SWEETENERS

1. Introduction

Sugar [57-50-1] (sucrose) imparts a sweet taste that is quick, clean, and short lived. These desirable qualities render sugar (qv) the gold standard for sweet taste. Sugar is also an important functional ingredient for preparing attractive foods. It provides the support for bulkiness, texture, preservation, flavor, and color. However, sugar is a nutritive sweetener. It is easily metabolized, yielding an energy of ~4 kcal/g (16.7 kJ/g), a fact welcome by some people, but disliked by others. Furthermore, metabolism of sugar and other fermentable carbohydrates (qv) by the microorganisms in the oral cavity contributes to tooth decay. Thus, for obvious reasons, there is a strong demand for alternative sweeteners that possess all the advantages of sugar, but do not demonstrate the disadvantages. As society's attitude continues to shift toward slimness and increased health, this demand for good alternative sweeteners is expected only to intensify.

As of this writing (~2005), an ideal alternative sweetener does not exist. There are, however, many sweet compounds in use, which generate less calories than sugar, albeit without all the advantages of sugar. Nonnutritive sweeteners are potently sweet in general and only minute quantities are required for sweetening foods. As such, foods containing nonnutritive sweeteners generate no or negligible calories from the sweeteners themselves, regardless of whether or not these sweeteners are caloric.

Sweetness potency (sucrose potency = 1X) denotes how many times a given compound is more potent than sugar on the same weight basis. When compared to a lower concentration of sugar solution, the sweetness potency is usually much higher than comparing with a high concentration of sugar solution. Therefore, reported sweetness potencies must be interpreted carefully, preferably with the percentage concentration of the matching sucrose solution also indicated. Because sweet beverages commonly employ ~ 10% sucrose, sweetness intensity matching this sucrose solution is commonly used. Determination of sweetness potency can be greatly affected by the sensitivity and experience of tasters; other ingredients in the solution, eg, pure water versus flavored beverage; texture of the food; pH; temperature of the samples. Therefore, the published potency should be used only as a guideline and a food technologist should optimize the sweetener level in each product. The potency of major sweeteners denoted throughout this article is reported by the manufacturer, accompanying without indication of the comparative sugar solution concentration.

In addition to being both sweet and safe, a good alternative sweetener should have other qualities similar to those of sucrose. These include stability as a function of temperature and pH, clean sweet taste, quick onset, no lingering aftertaste, compatibility with other food ingredients, high water solubility, high dissolution rate, and ease of handling. It should also be nonhygroscopic, synergistic with other sweeteners, economical (same or cheaper than sugar based on sweetness equivalence), and have a high degree of consumer acceptance, eg, no perceived toxicity. Even an extremely potent sweetener must possess these qualities in order to be developed as a commercial product. Homogeneous distribution of a very potent sweetener in solid products can be a challenge for food technologists.

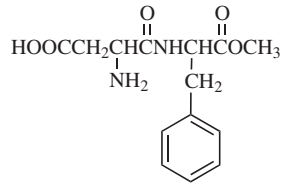
Nonnutritive sweeteners are highly regulated in United States and abroad. The regulation can be complex. Not only the limits in different food catagories vary, in some countries combination of nonnutritive and nutritive sweeteners is not allowed. Definition for diet, zero calories, low calories, and reduced calories also vary among different countries. Food technologists are advised to check out local sweetener regulations before formulating products for a particular country.

Blending of nonnutritive sweeteners has gained popularity in beverage and the food industry because of better taste and cost savings. A blend of sweeteners tends to impart a more rounded aftertaste with reduced shortcomings of individual sweeteners. This is referred to as qualitative synergy. A combination of sweeteners may also impart a total sweetness higher than the sum of sweetness from the respective sweeteners. This is called quantitative synergy. The most significant example of quantitative synergy is a blend of aspartame and acesulfame-K.

2. Nonnutritive Sweeteners

The worldwide total manufacturers's sales of high potency sweeteners in 2003 was estimated to be $\sim$ $1 billion, dominated by six sweeteners. In descending order, these were aspartame ($550 million), sucralose ($130 million), acesulfame-K ($120 million), cyclamate ($110 million), saccharin ($80 million), and stevioside ($10 million) (1). Sucralose has experienced significant growth since its approval by the U.S. Food and Drug Administration (FDA) in 1998. Neotame was approved by the FDA in 2002. Alitame appears to be dormant as there was no commercial development activity since $\sim$ 1996. Thaumatin, neohesperidin dihydrochalcone, and glycyrrhizin are flavor modifiers, but not approved as sweeteners in the United States. They can be used as flavors with GRAS (generally recognized as safe) status affirmed by Flavor and Extract Manufacturers Association (FEMA) of the United States.

2.1. Aspartame. Aspartame [22839-47-0]; [53906-69-1] (APM, L-α-aspartyl-L-phenylalanine methyl ester) (**1**), is the most widely used nonnutritive sweetener worldwide. This dipeptide ester was synthesized as an intermediate for an antiulcer peptide at G. D. Searle in 1965. Although this compound was known in the literature, its sweet taste was serendipitously discovered when a chemist licked his finger, which was contaminated with it. Many analogues, especially the more stable esters, were made (2) and their taste qualities and potencies determined. It was the first compound to be chosen for commercial development. Following the purchase of G. D. Searle by Monsanto, the aspartame business was split off to become a separate Monsanto subsidiary called NutraSweet. The J.W. Childs Associates purchased NutraSweet in 2000. NutraSweet is a tradename of aspartame produced by NutraSweet. Tradenames for tabletop products produced by Merisant include NutraSweet, Equal, Canderal, and Sucaryl. The other principal producers of aspartame include Ajinomoto (trade name: Pal Sweet), Holland Sweetener Company (trade name: SANECTA), a joint venture of Dutch (DSM) and Japanese (Toyo Soda) companies, and Dae Sang (formerly Miwon), which established a business relationship with NutraSweet in 2003.



(1)

Aspartame was approved by the FDA in 1981 for use in dry goods. Two years later it was approved for use in carbonated beverages (qv). Additional approvals came in 1993 for baked goods, candies, and still beverages. Aspartame can be used legally in just about all food categories. Economically, the most important use of nonnutritive sweeteners is in carbonated beverages. Aspartame has enjoyed great success in this category. The top five diet beverages in the United States in 2004 use aspartame as a stand alone sweetener. The U.S. patent for aspartame expired in December, 1992. Subsequently, the price has declined.

Aspartame is caloric. As a dipeptide, it yields ~4 kcal/g (16.7 kJ/g). However, because of its high sweetness potency (200X), only a minute quantity is consumed, resulting in negligible caloric contribution. Aspartame is soluble in water at room temperature (~ 1 g/100 mL at pH 3) (3). The solubility is a function of pH and temperature. Higher temperature and lower pH increase the solubility. These conditions also have impact on the rate of decomposition. Aspartame, in its common fine powder form, does not have a high dissolution rate in water. This can be a problem for manufacturing diet beverages. Methods for improving the dissolution rate of aspartame by cogrinding it with food acids (4–7) or polysaccharides (5,8–10), cospray drying with edible bulking agents (11–13), agglomeration (14), and complexation with metal ions (15) have been described in patents.

The methyl ester group of aspartame is very susceptible to deesterification. When this reaction takes place, the resulting deesterified dipeptide is not sweet, but is otherwise tasteless. Avoidance of excessive heat exposure to aspartame is therefore desirable. The impact of heat degradation can be reduced by the encapsulation of aspartame in maltodextrin; fatty acids, eg, hydrogenated cottonseed oil; or other coatings for baking purposes (16). In tropical climates, addition of excess aspartame (a costly option) and rapid supermarket shelf turnover have been employed to maintain product quality. For the same reason, most diet drinks dispensed from a beverage dispenser, ie, soda fountain, contain a blend of aspartame and saccharin or aspartame, acesulfame-K and saccharin, instead of 100% aspartame.

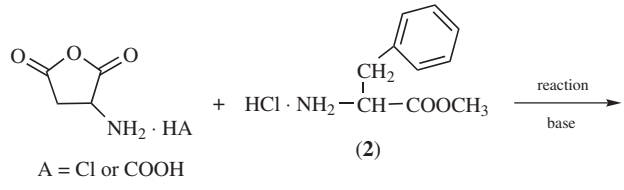
In aqueous solution, the relationship between pH and stability of aspartame is a bell curve having maximum stability at pH 4.3 (Fig. 1). At higher or lower pH, the half-life of aspartame in water diminishes quickly. Most soda and fruit-flavored ready-to-drink beverages are formulated at a pH centered ~ 3.0. To convert these beverages to aspartame-containing diet products, it would be advantageous to adjust the pH as close to 4.3 as possible without changing the original flavor and taste. Otherwise, overage of aspartame may be required to compensate anticipated decomposition during beverage shelf-life.

The principal pathway for the decomposition of aspartame begins with the cleavage of the ester bond, which may or may not be accompanied by cyclization (Fig. 2). The resultant diketopiperazine and/or deesterified dipeptide can be further hydrolyzed into individual amino acids (qv).

The rate of aspartame degradation in dry mixes is more dependent on the water activity than on the temperature (18). In dry mixes, aspartame may also engage in Maillard reactions with the aldehyde moieties of flavoring agents, resulting in the loss of sweetness and flavor. Use of the corresponding acetals of the flavor compounds to avoid this reaction has been reported (19).

