarto 1986). MOGV sweetness is accompanied by noticeable bitter "off" taste (I. Prakash & R. I. San Miguel, unpublished results).

It seems probable that MOGV as well as crude MG preparations will find greatest utility in blends with other natural non-caloric sweeteners (e.g., SGs and erythritol) for zero-cal formulations or with caloric sweeteners (e.g., sucrose, HFSS, fructose, glucose, etc.) for reduced-calorie food and beverage products. In 2009, for the U.S. market, the PureLo® LHGFC product was affirmed as GRAS and BioVittoria received a letter of no objection from the FDA (GRN No. 301). In 2011, the LHGFC product of Guilin Layn Natural Ingredients Corporation (Go-Luo® brand) was affirmed as GRAS with receipt of an FDA letter of no objection (GRN No. 359). An ADI for LHGFC of approximately 25 mg kg^{-1} body weight day^{-1} was established.

Erythritol. The polyol erythritol (ERY) (**Figure 2*d***) is found in a variety of foods, such as grapes, pears, melons, and mushrooms. The natural form of ERY is the meso isomer. The use of ERY as a sweetener has been reviewed in Embuscado & Patil 2001.

Physical and chemical properties. ERY is a crystalline solid with water solubility of 37% at 25°C. In solid form and in solution as well as on light exposure, ERY is very stable. Owing to its stability at higher temperatures, it can be used in cooking, baking, and confectionery.

Sensory properties and applications. The sweetness potency of ERY has been reported to be 0.6–0.7 times that of sucrose, and its flavor profile is similar to sucrose. ERY exhibits a cooling effect when tasted as a solid and in solid food products because of its negative heat of solution. ERY can be blended with HP sweeteners (e.g., APM, NTM, REBA, MOGV, etc.) with significant improvement in taste because of a reduction of sweetness linger and acceleration of sweetness onset. ERY was self-affirmed as GRAS in the United States in 1997 by Cerestar.

Glycyrrhizic acid. Glycyrrhizin, a mixture of calcium, magnesium, and potassium salts of glycyrrhizic acid (GA) (**Figure 2*e***), occurs at a level of 6% to 14% in the roots of the European and Central Asian shrub *Glycyrrhiza glabra* Linn (Fabaceae). The crude extract of the plant is well known as licorice. GA is a triterpenoid glycoside and its structure determination was completed by Lythgoe & Trippett (1950). It is not in use as a sweetener but is used as a flavor. The form most commonly used is the salt monoammonium glycyrrhizinate (MAG). MAG applications have been reviewed in DuBois 2000.

Physical and chemical properties. MAG is a white to brown colored solid and is freely soluble in hot water (Ponakala et al. 2006).

Sensory properties and applications. The C/R function in water determined for MAG is $R = 7.3C/(210 + C)$ (DuBois et al. 1991). Given the low R_m of 7.3% SE, MAG applications are clearly limited to blends with other sweeteners and where the MAG contribution is limited to ≤5% SE. From the C/R function, $P_w(5) = 110$. At all concentrations, however, MAG exhibits bitter and licorice-like "off" tastes as well as a significantly delayed onset of sweetness and a pronounced sweetness linger (DuBois & Lee 1983). As a consequence of these strong deviations from sucrose-like sweetness, MAG is not useful as a sweetener (DuBois 2000). MAG and related glycyrrhizin compounds were approved by FEMA for use in the United States as natural flavors in 1985. This approval does not include their use as sweeteners.

Thaumatin. The sweet component of the fruit of the West African plant *Thaumatococcus daniellii* is known as thaumatin (THM) (**Figure 2*f***) and was determined to be a single chain protein of

207 amino acids with eight disulfide bridges and a molecular weight of 22,209. Van der Wel and colleagues at Unilever carried out classical structure determination work on THM (Higginbotham 1983), and Kim and coworkers at the University of California-Berkeley (Kim et al. 1988) completed an X-ray crystal structure determination. THM is currently marketed under the trademark Talin®.

Physical and chemical properties. THM is a solid with water solubility of 60% at 25°C. It is stable as a dry powder and very stable in aqueous solution in the pH range 3.0–6.0 (Etheridge 1994).

Sensory properties and applications. THM's C/R function in water is $R = 10.1C/(3.6 + C)$ (DuBois et al. 1991). Given the low R_m of 10.1% SE, THM applications are clearly limited to blends and where the THM's contribution is limited to ≤5% SE. From the C/R function, $P_w(5) = 9{,}800$. At all concentrations, however, THM exhibits bitter and licorice-like "off" tastes as well as a markedly delayed onset of sweetness and very pronounced sweetness linger. On a molar basis, THM is the most potent sweetener ever found. Conversion of THM's weight basis potency [i.e., $P_w(5) = 9{,}800$] to a molar basis provides $P_m(5) = 910{,}000$. THM has not found application as a sweetener in foods or beverages. It has, however, found application as a flavor (Ochi 1980). In the United States, THM has FEMA approval as a natural flavor (Oser et al. 1984).

Brazzein. The sweet component of the fruit of the West African plant *Pentadiplandra brazzeana* Baillon is known as brazzein (BRZ) (**Figure 2*g***) and was determined to be a single polypeptide chain protein composed of 54 amino acids with four disulfide bridges and a molecular weight of approximately 6,500 (Ming & Hellekant 1994, van der Wel et al. 1989, Assadi-Porter et al. 2006) and is found in the pulp surrounding the seeds.

Physical and chemical properties. BRZ is a white solid with good water solubility. It is stable as a dry powder and over a broad pH range from 2.5 to 8.

Sensory properties and applications. The C/R function for BRZ is not available. However, the $P_w(2)$ and $P_w(10)$ values for BRZ have been reported to be 2,000 and 500, respectively (Pfeiffer et al. 2000). In preliminary testing, BRZ has been observed to exhibit clean sweet taste without bitterness and a high R_m but to be disadvantaged by sweetness with slow onset that significantly lingers (G. E. DuBois, unpublished results). Brazzein is not approved for any food or beverage applications.

Monatin. The bark of the roots of the South African spiny-leafed hardwood shrub known as *Sclerochiton ilicifolius* contains low levels of a sweet compound that has been determined to be (*2S,4S*)-2-amino-4-carboxy-4-hydroxy-5-(3-indolyl)-pentanoic acid (Vleggaar et al. 1992), which is commonly known as monatin (MON) (**Figure 2*b***). Recently, the (2S,4R), (2R,4S), and (2R,4R) diasteromeric forms of MON have also been found to occur in this plant (Bassoli et al. 2005). The four diastereoisomers of MON exhibit different sweetness potencies, with the (2R,4R) diastereoisomer being the most potent (Amino & Hirasawa 2004).

Physical and chemical properties. MON is a water-soluble crystalline solid and is stable as a dry powder but unstable under sunlight, particularly in solution. It produces several degradation products on exposure to sunlight, including skatole and 3-formyl indole. Sunlight-exposed MON solutions exhibit a malodor (Upreti et al. 2011).

Sensory properties and applications. The C/R function for MON is not available. However, $P_w(5) = 2{,}700$ has been reported for (2R,4R) MON, the most potent isomer (Amino & Hirasawa 2004). MON exhibits a clean sweet taste but is disadvantaged by a slow onset sweetness that significantly lingers (G. E. DuBois, unpublished results). MON is not approved for any food or beverage applications.

Taste receptor type 2 subtype undefined (T2R): the class of 25 human G protein–coupled receptors T2R1–T2R25 that are the bitterant receptors; the T2Rs are generally thought to exist in homo- and heterodimeric form and thus may be as many as 325 in number

SWEETNESS MODULATORS

Many HP sweeteners are disadvantaged by bitter and other negative taste attributes. An even more serious issue is the temporal problem of delay in sweetness onset and lingering sweet aftertaste. Progress in addressing these challenges, enabled by current views of their likely mechanistic rationale, is reviewed here.

Flavor Profile Modulation

As discussed above, SAC, the first commercialized HP sweetener, was not widely used in the first half of the twentieth century because of bitter and metallic "off" tastes. However, its usage rapidly increased in the 1960s following the discovery (Helgren 1957) that a SAC/CYC blend exhibited negligible bitterness. Recently, advantaged by knowledge of the T2Rs responsible for SAC bitterness (Kuhn et al. 2004, Pronin et al. 2004), Givaudan scientists conducted a cell-based HTS program to identify 4-[(1R,3S/1S,3R)-cis-2,2,3-trimethylcyclopentyl]butanoic acid (TCBA) (**Figure 3*a***) as a SAC bitterness inhibitor (Slack et al. 2010). Disappointingly, however, although highly effective in vitro, TCBA was not as effective in sensory panel testing. The authors suggest that genetic diversity may limit its effectiveness in sensory testing. Interestingly, TCBA is also claimed (Slack & Evans-Pennimpede 2009) to inhibit the bitterness of REBA. A related compound 4-[(R,S)-2,2,3-trimethylcyclopent-3-enyl]-2-butenoic acid (TCBE) (**Figure 3*b***) was reported (Brune et al. 2008) to inhibit SUL bitterness. In addition, (R,S)-2-[(ethyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy]propanoic acid (EMCC) (**Figure 3*c***) was claimed to be effective in the inhibition of the bitterness of REBA as well as of APM and SUL (Ungureanu & Van Ommeren 2010). Recently, evidence was provided that the 25 human T2Rs heterodimerize and homodimerize, in which case the actual population of functional bitterant receptors in taste bud

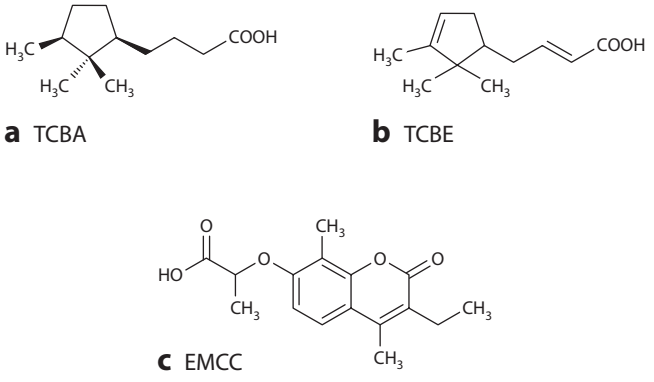


Figure 3

Inhibitors of high-potency sweetener bitterness: (*a*) 4-[(1R,3S/1S,3R)-cis-2,2,3-trimethylcyclopentyl]-butanoic acid (TCBA), (*b*) 4-(R,S)-(2,2,3-trimethylcyclopent-3-enyl)-2-butenoic acid (TCBE), and (*c*) (R,S)-2-[(ethyl-4-methyl-2-oxo-2H-chromen-7-yl)oxy]propanoic acid (EMCC).

cells could be as many as 325 (Kuhn et al. 2010). It seems quite possible that an inhibitor highly effective against a homodimeric species present in a cell-based assay may have different efficacies against a diverse population of receptors in human taste bud cells. Of course, this difference could explain the limited efficacy of TCBA in sensory testing. Thus, although discovery of potent inhibitors against individual T2Rs may be routine, it seems likely that the challenge will remain to discover inhibitors that are highly effective in human taste.

Over the past century, a great deal of effort by many groups has been applied to the search for inhibitors of the bitter "off" taste of SAC. It is clear that today as well there is a substantial effort underway to find bitterness inhibitors for natural HP sweeteners (e.g., SGs, MGs, etc.). However, before doing more work of this type, it may be useful to consider the likely value of such inhibitors, even if fully effective in sensory testing. SAC, REBA, and other SG sweeteners, as with many HP sweeteners, exhibit low R_m. As discussed above, SAC has an R_m of 10.1 SE, with the practical ramification being that it is nonsensical to try to reach a 10% SE level of sweetness with SAC alone because this equation predicts that an absurdly high concentration of 11.5 g L^{-1} would be needed. However, a practical approach to good-tasting sweetener systems when using sweeteners with low R_ms and bitter "off" tastes is to blend them, with each sweetener used below its bitterness detection threshold.