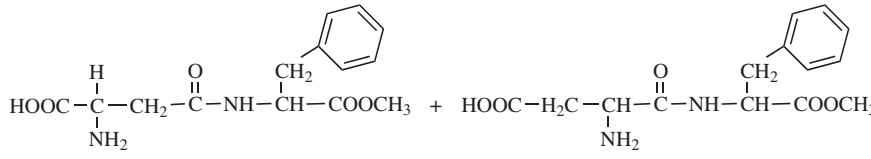
In principle, aspartame is produced through the coupling of two amino acid moieties. One moiety consists of L-phenylalanine methyl ester hydrochloride (**2**) made by treating the amino acid in methanol and hydrochloric acid; the other is aspartic acid anhydride hydrochloride or formic acid salt. The coupling reaction generates two positional isomers, α and β.

Methods (20,21) to increase the ratio of the desired α-isomer (**1**) versus the unsweet β-isomer [22839-61-8] (**3**) exist and are proprietary. The isomers can be separated by subjecting the solution of the final step to hydrochloric acid. The desired α-isomer hydrochloride salt crystallizes out of the solution; the β-isomer remains. There are many patented synthetic processes. The large-scale synthesis of aspartame has been discussed (17,22–40).



A = Cl or COOH

(**2**)

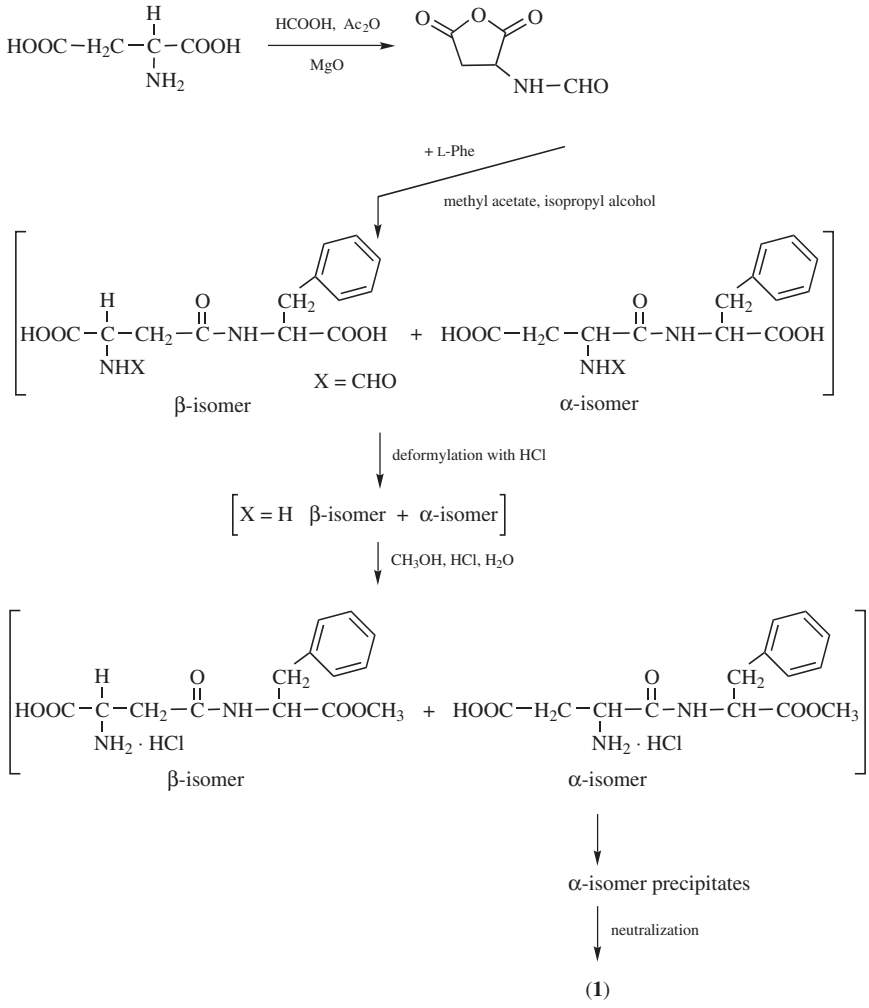


β-isomer (**3**)

α-isomer (**1**)

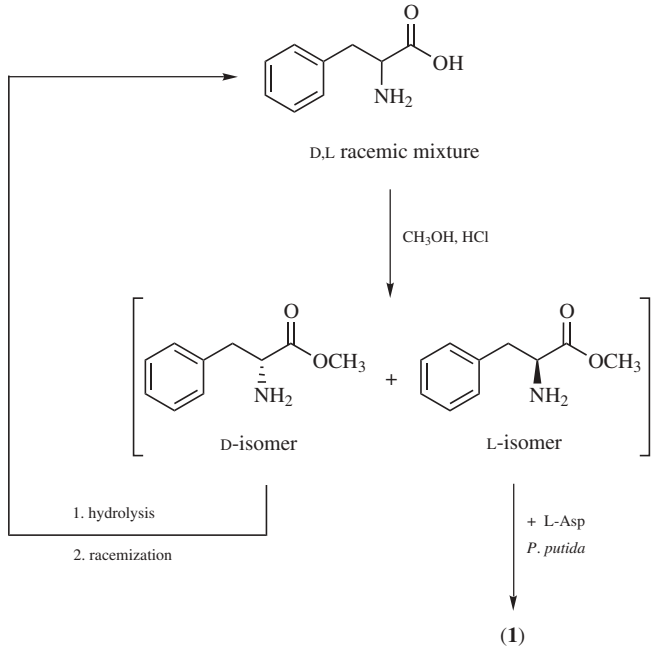
NutraSweet has streamlined the synthesis of aspartame into a more cost-effective one pot process (41). Their process begins by mixing L-aspartic acid with a minimal amount of formic acid and acetic anhydride in the presence of magnesium oxide, resulting in the formation of *N*-formyl-L-aspartic anhydride. L-phenylalanine is coupled with *N*-formyl-L-aspartic anhydride in equimolar amounts in the presence of methyl acetate and/or isopropyl alcohol. These two solvents increase the α/β ratio. The propensity for the reaction mixture to solidify can be reduced by adding acetic acid, raising the temperature to 35°C, and having slow, periodic agitation. The α and β isomers of *N*-formyl-L-aspartyl-L-phenylalanine are then deformylated with hydrochloric acid. After distilling off methanol, methyl acetate, and methyl formate by-products, the resulting mixture of α- and β- L-aspartyl-L-phenylalanine and their various methyl ester is

then esterified by adjusting the concentration of hydrochloric acid, methyl alcohol, and water to produce a high yield of α-aspartame hydrochloride. The esterification reaction, at ambient temperature with gentle agitation, completes in ∼6 days. The desirable α-aspartame hydrochloride salt is easily separated from the β-isomer since α-aspartame hydrochloride dihydrate has a lower solubility in water. The precipitated α-isomer is filtered off and is then neutralized with a base to form aspartame.



Toyo Soda Manufacturing Co. holds a number of patents related to the synthesis of aspartame with microorganisms (39,42). Their technology is the backbone of manufacturing process employed by Holland Sweetener Company—a joint venture between DSM and Toyo Soda. The process starts with methylation of a synthetic racemic mixture of phenylalanine, instead of a more expensive L-phenylalanine. The D- and L-phenylalanine methyl esters and L-aspartic acid are contacted with a culture or treated cultured product of a micro-

organism belonging to the genus Pseudomonas (eg, *Pseudomonas putida* FERM BP 159) to produce aspartame. The microbial coupling does not form the β-isomer of aspartame. The unreacted D-phenylalanine methyl ester is recovered, deesterified, isomerized back to racemic mixture, and recycled into the process.



To reduce the manufacturing cost, aspartame companies tend to produce L-phenylalanine via fermentation themselves. Effort to improve the yield of microorganism via genetic engineering technology has been reported (43).

The safety of aspartame for human consumption has been studied extensively. The results of these studies have satisfied the FDA. However, because phenylalanine is a metabolite of aspartame, people who lack the ability to metabolize this amino acid should refrain from using aspartame. Any aspartame-containing diet food must indicate that the product contains phenylalanine.

In addition to aspartame, two dipeptide sweeteners, neotame and alitame, have been commercialized.

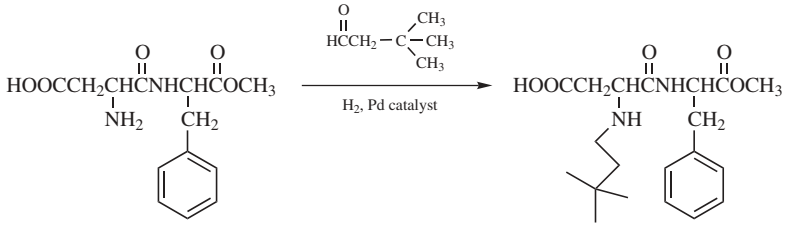
2.2. Neotame. Neotame [165450-17-9] (**4**), *N*-[*N*-(3,3-dimethylbutyl)-L-α-aspartyl]-L-phenylalanine 1-methyl ester, is the most potent commercial sweetener to date (∼2005). It is ∼8000 times sweeter than sucrose or ∼40 times sweeter than its analogue, aspartame (44). The very high potency means that a much lower dose of neotame is required to achieve equivalent sweetness by other sweeteners. Low dosage translates into significant cost savings and reduced logistic resources. Because the molecule is not metabolized into free amino acids in the body, an information statement for phenylketoureic consumers is not required for neotame-containing products.