Temporal Profile Modulation

The major problem in replication of CHO sweetener taste with HP sweeteners is delay in sweetness onset and sweetness linger. Consumers generally object to lingering sweetness but do not mention delay in sweetness onset as noticeable for commercial zero-cal products. Sweetness linger is a problem with synthetic HP sweeteners and a major problem with natural HP sweeteners. Methods have been developed to quantify temporal aspects of sweetener taste (DuBois & Lee 1983).

In 2003, a program was initiated for the development and commercialization of the stevia sweetener known as REBA (Prakash et al. 2008) (**Figure 4**). In this program, intensive effort was focused on identification of REBA formulations with more sucrose-like temporal profiles. REBA exhibits a notable delay in sweetness onset as well as significant sweetness linger. To address the temporal profile challenge, the approach employed was based on consideration of the most likely mechanistic rationale for slow sweetness onset and sweetness linger (DuBois 2011). It was accepted that as a class, HP sweeteners bind with higher affinity to the receptor than do CHO sweeteners. However, given that REBA is only approximately 200 times more potent than sucrose, this explanation is inadequate to explain a sweetness that persists for many minutes. The apparent

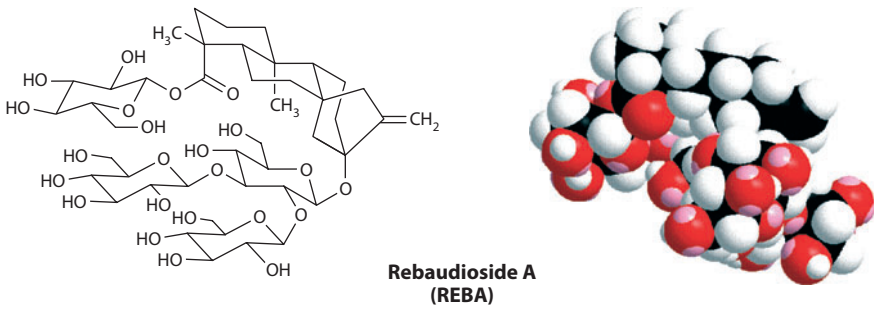


Figure 4

Rebaudioside A (REBA) shown in three-dimensional structural representations.

equilibrium constant for REBA-receptor dissociation (K_d) may be calculated to be 210μM (DuBois et al. 1991). Given that (*a*) K_d is the quotient of the rate constants for REBA-receptor dissociation (k_d) and association (k_a), and (*b*) rate constants for binding of small molecules to receptors reflect diffusion-controlled kinetics (i.e., $k_a \sim 10^{10}\ M^{-1}\ sec^{-1}$), then k_d for REBA-receptor dissociation is estimated to be approximately $2 \times 10^6\ sec^{-1}$. Thus, assuming first order kinetics for REBA-receptor dissociation, at a time 60 sec after the time of maximal sweetness, the percentage of REBA still bound is less than 1%. Therefore, the potency of REBA cannot explain sweetness that lingers for many minutes. Also considered was a proposal by Michael Naim and colleagues (Hebrew University) that sweetness linger may be a consequence of sweetener inhibition of G protein receptor kinases involved in termination of G protein–coupled receptor (GPCR) signaling (Zubare-Samuelov et al. 2005). However, this rationale is unlikely because it does not explain slow sweetness onset that always co-occurs with sweetness linger. Other explanations were considered as well. However, it was concluded that the most probable rationale for sweetness linger, which also accounts for slow onset of sweetness, is that HP sweeteners, particularly large saponin-like molecules like REBA, engage in extensive nonspecific binding to cell membrane sites throughout the oral cavity. If this is the case, one would expect that much of the sweetener in a solution entering the mouth binds to nonreceptor sites and the concentration on the receptor only reaches its maxima after a delay. At the same time, when an HP sweetener dissociates from the receptor, it likely binds nonspecifically to nearby nonreceptor sites and thus is available for rebinding to the receptor over and over, thus resulting in lingering sweetness. This rationale for the atypical temporal profiles of HP sweeteners is known as the nonspecific binding (NSB) hypothesis.

G protein–coupled receptor (GPCR): membrane protein that transduces molecular signals from extracellular space to cellular interiors

Nonspecific binding (NSB): the binding of sweeteners at sites in the oral cavity other than the receptor site and hypothesized to cause delay in sweetness onset and a lingering sweetness perception

If the NSB hypothesis is accurate, then the challenge becomes one of formulation to reduce or eliminate nonspecific binding. It is well known that the tonicity of biological media dramatically affects cells and for this reason, living cells are normally incubated in isotonic buffered saline systems. If cell medium tonicity is reduced, cells swell and even lyse if the medium is sufficiently hypotonic. Conversely, if tonicity is increased beyond cytosolic osmolarity, the cells shrink. Recognizing that hypertonic formulations of sweeteners may have such an effect on the oral epithelium, it was speculated that cell shrinkage may cause changes in exposed membrane structures, thus attenuating HP sweetener nonspecific binding and thereby accelerating sweetness onset and attenuating the sweetness linger.

Support for the NSB hypothesis was found in the observations of Maruzen Pharmaceutical Company (MPC) scientists (Crammer & Ikan 1987) that hypertonic NaCl enhanced the sweetness potency of an SG composition by approximately threefold and of a SG/GA blend by fourfold to fivefold. The precedent for action of NaCl and other salts as PAMs for a number of GPCRs (Christopoulos & Kennakin 2002) was understood. However, NaCl functioning as a threefold to fivefold sweetener receptor PAM seemed unlikely. SG and GA sweeteners normally exhibit slow sweetness onsets and pronounced sweetness lingers. However, in hypertonic NaCl their temporal properties were observed to be similar to sucrose. To explain the apparent enhancement effects, earlier findings on the sensory properties of SG and GA sweeteners were considered. Thus, it had been demonstrated that STV, REBA, and GA, when evaluated in water, exhibit R_ms of 9.9%, 10.0%, and 7.3% SE, respectively (DuBois et al. 1991). It had been demonstrated that STV and GA in water require 22 and 69 seconds, respectively, for decrease of maximal sweetness to a 2% SE level, whereas sucrose sweetness reaches 2% SE in 13 seconds (DuBois & Lee 1983). However, SGs and GA in hypertonic NaCl exhibit sucrose-like temporal profiles. Thus, the entire sweetness signals for SGs and GA, when formulated in hypertonic NaCl, are observed over a short window of time, as is the case for sucrose, and this leads to an increase in their R_ms, an observation interpreted as enhancement by the MPC scientists. In subsequent work (Prakash et al. 2007), it was demonstrated that NaCl was not unique in modulation of the temporal profile of REBA

and other HP sweeteners. In fact, it was shown that osmolytes, in general, exhibit this effect when formulated with REBA. As examples, 500 mOsM NaCl, 500 mOsM KCl, and 500 mOsM erythritol compositions were found to be equally effective in accelerating the sweetness onset of 500 mg L^{-1} REBA and in attenuating its sweetness linger.

Information presented above generally supports the NSB hypothesis as the mechanism for HP sweetener slow sweetness onset and sweetness linger. Further support is derived from the comparative behaviors of CHO and HP sweeteners in reverse-phase chromatography (Briciu et al. 2010) and on interaction with solid-phase extraction sorbents (Zygler et al. 2010), on which HP sweeteners are retained and the more hydrophilic CHO sweeteners are not. These retention behaviors correlate with calculated log P values and also with observed levels of delay in sweetness onset and sweetness linger. Direct support for the interactions of sweeteners with biological membranes was recently provided by a surface plasmon resonance study of CHO sweeteners and HP sweeteners (i.e., APM, STV, and proteins sweeteners THM and single-chain monellin) (Miyano et al. 2010). In general, the level of interaction of these sweeteners with the model membranes correlates well with observed levels of sweetness linger.

SWEETENER RECEPTOR POSITIVE ALLOSTERIC MODULATORS

The Concept of Sweetener Receptor Positive Allosteric Modulators and Proof of Concept

The challenge in the development of new sweetness technologies is accurate replication of sucrose taste in zero- and reduced-calorie systems. Although sweetness modulators, which (*a*) increase R_m, (*b*) inhibit bitter, metallic, and licorice-like "off" tastes, (*c*) accelerate sweetness onset and attenuate sweetness linger, and (*d*) reduce sweetness adaptation, can improve the tastes of HP sweeteners, there is need for a simpler approach. One option is the continued use of sucrose as the sweetener, thus ensuring great taste, but formulation with inhibitors of metabolism or absorption, thus reducing caloric contribution. For example, a sucrose-sweetened food or beverage formulated with an α-glucosidase inhibitor (e.g., acarbose) could, in principle, yield great-tasting foods or beverages without sucrose-derived calories. The normal conversion of sucrose into glucose and fructose that occurs in the gastrointestinal system would be blocked, and the sucrose would be excreted unchanged. However, α-glucosidase inhibitors, when used as drugs in diabetic patients, are known for the unpleasant side effects of flatulence and diarrhea (Sleevi 2003). Other similar approaches (e.g., glucose and fructose transport inhibitors) formulated with CHO sweeteners are anticipated to have similar effects. A second option to improve the tastes of HP sweeteners is based on the concept that molecules may exist that bind to the sweetener receptor without activating it, but do so in a manner that they cause CHO sweeteners (e.g., sucrose, fructose, and glucose) to bind with higher affinity, as illustrated in **Figure 5**. Thus, such compounds would be enhancers, or more properly, PAMs, of the sweetener receptor. Because PAMs would have no sweetness activities, these CHO sweetener/PAM formulations should accurately replicate CHO sweetener taste. Of course, given that sucrose, or another CHO sweetener (e.g., fructose or glucose), is still present, albeit at lower concentrations, in PAM/CHO sweetener formulations, some calories remain. However, if PAMs could be identified with ≥20-fold enhancement effects, then foods and beverages could be sweetened with sucrose, providing great taste, but with ≤5% of the sucrose-derived calories of normal products. For many such beverages, they could carry zero-cal labeling. The foregoing rationale provided the impetus for a program in search of sweetener receptor PAMs (DuBois 2011). At the time this work was initiated, it was generally accepted that sweetness is mediated by multiple GPCRs and that CHO sweeteners activate more than one receptor.

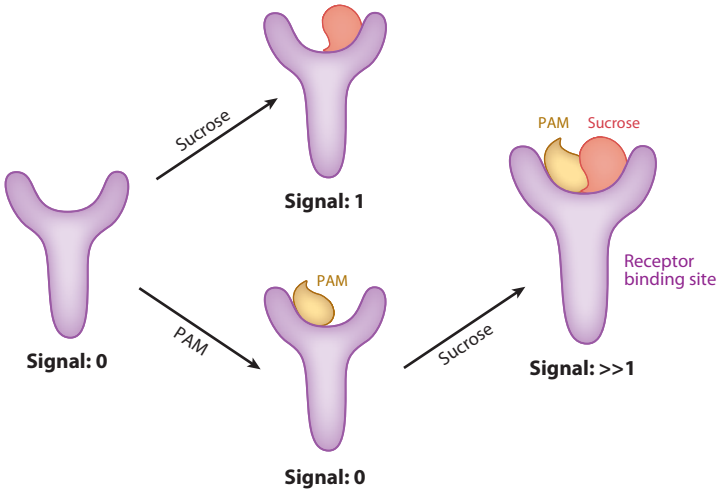


Figure 5

Early conceptual model of positive allosteric modulator action at a sweetener receptor.

Nonetheless, the program was initiated with the hope that PAMs could be identified for the key CHO sweetener GPCRs.