Neotame follows similar degradation kinetics as that of aspartame. The main difference is that neotame does not generate diketopiperazine (DKP). As

in the case of aspartame, once the methyl ester of neotame is hydrolyzed, the sweetness is lost.

Neotame was approved by the FDA as a sweetener for general purposes in 2002. Unlike aspartame, neotame is more commonly used as a partial substitution of sugar or high fructose corn syrup, and in blends with other high potency sweeteners, instead of a stand alone sweetener.

Neotame is manufactured via a reductive alkylation of aspartame with 3,3-dimethylbutyaldehyde [2987-16-8] (45).



(**4**)

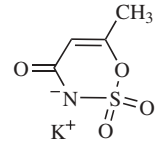
2.3. Alitame. Alitame [80863-62-3], L-α-aspartyl-D-alanine *N*-(2,2,4,4-tetramethylthietan-3-yl)amide (**5**), was developed by Pfizer, but is owned by Danisco now. In 1986, Pfizer filed a food additive petition with the FDA. As of 2005, it was still pending. Alitame was approved for use as a sweetener by Australia in 1993, by China, Mexico, and New Zealand in 1994, by Indonesia in 1995, and by Colombia in 1996.

Alitame (trade name: Aclame) is a water-soluble, crystalline powder of high sweetness potency (2000X). The sweet taste is clean, and the time–intensity profile is similar to that of aspartame. Because it is a sterically hindered amide rather than an ester, alitame is expected to be more stable than aspartame. At pH 2–4, the half-life of alitame in solution is reported to be twice that of aspartame. The main decomposition pathways (Fig. 3) include conversion to the unsweet β-aspartic isomer (**6**) and hydrolysis to aspartic acid and alanine amide (46). No cyclization to diketopiperazine or hydrolysis of the alanine amide bond has been reported.

Although the exact pathway for manufacturing alitame is proprietary, one of the routes for small-scale synthesis has been given (46). This 1983 Pfizer patent lists many active analogues and serves as a good reference for the structure–activity relationship.

2.4. Acesulfame-K. Acesulfame-K [55589-62-3] (**7**), the potassium salt of acesulfame [33665-90-6] (6-methyl-1,2,3-oxathiazin-4(3*H*)-one 2,2-dioxide), is a sweetener that resembles saccharin in structure and taste profile. 5,6-Dimethyl-1,2,3-oxathiazine-4(3*H*)-one 2,2-dioxide, the first of many sweet compounds belonging to the dihydrooxathiazinone dioxide class, was discovered accidentally in 1967 (47). From these many sweet compounds, acesulfame was chosen for commercialization. To improve water solubility, the potassium salt was made. Acesulfame-K (trade name: Sunett) was approved for dry product

use in the United States in 1988 and in Canada in October, 1994. In 2003, acesulfame-K was approved as a general purposes sweetener by the FDA.

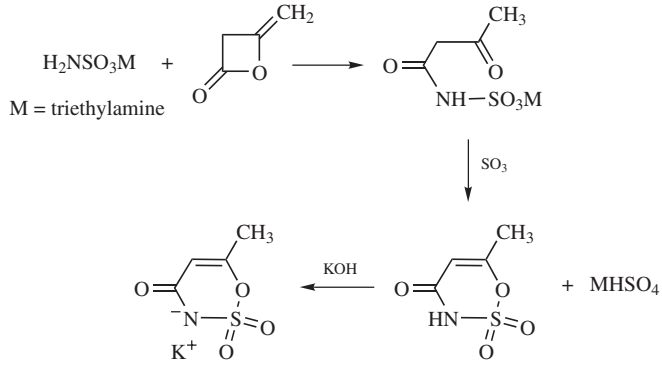


(**7**)

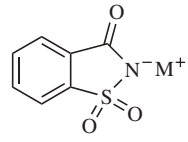
Acesulfame-K is a white crystalline powder having a long (6 years or more) shelf-life. It readily dissolves in water (270 g/L at 20°C). Like saccharin, acesulfame-K is stable to heat over a wide range of pH. At higher concentrations, there is a detectable bitter and metallic off-taste similar to saccharin. Use of the sodium salt of ferulic acid [437-98-4] (FEMA no. 3812) to reduce the bitter aftertaste of acesulfame-K has been described (48). The sweetness potency of acesulfame-K (100–200X, depending on the matching sucrose concentration) (47) is considered to be about one-half that of saccharin, but is about the same as that of aspartame.

Acesulfame–K-aspartame blends exhibit a significant synergistic effect (Fig. 4) (49). This synergy provides large cost savings for the diet foods industry. The blend also has a more rounded sweetness profile.

The principal commercial process for acesulfame-K is depicted below (50).



2.5. Saccharin. Saccharin [81-07-2], 3-oxo-2,3-dihydro-1,2-benzisothiazole 1,1-dioxide (*o*-sulfobenzimide or *o*-benzosulfimide) (**8**), was accidentally discovered to be a sweet compound in 1878. A pilot plant was set up in New York to manufacture saccharin, which was displayed in a London exposition in 1885 (51). Since that time, saccharin has been used in many parts of the world.



(**8**)

M = H	saccharin	[81-07-2]
M = Na^+	sodium saccharin	[128-44-9]
M = $(Ca)^+_{½}$	calcium saccharin	[6485-34-3]

In 1969, a chronic toxicity study on a cyclamate/saccharin (10:1) blend indicated bladder cancer problems in rats. Cyclamate was soon banned by the FDA, but saccharin remained an approved sweetener. In 1977, the FDA proposed a ban on saccharin because of the discovery of bladder tumors in some male rats fed with high doses of saccharin. Because no other nonnutritive sweetener was available at that time, the proposed ban faced strong opposition. Legislation to stay the ban has been passed in the U.S. Congress periodically. In December, 1991, the FDA withdrew its proposed ban. All saccharin-containing packaged products were required to carry a warning label indicating that saccharin has been determined to cause cancer in laboratory animals. In 2001, the warning label requirement was lifted by the Congress. In 2003, saccharin was delisted from California Proposition 65 (the so-called carcinogen list).

The main utility of saccharin had been in beverages and as a tabletop sweetener. Upon the approval of aspartame for carbonated beverages in 1983, aspartame displaced saccharin in most canned and bottled soft drinks. However, saccharin is still used, usually blended with aspartame, in carbonated soft drinks dispensed from soda fountains.

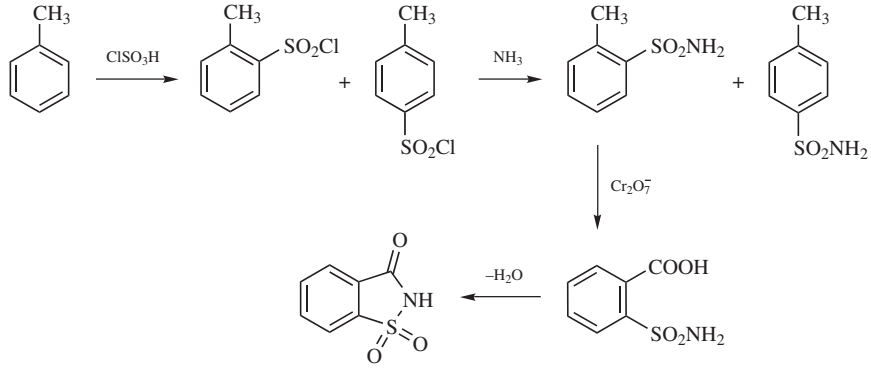
Saccharin is acidic and not very soluble in water. For improved solubility, the food industry prefers the sodium [128-44-9] or calcium [6485-34-3] salt. Sodium saccharin is so widely used that it is often referred to simply as saccharin. The aqueous solubilities of both salts are about the same, ie, 0.67 g/mL. Saccharin, stable to heat over a wide pH range, can withstand most food processing (qv) conditions. Interactions between saccharin and other food ingredients have not been reported.

Saccharin imparts a sweetness (300X) that is pleasant at the onset, but is followed by a lingering, bitter aftertaste. Sensitivity to this bitterness varies from person to person. At high concentration, however, most people can detect the rather unpleasant aftertaste. Saccharin is synergistic with other sweeteners of different chemical classes. For example, saccharin–cyclamate, saccharin–aspartame, saccharin–sucralose, and saccharin–alitame combinations all exert synergy to various degrees. The blends, as a rule, exhibit less aftertaste than each of the component sweeteners by themselves.

Saccharin is the most economical sweetener available. It is 300 times more potent than sugar and its price in 1998 was ~$2.75/lb, ~$0.009/(lb·sweet unit). Sugar, on the other hand, was ~$0.36/lb, which is 40 times more expensive than saccharin on equal sweetness basis (51). The low cost and high stability of sac-

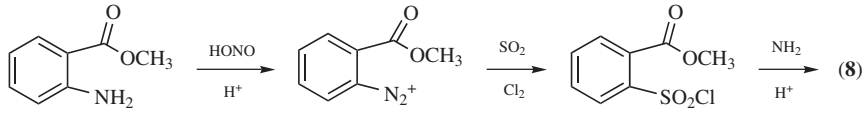
charin render it the sweetener of choice for dentifrices (qv), other toiletry products, and pharmaceuticals (qv).

The original Remsen–Fahlberg process (52) for saccharin synthesis requires the separation of ortho- and para-isomeric intermediates.



(**8**)

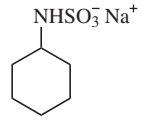
In early 1950, a newer process for saccharin production was developed and the Maumee Chemical Company was subsequently formed. The Maumee process uses anthranilic acid [118-92-3] as the starting material. After the merger of the PMC Specialties Group and the Maumee Chemical Company, the process was improved to a one-pot continuous process using methyl anthranilate [134-20-3] as the starting material (52). As of this writing, PMC is the sole producer of saccharin in the United States. Saccharin is also produced in Japan, China, Korea, and Taiwan. In 2003, PMC scored a victory in antidumping cases against multiple Chinese manufacturers.



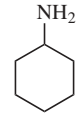
(**8**)

Many analogues of saccharin have been synthesized since its discovery. With the exception of one compound, thieno[3,4-*d*]isothiazolone dioxide [59337-79-0], 1000X, this effort has not generated more potent compounds. Acesulfame-K could be considered a ring-modification derivative of saccharin, however.

2.6. Cyclamate. Sodium cyclamate [139-05-9] (**9**), the sodium salt of cyclamic acid [100-88-9], was so widely used that it was often just called cyclamate. The other common salt, calcium cyclamate [139-06-0], is useful in low sodium diets.



(**9**)



(**10**)

Cyclamate was first synthesized in 1937. Like the other sweeteners, its sweet taste was accidentally discovered (53,54). The FDA in 1958 classified sodium cyclamate as a GRAS sweetener. In 1969, a 2-year chronic toxicity study with a sodium cyclamate–sodium saccharin (10:1) mixture found bladder tumors in rats. The FDA took cyclamate off the GRAS list, banning it from foods and beverages, but permitting its sale in pharmacies. In 1970, after a congressional investigation, the FDA banned the use of cyclamate entirely. Abbott Laboratories, which has conducted additional toxicity and carcinogenicity studies with cyclamate, a 10:1 mixture of cyclamate–saccharin, and cyclohexylamine [108-91-8], claimed to be unable to confirm the 1969 findings. Abbott then filed a food additive petition for cyclamate in 1973, which was denied by the FDA in 1980. In 1982, the Calorie Control Council and Abbott Laboratories filed a second food additive petition containing the results of additional safety studies (55). That petition remains active. Cyclamate is, however, allowed for use in any or all three categories, ie, food, beverage, and tabletop, in ∼50 countries. In 2004, the maximum beverage use level of cyclamate in the European Union was lowered to 250 ppm (as cyclamic acid). Sweet ′n Low, known in the United States as a saccharin-based tabletop sweetener, contains exclusively cyclamate in Canada.

Cyclamate is ∼30 times more potent than sugar. Its aftertaste is minor compared to saccharin and acesulfame-K. The mixture of cyclamate and saccharin, especially in a 10:1 ratio, imparts both a more rounded taste and a 10–20% synergy. Cyclamate (**9**) is manufactured by sulfonation of cyclohexylamine (**10**). Many reagents can be used, including sulfamic acid, salts of sulfamic acid, and sulfur trioxide (56–60).

2.7. Sucralose. Sucralose [56038-13-2], 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactopyranoside, is a trichloro-*galacto*-sucrose sweetener developed by the British sugar company Tate & Lyle during the 1970s (61–63). It was licensed to McNeil-PPC, Inc., a Johnson & Johnson subsidiary, in the United States until a new agreement took place in February, 2004. McNeil Nutritionals retained ownership of SPLENDA Brand and the right for its worldwide retail and food service business. Tate & Lyle became the sole manufacturer of SPLENDA Brand sucralose and owned the right for its worldwide ingredient sales.

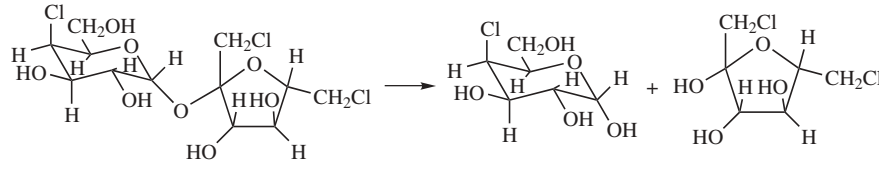
Sucralose was approved by the FDA in 1998. It is also allowed in Canada, EU, Japan, and many other countries.

The disaccharide structure of (**11**) (trade name: SPLENDA) is argued by the manufacturer as responsible for a taste quality and time-intensity profile closer to that of sucrose than any other high potency sweetener. Their favorite commercial slogan is “made from sugar, so it tastes like sugar”. The marketing strategy appears to be sucessful as sucralose shows strong growth since FDA approval.

The slogan was challenged in two lawsuits filed by Merisant in November, 2004 and by the U.S. Sugar Association in January, 2005.

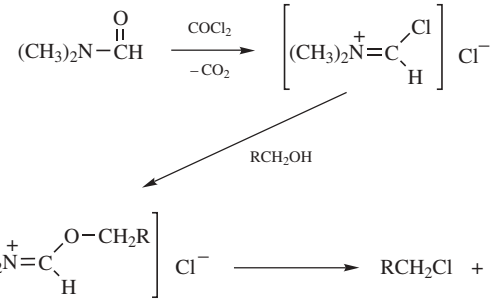
The sweetness potency of sucralose is reported to be 600X. A moderate degree of synergy between sucralose and other nonnutritive (64) or nutritive (65) sweeteners has been reported.

Sucralose in water is quite stable to heat over a wide range of pH. However, the pure white dry powder, when stored at high temperature, can decompose through, presumably, a dehydrochlorination reaction. Shipping and storage of pure sucralose must comply strictly with the conditions stipulated in the Material Safety Data Sheet (MSDS). The decomposition potential is greatly reduced after admixing with other ingredients, such as maltodextrin, fibers, carbohydrates, water, etc (66). In addition to powder, the commercial product can also be a 25% concentrate in water, buffered at pH 4.4, and is preserved with sodium benzoate and potassium sorbate. The shelf-life of this water solution is 5 years according to manufacturer's specification. The degradation of sucralose in aqueous systems, however slowly, yields respective chlorinated monosaccharides, 4-chloro-4-deoxy-galactose (**12**) and 1,6-dichloro-1,6-dideoxy-fructose (**13**) (67).

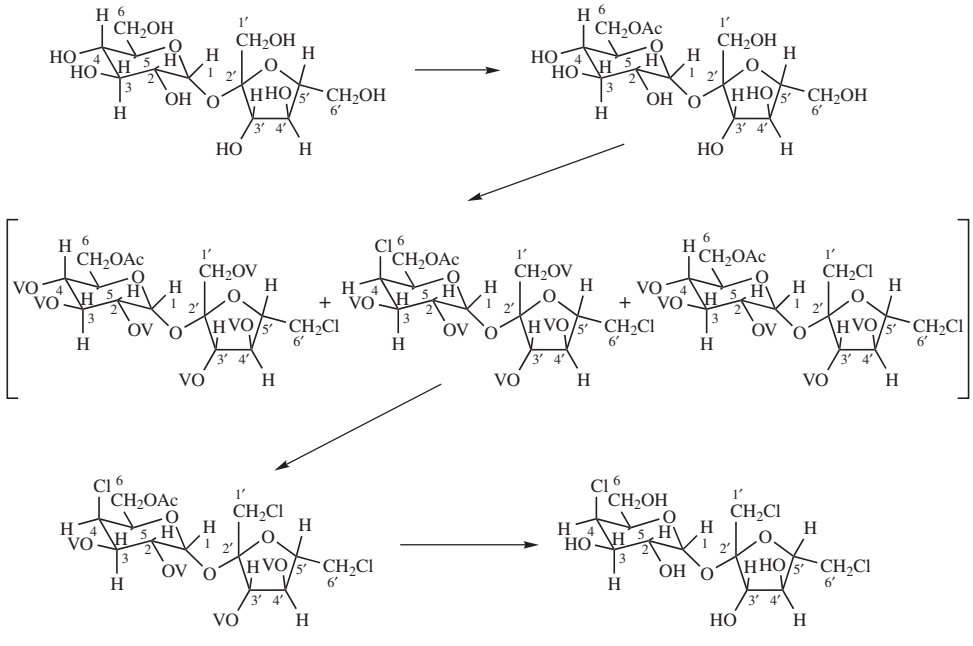


(**11**) (**12**) (**13**)

The synthesis of sucralose employs sucrose (glucosyl-fructose) as the starting material. The principle of a successful technology would require discriminative conversion of the eight hydroxy groups into desirable 4,1',6'-trichloro-*galacto*-sucrose. Protection and deprotection steps should be avoided, as they are costly. Based on information depicted in several patents, the following incremental chlorination scheme is believed to be close to current commercial synthetic process. Assisted by a tin-catalyst (eg, 1,3-diacetoxy-1,1-3,3-tetrabutyldistannoxane), sucrose 6-ester (acetate or benzoate) is made first from sucrose. The remaining hydroxy groups of the sucrose 6-ester form *O*-alkylforminium chloride adduct with a Vilsmeier reagent made from dimethylformamide (DMF) and phosgene. Upon heating to $< 85^{\circ}C$, monochloro (4- and 6'-) and dichloro (4,6'- and 1',6'-) sucrose 6-esters were made. The temperature of the reaction mixture is then elevated to $< 125^{\circ}C$ to produce the 1',4,6'-trichlorogalacto-sucrose 6-ester. After alkali hydrolysis of the nonchlorinated *O*-alkylforminium chloride complexed hydroxy groups at 2,3,3',4' followed by neutralization with acid, the desired 4,1',6'-trichlorogalactosucrose 6-ester was extracted into a water misciscible organic solvent followed by crystalization. Removal of the ester group at the C-6 position via alkali hydrolysis yields sucralose (68,69). Because chlorination at the C-4 position reverses the stereochemistry (ie, from α-OH to β-Cl), the nomenclature for sucralose is chlorinated *galacto*-fructose instead of chlorinated *glucosyl*-fructose.