Many substances have been claimed to enhance the sweetness intensities of CHO sweeteners. Among those reported are soluble starch (Kanemaru et al. 2002), d-catechin (Kashket 1990), alapyridaine (Ottinger et al. 2003), and NDC (Beerens 1981). However, formulations of these and other claimed CHO sweetener enhancers with sucrose, fructose, and glucose showed no significant enhancement (G. E. DuBois, G. A. King, unpublished results). In continuation of this search for PAMs, 2,4-dihydroxybenzoic acid (DHB) (**Figure 6*a***), a compound with approval by FEMA as an artificial flavor, was evaluated based on a report by Lindley and coworkers (Holland Sweetener Company) that it enhances the sweetness of APM (Britton et al. 1999). The Holland Sweetener Company researcher's primary interest in DHB was improvement of APM taste, but not enhancement. Interestingly, formulation of 6% sucrose with DHB showed, for the first time, a clear sweetness enhancement effect. At a level having no sweetness (i.e., 375 mg L^{-1}), 6% sucrose was enhanced by DHB to exceed 8% sucrose in sweetness (i.e. >33% enhancement) and, importantly, without any "off" taste. In evaluation of DHB with other CHO sweeteners, selective

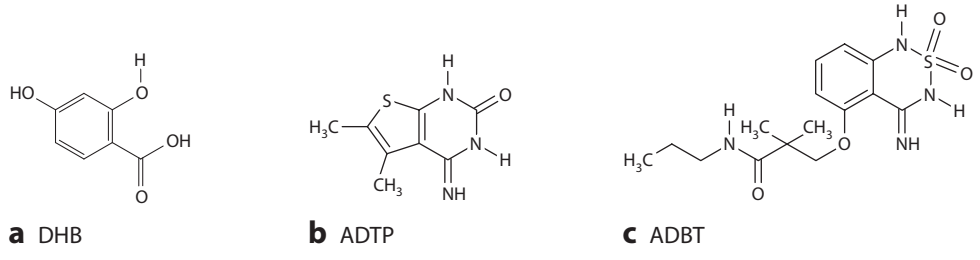


Figure 6

Positive allosteric modulators of the human sweetener receptor: (*a*) 2,4 dihydroxybenzoic acid (DHB), (*b*) 4-amino-5,6-dimethylthieno(2,3-D)pyrimidin-2(1H)-one (ADTP), and (*c*) 3-[(4-amino-2, 2-dioxido-1H-2,1,3-benzothiadiazin-5-yl)oxy]-2,2-dimethyl-N-propylpropanamide (ADBT).

action was observed. The effect was strongest with sucrose, weaker with fructose, and absent with glucose. This selectivity suggests that the observed effect may be PAM-mediated because by such a mechanism, equivalent effects for different sweeteners would not be anticipated. In this work, structural analogs of DHB (e.g., salicylic acid and derivatives) also showed sweetness enhancement activity.

Sweetener receptor PAM discovery, using human taste as the assay, is a very slow and arduous process. Thus, progress in identification of PAMs more potent than DHB, and with activity against other CHO sweeteners (e.g., fructose and glucose), was unacceptably slow. However, in 2002, Li and coworkers reported the discovery of the human sweetener receptor (Li et al. 2002), and this provided hope that the rate of PAM discovery could be markedly accelerated by use of cell-based assays and HTS. They demonstrated the sweetener receptor to be a heterodimer of two 7-transmembrane domain (7-TMD) proteins named T1R2 and T1R3, and named the receptor T1R2/T1R3. They also reported the discovery of the human savory receptor, most often referred to as the umami receptor. Interestingly, the umami receptor was also found to be a heterodimer of two 7-TMD proteins, T1R1 and T1R3, where T1R3 is the same 7-TMD protein as in the sweetener receptor. It is noteworthy that these three T1R 7-TMD proteins are members of the small GPCR family known as Class C. Class C of the GPCR superfamily of approximately 800–1,000 GPCRs is known to have 12 members, eight metabotrophic glutamate receptors (mGluRs), one GABA receptor ($GABA_B$), one calcium receptor (CaR), and the two taste receptors identified by Li and coworkers (2002). Class C GPCRs are unique in the GPCR family in that they possess large N-terminal domains that appear to close on binding ligands like a Venus flytrap closes on its prey, thus leading to their reference as venus flytrap domains (VFDs). Ligands binding in the VFDs are thought to initiate a conformational change on an intracellular domain of the receptor, thus exposing determinants that initiate G-protein coupling and a cascade of reactions en route to cell excitation.

Transmembrane domain (TMD): the domain of a G protein–coupled receptor, which crosses the membrane of the taste bud cell seven times and makes contact with the G protein in the cytosol

Venus flytrap domain (VFD): the domain of the T1R2/T1R3 G protein–coupled receptor in which binding of native ligands (e.g., sucrose) occurs, leading to closure and receptor activation

Negative allosteric modulator (NAM): compound that inhibits the activity of the sweetener receptor through binding at an allosteric site

Discovery of Positive Allosteric Modulators of Other G Protein–Coupled Receptors Related to the Sweetener Receptor

Around the time that the human sweetener receptor was identified, reports began to appear of the discoveries of PAMs for several of the other 11 Class C GPCRs. The most noteworthy of these reports, given interest in sweetener receptor PAMs, was the report (Li et al. 2002) on the umami taste receptor that nucleotide monophosphates, such as inosine monophosphate (IMP), strongly potentiate the activity of glutamate (GLU), the normal agonist. Up until this time, it was accepted that the effect in GLU/IMP mixtures was synergistic with both GLU and IMP being agonists. However, it was demonstrated that IMP is not an agonist, but rather a PAM, with enhancement effects on GLU of up to 30-fold. Work aimed at the identification of allosteric modulators of the mGluRs, $GABA_B$ and CaR preceded efforts to find sweetener receptor PAMs by a few years. The impetus for this research was the expectation of high selectivity and low off-target activity for drugs that act at allosteric rather than orthosteric receptor sites (Werner 2009, Rocheville & Garland 2010). Thus, beginning in the late 1990s, reports began to appear on PAMs as well as negative allosteric modulators (NAMs) for these receptors, such that today PAMs and/or NAMs are known for all of the Class C GPCRs (Urweyler 2011). One such drug known as cinacalcet is now on the market as a treatment for hyperparathyroidism (Nagano 2006). PAMs with EC_{50}s in the low nanomolar range and with enhancement effects of >10-fold for some Class C GPCRs are now known and as expected, both PAMs and NAMs exhibit high selectivity for their targets. Thus, there is a great deal of optimism today about allosteric modulators as a class of therapeutic drugs with an enhanced likelihood of safety. An interesting finding in the research on allosteric