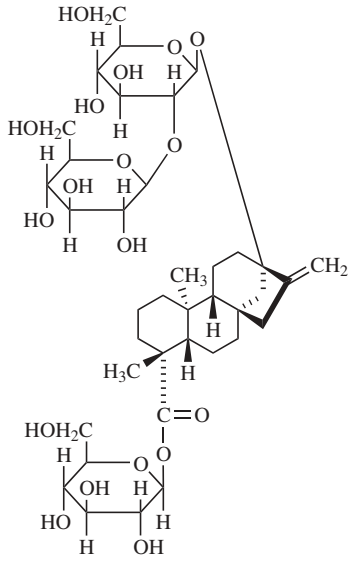
Chlorination of hydroxy group with Vilsmeier-type salt



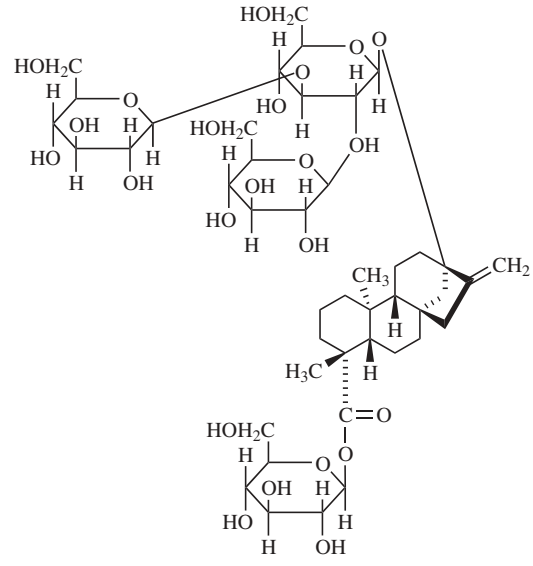
V denotes Vilsmeyer-type adduct

(**11**)

2.8. Stevioside. Stevioside [57817-89-7] (**14**) is a naturally occurring sweetener (~200X) extracted from a South American plant, *Stevia rebaudiana* Bertoni. The dried leaves, the water extract of leaves, and the refined chemical ingredients, eg, (**14**) and Rebaudioside A [58543-16-1] (~300X) (**15**), can all be used as sweetening agents. These are collectively referred to as stevia. Discovered in Paraguay and Brazil, the plant was identified in the early 1970s as a plant of high economical value and transported to Japan for cultivation, where the commercialization of stevia leaves extract as a natural sweetener became a success. Today Stevia plant is cultivated primarily in China.



(**14**)



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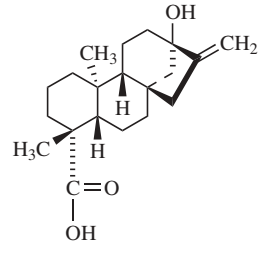


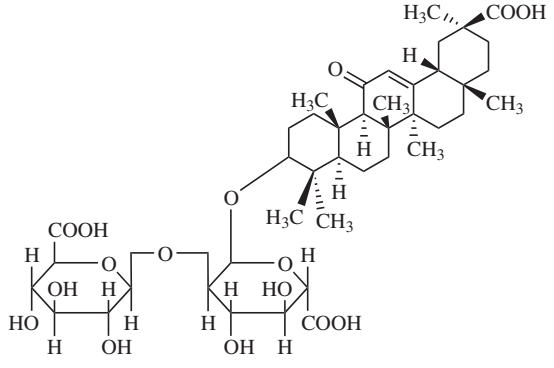
(**16**)

Stevioside and rebaudioside A are diterpene glycosides. The sweetness is tainted with a bitter and undesirable aftertaste. The time–intensity profile is characteristic of naturally occurring sweeteners: slow onset, but lingering. The aglycone moiety, steviol [471-80-7] (**16**), which is the principal metabolite, has been reported to be mutagenic (70). Wide use of stevia in Japan for >30 years did not produce any known deleterious side effects. In Japan, enzymatically glycosylated blend of stevioside and rebaudioside A, which appears to impart a cleaner taste profile, is also available commercially.

Because no food additive petition has been presented to the FDA, stevioside and related materials cannot be used in the United States as food ingredients. An import alert against stevia was issued by the FDA in 1991. In 1995, however, the FDA revised this import alert to allow the importation and use of stevia as a dietary supplement (71), but not as a sweetener or an ingredient for foods. In 2004, JECFA (Joint Expert Committee of Food Additives) issued a preliminary ADI (Allowed Daily Intake) of 2-mg/kg body weight (based on steviol) for stevia extract. This action may impact favorably on worldwide regulatory approval. As consumer's demand on naturally sweetened products increases, interest in

Stevia, and naturally occurring potent sweeteners in general, is expected to grow. A comprehensive review of stevia is available (72).

2.9. Glycyrrhizin. Glycyrrhizin (**17**), also known as glycyrrhizic acid [1405-86-3], is a glycoside isolated from the roots of licorice, *Glycyrrhiza glabra* L. For improved water solubility, an ammoniated salt is commonly used. This can be in the form of either ammonium glycyrrhizinate or monoammonium glycyrrhizinate.



(**17**)

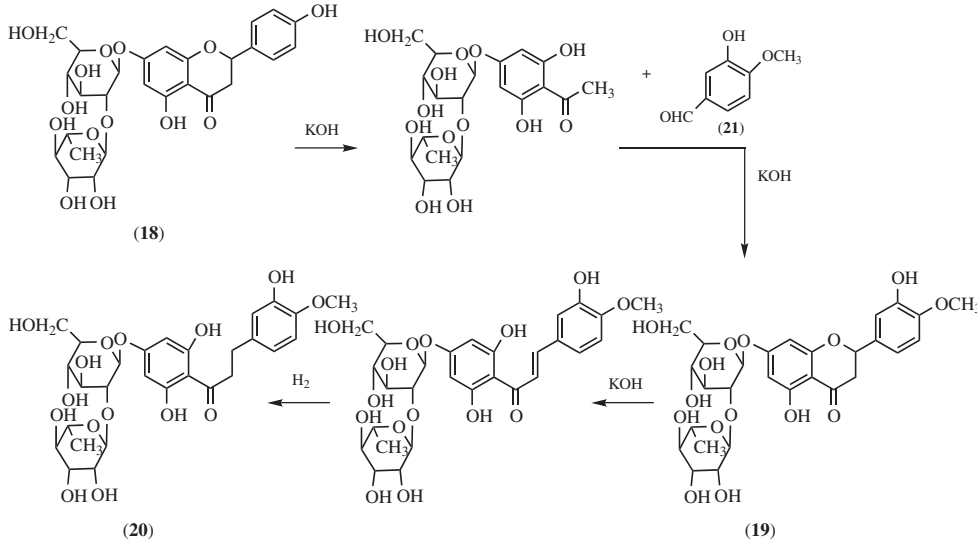
The sweetness potency of glycyrrhizin is ~33X. Its taste, however, is accompanied by a characteristic licorice flavor, making it incompatible with many other food ingredients. The time–intensity profile is similar to that of other naturally occurring high potency sweeteners: slow onset followed by lingering aftertaste. It is claimed to be heat stable. Ammonium glycyrrhizinate, which tends to precipitate < pH 4.5, is affirmed in the United States as a GRAS flavoring agent (FEMA no. 2528). It is not approved for use as a sweetener.

Glycyrrhiza root extracts containing at least 90 wt % pure glycyrrhizin are widely used in Japan, second only to stevia sweeteners. Glycyrrhizin exerts pharmacological effects, eg, edema and hypertension, on account of which the Japanese government has urged people to curtail consumption to < 200 mg/day of glycyrrhizin in drug formulations (73). Several reviews of glycyrrhizins are available (73–77).