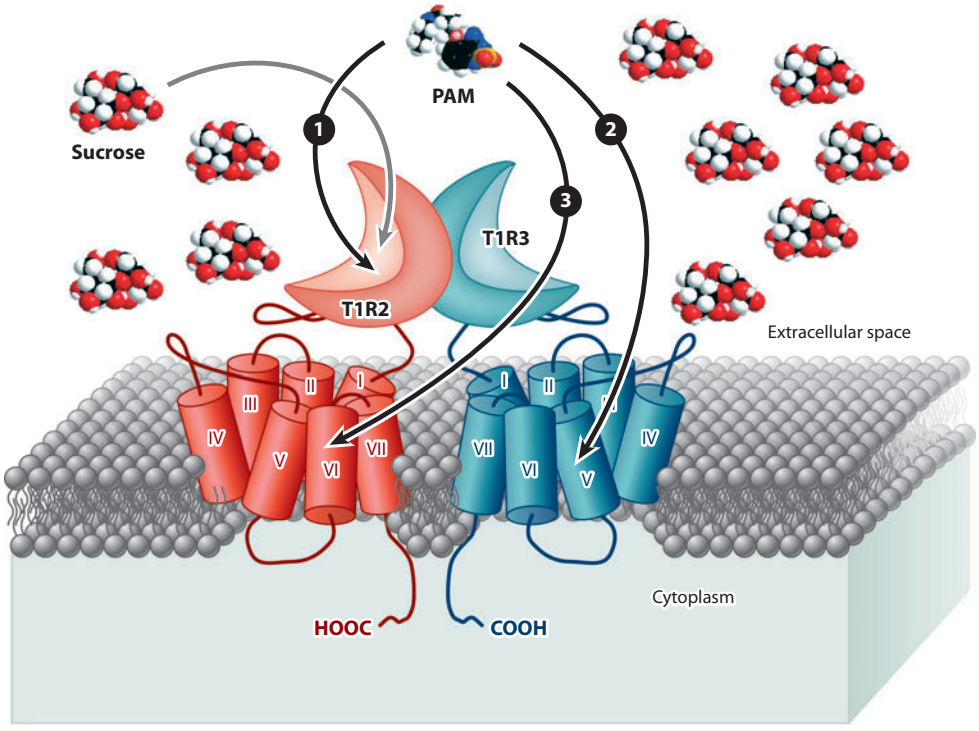


Figure 7

Potential loci of action of positive allosteric modulators (PAMs) at the sweetener receptor. Black arrows signify three potential sites for PAM binding to the sweetener receptor (adapted from Vigues et al. 2008).

modulators of mGlu, $GABA_B$, and Ca receptors is that their loci of action for all cases determined to date are in the receptor TMD, rather than in the proximity of the orthosteric sites in the VFDs. For the sweetener receptor, it was shown that the sweetness inhibitor lactisole is a NAM with a binding locus in the T1R3 TMD (Jiang et al. 2005). In contrast, the IMP binding site in the umami receptor was determined to be in the T1R1 VFD, adjacent to the GLU binding site (Zhang et al. 2008). Thus, as illustrated in **Figure 7**, in early sweetener receptor PAM discovery work, it was not clear as to the likely locus of PAM activity.

Discovery of Sweetener Receptor Positive Allosteric Modulators by High-Throughput Screening with a Cell-Based Assay

The discovery of the human sweetener receptor and its cloning into biological cells made possible HTS with large diverse chemical libraries. This advanced capability provided optimism for the discovery of potent (i.e., low EC_{50}s) and efficacious (i.e., large EC_{50} shifts) T1R2/T1R3 PAMs with good safety profiles. The optimism was fueled by (*a*) proof of concept for the existence of T1R2/T1R3 PAMs (i.e., DHB and other salicylic acid and derivatives), (*b*) discoveries of PAMs for many other Class C GPCRs, (*c*) the expectation of excellent safety profiles for PAMs, and (*d*) the expectation of PAM enhancements without sweetness temporal profile effects. It is noteworthy that reproduction of the temporal activity of endogenous ligands is now an accepted hallmark characteristic of PAMs (Wood et al. 2011).

The first intensive program targeted at T1R2/T1R3 PAMs, enabled by an efficient cell-based assay, was initiated in 2002 by Senomyx in collaboration with The Coca-Cola Company. This program first led to the SUL-selective enhancer 4-amino-5,6-dimethylthieno[2,3-D]pyrimidin-2(1H)-one (ADTP) (**Figure 6*b***) (Servant et al. 2010). In human sensory panel assays, ADTP at 9.7 mg L^{-1} was found to enhance the sweetness of a 100 mg L^{-1} SUL solution to equivalence with 600 mg L^{-1} SUL (i.e., sixfold). On a sucrose sweetness scale, this is equivalent to a 6.3% to 12.3% (i.e., twofold) enhancement. Also, at 19.5 mg L^{-1}, ADTP was observed to enhance the sweetness of 12.5 mg L^{-1} SUL to equal the sweetness of 100 mg L^{-1} SUL (i.e., eightfold). On a sucrose sweetness scale, this is equivalent to a 0.5% to 6% sucrose (i.e., 12-fold) enhancement. The discovery of the SUL enhancer was quickly followed by the discovery of sucrose-selective enhancer 3-[(4-amino-2,2-dioxido-1H-2,1,3-benzothiadiazin-5-yl)oxy]-2,2-dimethyl-N-propylpropanamide (ADBT) (**Figure 6*c***) (Shigemura et al. 2010). In human sensory panel studies, ADBT at 8.8 mg L^{-1} was observed to enhance 6% sucrose to equivalence with 10.6% sucrose (i.e., 1.8-fold). Early sensory studies of sucrose-ADBT formulations are consistent with the expectation that PAMs will enable dramatic calorie reduction with retention of the desirable temporal profiles of CHO sweeteners. Both PAMs ADTP and ADBT have been commercially developed with U.S. regulatory approvals by FEMA as artificial flavors.