2.10. Neohesperidin Dihydrochalcone. In the 1960s, there was a strong effort by the U.S. Department of Agriculture (USDA) to study the structure–activity relationship of citrus-derived chemicals. The goal was to reduce the bitter taste of citrus juices derived from bitter principles, such as naringin [10236-47-2] (**18**), neohesperidin [13241-33-3] (**19**), and limonin [1180-71-8]. Neohesperidin is a glycoside composed of a flavanone and a disaccharide (glucose and L-rhamnose [3615-41-6]). Upon treatment with potassium hydroxide, the flavanone ring opens up to yield a chalcone. Catalytic hydrogenation of this chalcone produces neohesperidin dihydrochalcone [20702-77-6] (NHDC) (**20**), which tastes sweet.

Many other dihydrochalcones have been made, but most of the toxicological studies have been conducted using NHDC and thus **20** has been petitioned and

allowed for use. Neohesperidin (**19**) is best isolated from the bitter orange (Seville orange), but it can also be synthesized from **18** and isovanillin [621-59-0] (**21**) (78).



NHDC imparts a sweetness that has a much slower onset and much greater lingering than sucrose. There is a slight aftertaste. The sweet potency at the 10% sucrose solution sweetness equivalence is ~300X. The most significant advantage of **20** is its ability to reduce the bitterness of the citrus bitter principles: naringin (**18**) and limonin. For example, at 5% sucrose equivalence, NHDC increased the threshold for **18** from 20 to 49 mg/kg (78).

NHDC is an off-white powder having low solubility in water (0.5 g/L at room temperature). It is quite stable over a broad range of pH and temperature. Under extreme conditions, hydrolysis of the ether linkage between the saccharides and the aglycone can take place. However, the aglycone itself is reported to be sweet. NHDC is allowed for use as a sweetener according to the European Union Sweeteners Directive in 1994. It has not been approved as a sweetener in the United States. However, in 1993, NHDC was affirmed by FEMA as a GRAS flavor modifier (FEMA no. 3811) for many food categories.

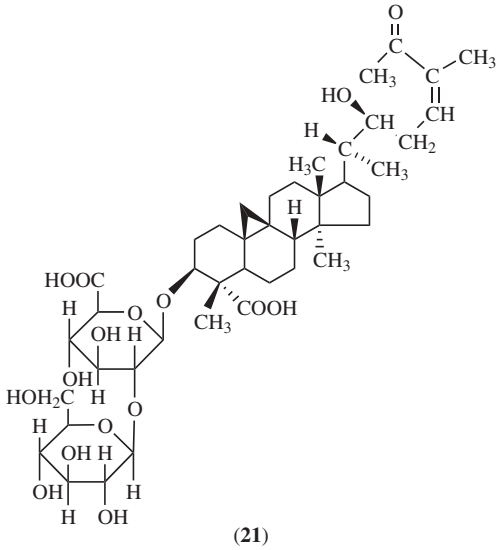
2.11. Thaumatin. Thaumatin [53850-34-3] is a mixture of proteins extracted from the fruit of a West African plant, *Thaumatococcus daniellii* (Bennett) Benth. Research at Unilever showed that the aqueous extract contains two principal proteins: thaumatin I and thaumatin II. Thaumatin I, mol wt 22,209, contains 207 amino acids in a single chain that is cross-linked with eight disulfide bridges. Thaumatin II has the same number of amino acids, but there are five sequence differences. Production of thaumatins via genetic engineering technology has been reported (79).

Thaumatin (trade name: Talin) is a very potent sweetener (~2000X). However, its potency is overshadowed by inferior taste qualities. The onset of sweetness is very slow, and after reaching maximum sweetness, a very long-lingering

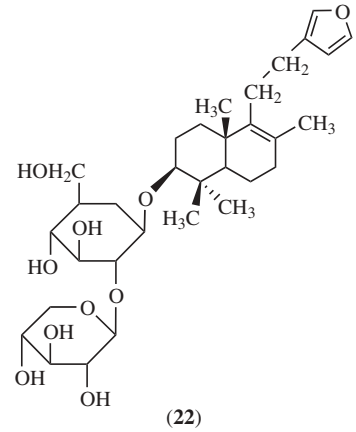
sweetness combined with an unpleasant aftertaste follows. Primarily owing to this poor taste quality, thaumatin is not considered a practically useful sweetener. It is, however, used as a flavor enhancer, especially in products such as chewing gum. Thaumatin and thaumatin B-recombinant were affirmed GRAS flavors (FEMA no. 3732 and 3814, respectively). They are not approved as sweeteners in the United States.

As a protein, thaumatin is remarkably water-soluble (up to 60%) and is stable to heat at low pH. It has been reported that a thaumatin solution at pH < 5.5 can be heated at 100°C for several hours without loss of sweetness. Comprehensive reviews on thaumatin as sweetener are available (80,81).

Table 1 compiles examples of other compounds reported to be sweet.



(**21**)



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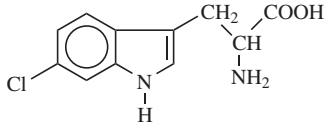

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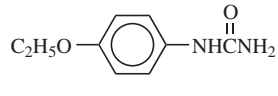

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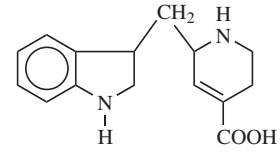

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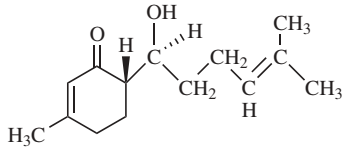

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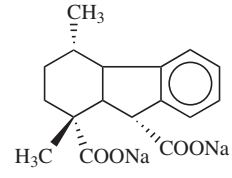

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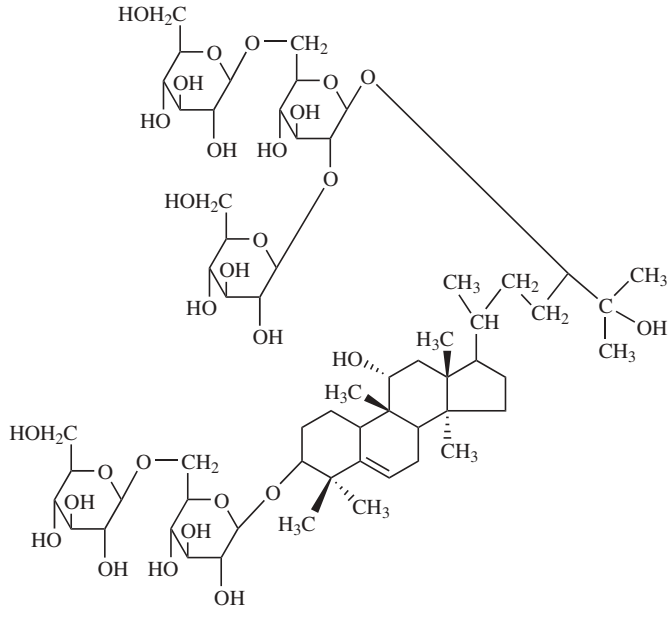

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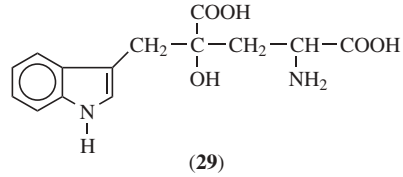

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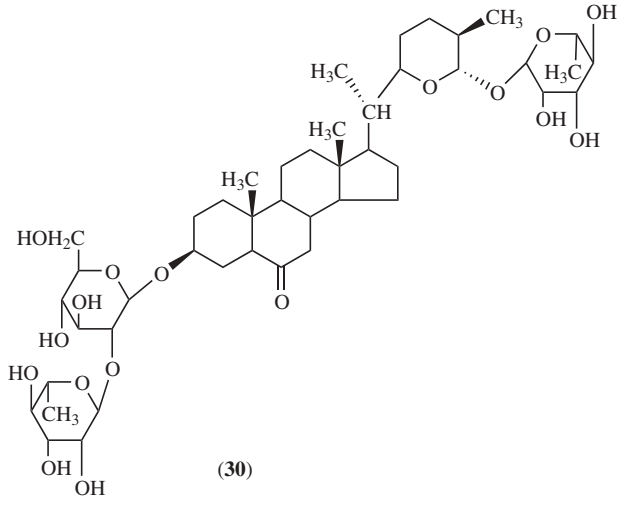

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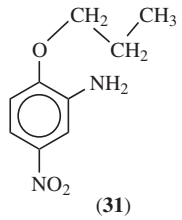

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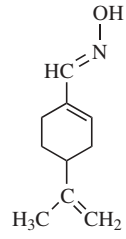

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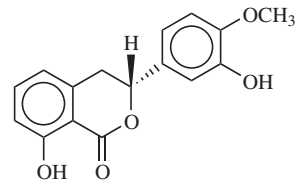
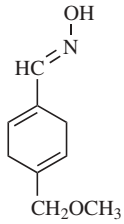


(**33**)



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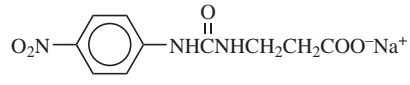
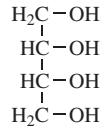


(**35**)

3. Bulking Agents

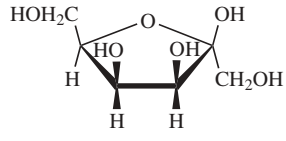
Although food technologists can use nonnutritive sweetener(s) to match the sweetness of regular caloric products, invariably they face the texture or mouthfeel issues in developing sugarless or sugar reduced products. Ideal bulking agents that impart no calories and cause no gastrointestinal side effects remain elusive. Principal examples of bulking agents are the sugar alcohols (qv), eg, sorbitol [50-70-4]; mannitol [69-65-8]; xylitol [87-99-0]; maltitol [585-88-6]; lactitol [585-86-4]; erythritol [149-32-6]; hydrogenated starch hydrolysate; and isomalt, a mixture of glucosyl sorbitol [534-73-6] and glucosylmannitol [20942-99-8]. These alcohols are reduced saccharides resulting primarily from catalytic hydrogenation and, for the most part, are less sweet and less caloric than sugar [$\sim$ 2.4 kcal/g (10.0 kJ/g)] (88) and mostly noncariogenic. The other examples include polydextrose [68424-04-4] [$\sim$ 1 kcal/g (4.18 kJ/g)] and D-tagatose [87-81-0].

3.1. Erythritol. Erythritol (**36**), a four-carbon sugar alcohol of 0.6X sweetness potency, is produced commercially via glucose fermentation by yeasts, such as *Moniliella pollinis* (97). Unlike other sugar alcohols, erythritol is largely absorbed into our body, but excreted out in urine intact. The unique metabolic profile renders it a very low calorie (0.2 kcal/g) sweetener. In 2001, erythritol was accorded GRAS status (GRN 000076) under FDAs GRAS notification policy. Maximum beverage use is 3.5%.

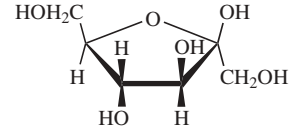


(**36**)

3.2. D-Tagatose. D-Tagatose (**37**) is a ketohexose that only differs from D-fructose [57-48-7] at the C-4 position. At this chiral carbon, it is a mirror image between the two ketohexoses. Commercial production of D-tagatose starts with lactose recovered from whey: A waste product from cheese making. Lactose is hydrolyzed into galactose and glucose via lactase enzyme isolated from microorganisms, eg, *Aspergillus oryzae*. After separation from glucose, D-galactose is isomerized into D-tagatose via calcium hydroxide complexation followed by neutralization with acid (98). D-Tagatose imparts a sweetness of same intensity as sucrose, but with only a fraction of calories [1.5 kcal/g (6.27 kJ/g)]. D-Tagatose was accorded GRAS status in 2001 (GRN 000078). The maximum usage in beverage is 1%.



(**37**) D-Tagatose



D-Fructose

4. Sweetness Enhancers, Inducers, and Inhibitors

4.1. Enhancers and Inducers. A sweetness enhancer is defined as a compound that imparts no sweet taste by itself, but when combined with a sweetener in small quantities, it increases sweetness intensity. A true sweetness enhancer has yet to be found. However, in 2005, Senomyx, a biotechnology firm in California, claimed to have identified up to three chemicals capable to cause x % sugar solution to taste like an $x + n$ % solution (99).

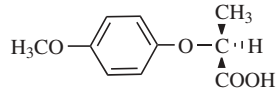
A good sweetness inducer, miraculin [143403-94-5] or [125267-18-7] (100), is known. Miraculin is a glycoprotein found in the fruit (called Miracle Fruit) of a West African shrub, *Richardella dulcifica*. By itself, miraculin imparts no sweetness. When activated in the mouth by acidic substances, however, a sucrose-like sweetness is perceived. Thus, sour lemon, lime, grapefruit, rhubarb, and strawberry taste sweet when combined with miraculin. The taste conversion effect can last an hour or longer.

In 1974, a petition for affirmation of the GRAS status of miracle fruit was submitted by the Miralin Company, mainly based on the fact that miracle fruits have been consumed by humans since before 1958. In 1977, the petition was denied by the FDA. However, miraculin remains a research curiosity. Its struc-

ture was elucidated in 1989 (101). Another protein, curculin [151404-13-6] (102), has also been reported to exert a sweet-inducing activity similar to miraculin.

4.2. Inhibitors. Sugar is used in large quantities in fruit jams as a preservative. The strong sweetness, however, prevents fruity flavors from being noticed. For these and other foods that must use a large amount of sugar for purposes other than sweet taste, there is need for a sweet-taste inhibitor.

Lactisole [13794-15-5], the sodium salt of racemic 2(4-methoxyphenoxy)propionic acid, is a sweet-taste inhibitor marketed by Domino Sugar. It was affirmed as a GRAS flavor (FEMA no. 3773). At a concentration of 100–150 ppm, lactisole eliminates the sweet taste of a 10% sugar solution. This inhibition appears to be receptor related because lactisole also inhibits the sweet taste of aspartame. The (*S*−) enantiomer [4276-74-8] (**38**), isolated from roasted coffee beans, is the active isomer; the (*R*+) enantiomer is inert (103).



(**38**)

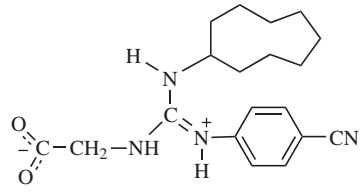
Several natural products, eg, gymnemic acid [122168-40-5] and ziziphin [73667-51-3], have also shown sweet-inhibiting activities. These are not allowed for foods in the United States, however.

5. Sweet-Taste Transduction Mechanisms

Scientific curiosity and commercial interests in developing new potent sweeteners have driven efforts to learn how sweet taste is initiated at the taste bud level on our tongues, to the biochemical events in the taste receptor cell that transduce the receptor–stimulus binding recognition process into a cellular depolarization. This cellular excitation releases neurotransmitter onto the innervating sensory nerve, changing its firing rate, sending the taste signal to the brain. For decades, scientists debated the existence of sweet receptors. On the one hand, the existence of potent sweeteners as well as a sweet inhibitor means there must be specific sweet receptors. On the other hand, low potency of carbohydrate sweeteners seems to indicate a sweet taste initiation without binding with a receptor. The recent discovery of a sweet taste receptor, namely, T1R2/T1R3, appears to give the receptor proponents an upper hand (104).

Starting in the 1960s and 1970s, the binding space of a putative sweet taste receptor was under empirical study. Schallenberger and Acree proposed the "A-HB" theory to explain the structural features of sweet carbohydrates (105). The "A" denotes a hydrogen acceptor and "HB" represents a hydrogen donor. Kier added a third site "Y", a hydrophobic site or hydrophobic cluster, to show enantiomeric specificity of more complex sweeteners (106). Since then, numerous modifications and additions have been made by other scientists. Tinti and Nofre's multipoint attachment theory would appear to have reached an entropic high point by suggesting an eight-point model shown in Figure 5 (107). Purportedly,

sucrononic acid [116869-55-7] (**39**), the most potent sweetener (200,000X) known to mankind and neotame were the fruits of this model. While these and other models were refined and tested, such activity did not bring us closer to discovering the actual receptor itself.



(**39**)

The advancements of molecular biology have led to the identification of the sweet taste receptor. In 1999, Hoon and coauthors from Zucker and Ryba's laboratories reported the cloning and characterization of two class C, family three proteins from rat. Their expression in taste buds and their topographical restriction to specific areas of the tongue led them to claim these as taste receptors, designated as TR1 and TR2 (108). As class C, family 3 receptors, both are members of the large superfamily of G (guanosine triphosphate)-protein coupled receptors (abbreviated as GPCR), characterized by the presence of seven transmembrane segments, a number of conserved cysteine residues in the extracellular domain and several short sequence motifs dispersed throughout the molecule. The fact that the gene for TR1 was expressed almost exclusively in cells of fungiform papillae on the anterior tongue surface and that it was at least near to a region of a chromosomal locus known to affect taste preference for sweet (called the Sac locus) led the authors to claim that TR1 was the sweet receptor. In addition, since they reported that TR2 was expressed exclusively in the posterior taste buds, it was assumed to be a bitter receptor. These specificity judgments were made on the false assumption that the posterior tongue was almost exclusively sensitive to bitter while the anterior was sensitive to sweet. The claims for modality specificity were soon found to be false. In fact, it would be found that TR1 (later renamed as T1R1) is part of an amino acid taste receptor while TR2 (later renamed as T1R2) is part of the sweet taste receptor.

As discovery of more taste receptors was reported by many other groups, nomenclatures changed to avoid confusion and to give a common abbreviation designation to receptors of related structural classes. The taste receptors first identified by Hoon and co-workers, having long extracellular domains, were hereafter to be referred as the T1R family of taste receptors, falling into family 3 of Class C proteins. The bitter receptors reported by Adler and co-workers (109) all have short extracellular domains, are known as the T2R family. They are Class C receptors of family 1.

The third member of the T1R family, T1R3, was identified and characterized by seven laboratories soon after the Sac locus of the human genome was sequenced (110–116). T1R3 was found to be the pivotal receptor, as it was soon realized that both the sweet receptor and the amino acid "umami" receptor were, presumably, heterodimers of members of the T1R family. The sweet receptor is

T1R2/T1R3, and the umami receptor is T1R1/T1R3. The discovery of receptors for sweet, umami, and bitter tastes will undoubtedly propel more research toward better understanding of how human taste transduction is initiated.