With the discovery of PAMs ADTP and ADBT, the question returns as to loci of action (see **Figure 7**). Do the SUL and sucrose enhancers act at a locus in the T1R2 VFD, as is the case for IMP action in the umami receptor (i.e., pathway 1)? Or do they bind in the TMD of either T1R2 or T1R3 as in mGluR, $GABA_BR$, and CaR PAMs (i.e., pathways 2 or 3)? It is noteworthy that the binding loci of the synergistic sweeteners CYC and APM have been determined to be in the TMD of T1R3 and in the T1R2 VFD, respectively (Xu et al. 2004). Mechanistically, this well-known CYC/APM sweetness synergism phenomenon seems best interpreted as a PAM effect induced by CYC acting as an APM-selective PAM while also acting as a sweetener (DuBois 2004). Thus, there is additional precedent to expect CHO sweetener PAMs to act at a locus in the TMD of T1R3 because CHO sweeteners, such as APM, act at a T1R2 VFD locus. However, in recent work (Zhang et al. 2010), it was demonstrated that SUL enhancer ADTP acts in the VFD of T1R2 at a site in which it binds concurrently to the receptor protein and to SUL as illustrated in **Figure 8**. It seems likely that sucrose and enhancer ADBT bind at the same locus.

It is now apparent that many programs targeted at T1R2/T1R3 PAMs are underway. Exemplary of these efforts are the following:

1. Cadbury Adams USA: 3-Hydroxybenzoic acid (3HB) and especially mixtures of 3HB with DHB are reported to enhance the sweetness of sucrose as well as other CHO sweeteners and some HP sweeteners (Bingley et al. 2007). As an examples, 3HB, DHB, and a 1:1 mixture of 3HB and DHB when employed at 500, 500, and 500/500 mg L^{-1}, respectively, caused 1.4-, 1.3-, and 1.7-fold enhancements of the sweetness of 5% sucrose.

2. Givaudan: Multiple examples of sweetness enhancers have been reported. In the first example, it is claimed that sweeteners at sub-threshold concentrations can be used in combination with CHO sweeteners to achieve small increases in sweetness (Slack et al. 2007). However, the sweetness increases realized by this approach are not PAM effects. In a second example, 4-O-β-D-glucosyl-hesperetin dihydrochalcone at 20 mg L^{-1} with 7% sucrose is claimed to cause a 1.1-fold enhancement (Jia et al. 2008). In a third example, the naturally occurring MG isomogroside V at 10 mg L^{-1} with 7% sucrose is claimed to cause a 1.1-fold enhancement (Jia & Yang 2009). In a fourth example, a mixture of 10 mg L^{-1} 4-hydroxycinnamic

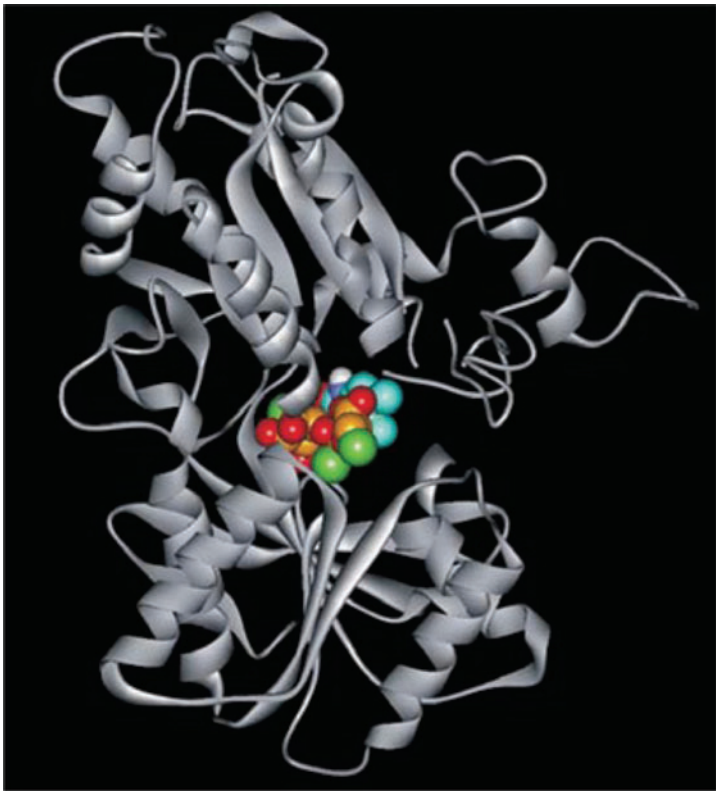


Figure 8

A molecular model illustrating the likely binding loci for SUL (C, gold; O, red; and Cl, green) and sucralose positive allosteric modulator ADTP (C, cyan) in the T1R2 VFD of the sweetener receptor in a closed conformation (Zhang et al. 2010). Abbreviation: ADTP, 4-amino-5,6-dimethylthieno(2,3-D)pyrimidin-2(1H)-one.

acid and 10 mg L^{-1} 4-methoxycinnamic acid is claimed to increase the sweetness of sucrose-sweetened milk (Bouter et al. 2009). In a fifth example, 1-(2-hydroxyphenyl)-3-(4-pyridyl)-1-propanone at 20 mg L^{-1} with 7% sucrose was reported to exhibit a 1.1-fold enhancement (Wang & Daniher 2009). In a sixth example, 4-ethoxybenzonitrile at 20 mg L^{-1} with 7% sucrose was reported to exhibit a 1.1-fold enhancement (Flamme et al. 2011). And finally, in a seventh example, 2-methoxy-5-(phenoxymethyl)-phenol at 5 mg L^{-1} was reported to cause a 1.3-fold sweetness enhancement effect (Flamme & Bom 2011).

3. Nutrinova: The tripeptide aladapcin, a natural microbial metabolite, is claimed to be a strong fructose enhancer in a cell-based assay (i.e., 6.8-fold on signal of 0.45% fructose with aladapcin at 8.3 mg L^{-1}) (Krohn & Zinke 2009a). Also, surfactin, a natural cyclic lipopeptide, is claimed to strongly enhance fructose activity in a cell-based assay (i.e., sixfold enhancement of 0.54% fructose) (Krohn & Zinke 2009b).
4. Redpoint Bio Corporation: Rebaudioside C, a natural product, is claimed to have enhancement activity on a broad range of CHO and HP sweeteners (Salemme et al. 2010). As an example, rebaudioside C, at 285 mg L^{-1}, reportedly causes a 1.4-fold enhancement of 5% sucrose.

5. Symrise: Multiple examples of compounds with sweetness enhancement activity have been reported. As a first example, 1-(2, 4-dihydroxyphenyl)-2-(3-methoxy-4-hydroxyphenyl)-ethanone is reported (Ley et al. 2006) to enhance sucrose (i.e., at 100 mg L^{-1}, a 1.2-fold enhancement of 5% sucrose). As a second example, hesperetin is reported (Ley et al. 2007) to enhance sucrose (i.e., at 30 mg L^{-1}, a 1.2-fold enhancement of 8% sucrose). As a third example, 2,3′,6-trihydroxy-4′-methoxydihydrochalcone is reported (Krammer et al. 2007) to enhance sucrose (i.e., at 100 mg L^{-1}, a 1.2-fold enhancement of 5% sucrose). As a fourth example, N-(3′-methoxy-4′-hydroxybenzyl)-2,4,6-trihydroxybenzamide is reported (Ley et al. 2008) to enhance sucrose (i.e., at 100 mg L^{-1}, a 1.3-fold enhancement of 5% sucrose). And as a fifth example, 3′-7-dihydroxy-4′-methoxyflavan is reported (Wessjohann et al. 2010) to enhance sucrose (i.e., at 50 mg L^{-1}, a 1.6-fold enhancement of 5% sucrose).

The enhancement effects in the examples from the five companies listed above are quite modest and in all cases, the enhancers exhibit sweetness themselves. Compounds exhibiting both agonist and enhancement activities have been termed agoenhancers (Schwartz & Holst 2006). It remains to be demonstrated, for many of these compounds, that the effects observed are truly enhancement effects and not principally due to additive sweetness.

In summary, proof of concept is now in place for sweetener PAM technology and although it is too soon to tell, this new technology just might be a breakthrough technology for the enablement of reduced-calorie foods and beverages with qualities of sweetness not possible with HP sweeteners. For beverages and some other food applications, the intensive efforts presently underway will likely lead to PAMs that make possible, for the first time, CHO sweetener taste quality at significantly lower levels of calories.

SUMMARY AND OUTLOOK

Because of increasing consumer desire for products that deliver great taste with fewer calories, the need for improved zero- and reduced-calorie sweetener technologies will continue. Clearly, although consumer interest in all-natural ingredient systems increases, compromise on taste quality is unlikely. Today, enabled by the cloning of the human sweetener receptor, rapid discovery of novel synthetic and natural HP sweeteners is possible. However, given the thousands of compounds that chemists have synthesized and discovered in nature over the past two centuries, the identification of novel HP sweeteners that accurately replicate the taste of sucrose seems unlikely. If this is the case, then advances in sweetness technologies must derive from either taste modulation on existing HP sweeteners or by PAM-enabled enhancement of CHO sweeteners. However, modulation of existing HP sweetener tastes, particularly in the case of natural HP sweeteners, is very challenging. Scientists have been trying to fix the tastes of SAC and APM (i.e., eliminate bitter and metallic tastes and attenuate sweetness linger) for decades with very limited success. In this review, we have discussed mechanism-of-action based approaches to modulate sweetness, a route more effective than the empirical approaches that have been the norm for many years. However, even though ingredients are now known that eliminate/attenuate HP sweetener "off" tastes, their use significantly increases food and beverage formulation costs. This cost increase is a serious drawback, especially for already-expensive natural HP sweeteners. Commercial success in taste modulation will require a breakthrough. Sweetener PAM technology is an exciting, novel approach to calorie reduction with breakthrough potential. PAMs ensure CHO sweetener taste and can do so in a cost-effective manner. However, the challenges with PAM technology will be identification of more efficacious PAMs (i.e., 10- to 20-fold enhancers) and, ultimately, natural PAMs.

FUTURE ISSUES

1. The Senomyx sucrose-selective PAM is only a twofold enhancer, whereas beverages that may be labeled as zero-cal require a ≥20-fold enhancer. However, optimism for the discovery of ≥20-fold CHO sweetener PAMs is provided by the recent report by Merck Pharmaceutical Company scientists (Ma et al. 2009) on discovery of >100-fold PAMs for the muscarinic acetylcholine receptor. What is the maximal enhancement effect possible with T1R2/T1R3 PAMs for CHO sweeteners?
2. The T1R2/T1R3 PAMs reported to date that are pure PAMs and with little or no agonist activity exhibit sweetener selectivity. Is it possible to identify PAMs with broad sweetener enhancement capability?
3. Consumers have expressed preference for natural flavors. Will it be possible to identify natural enhancers with strong enhancement effects (i.e., ≥2 fold) that are cost effective?
4. In principle, PAMs for HP sweeteners with "off" tastes or atypical temporal profiles (e.g., REBA, MOGV, brazzein, etc.) could benefit by a PAM. Is it possible to identify PAMs for synthetic and natural HP sweeteners, and will PAM formulations significantly improve their taste qualities?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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