There is evidence to suggest that the binding of sugars to the sweet GPCR activates several secondary messenger cascades including those generating the rapid production of cyclic-GMP and the inositolpolyphosphate/diacetylglycerol family of compounds, and the slower accumulation of cyclic-AMP (117). This generation of second messengers presumably activates protein kinases that, in turn, alter the activity of potassium channels and allow the influx of calcium ions from extracellular space. These events lead to the depolarization of the cell and the release of neurotransmitter (118). It was reported that some sweeteners were able to block their own sweet taste signal termination mechanism, which might explain their sweet aftertaste lingering effect (119).

The nerve fibers that innervate taste bud cells respond to sweet stimuli also respond to sour or bitter or salty. The arguments between those scientists who favor a direct, or "labeled line" theory, and those favoring a mixed, or "across fiber pattern" theory continue to enliven the field. It is hoped that molecular biology can help resolve this division as well as many unknowns in the complicated sweet, and indeed all tastes, system.

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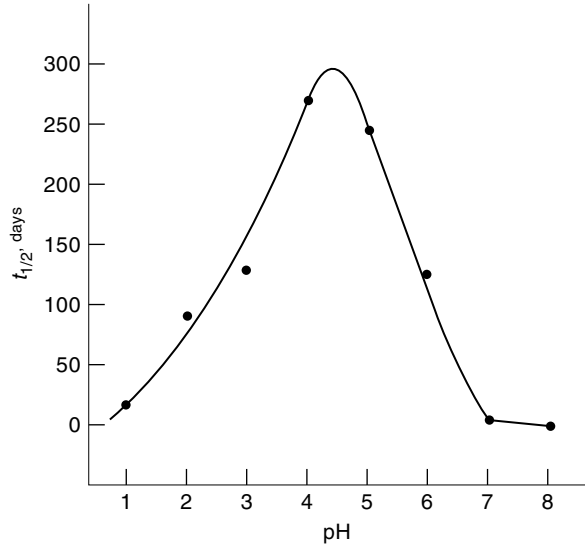

Fig. 1. Stability of aspartame in water at 25°C, where $t_{1/2}$ is the half-life in days (17).

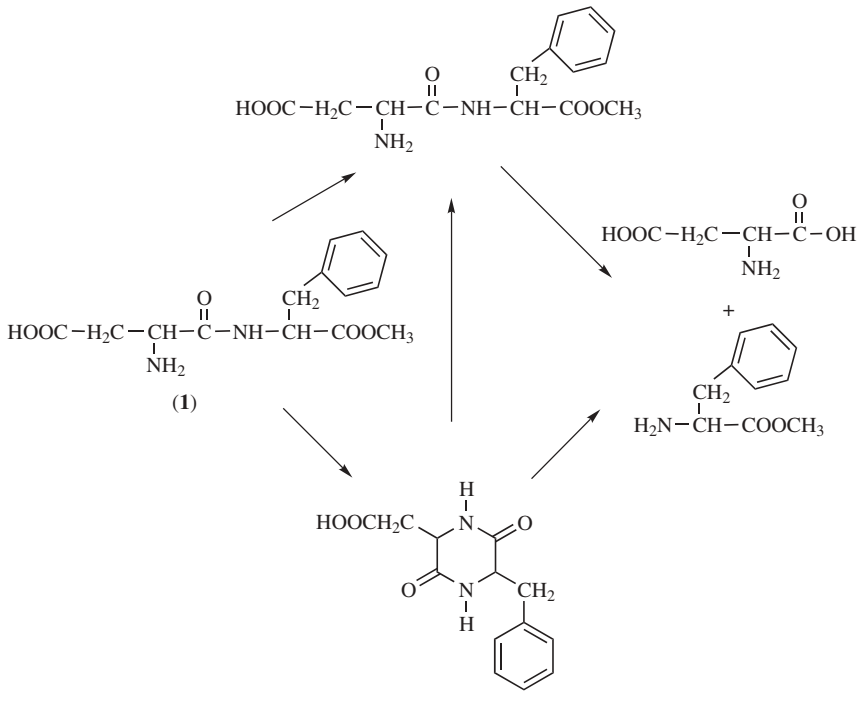

Fig. 2. Decomposition of aspartame to diketopiperazine and/or aspartyl-phenylalanine, and then to the amino acids, aspartic acid and phenylalanine.

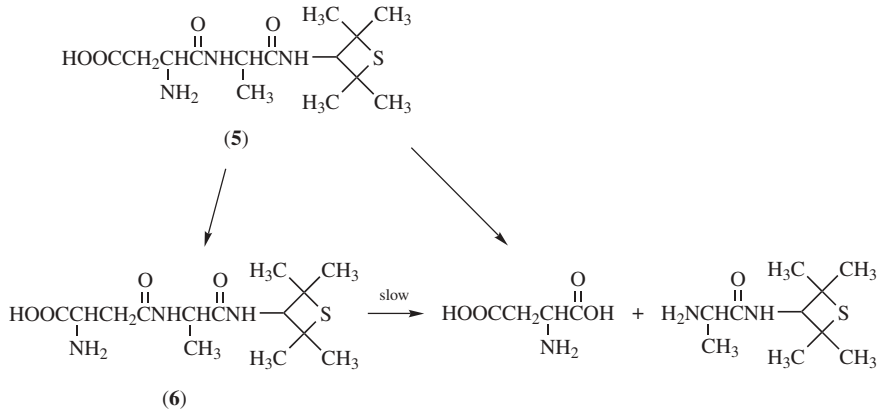

Fig. 3. Main degradation pathways of alitame. (Courtesy of Marcel Dekker, Inc.)

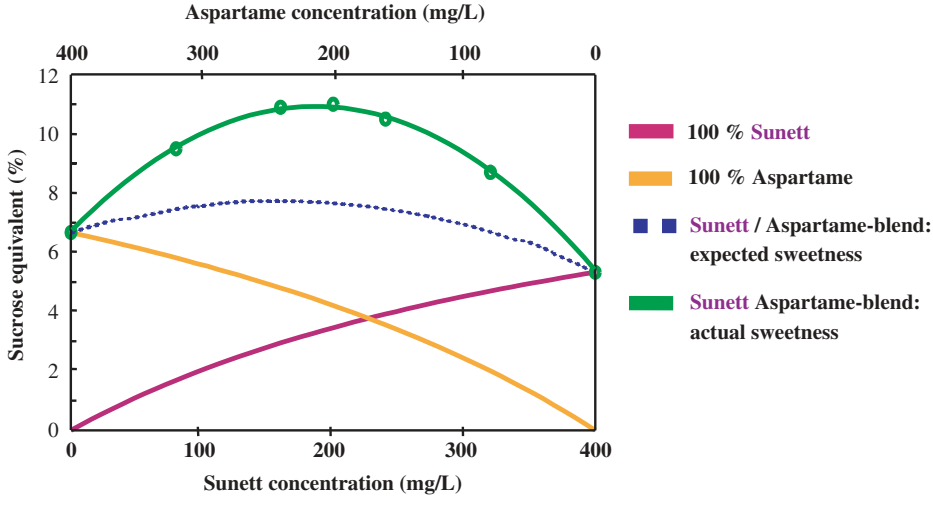

Fig. 4. Quantitative synergy between aspartame and acesulfame-K. (Courtesy of Nutrinova.)

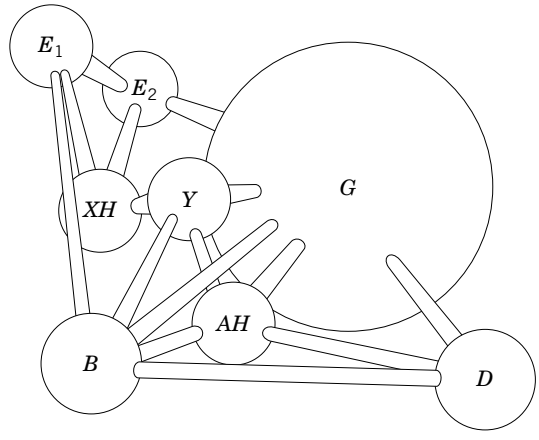

Fig. 5. Sweetener receptor binding sites postulated by Tinti and Nofre, where *B* is an anionic group, eg, CO_2^-, SO_3^-, or CN_4^-; *AH*, a hydrogen-bond donor group, eg, NH or OH; *G*, a hydrophobic, hydrocarbon group; *D*, a hydrogen-bond acceptor group, eg, CN, NO_2, or Cl; *Y*, E_1, and E_2 are hydrogen-bond acceptors, eg, CO or halogen atoms; and *XH* is a hydrogen-bond donor group, eg, NH or OH (100). (Courtesy of American Chemical Society.)

Table 1. **Other Compounds Reported to be Sweet**

Compounds	CAS Registry Number	Structure number	Potency	Reference
Abrososide D	[125003-001]	(**21**)	75X	82
Baiyunoside	[86450-75-1]	(**22**)	500X	83
Brazzeina[a]	[160047-05-2]		200X	84
Chloroform	[67-66-3]		40X	85
6-Chloro-D-tryptophan	[17808-35-4]	(**23**)	1300X	86
Dulcin	[150-69-6]	(**24**)	250X	87
Glycergic acid	[84215-86-1]	(**25**)	500X	88
Hernandulcin	[108944-70-3]	(**26**)	1000X	89
Hydrofluorene sweeteners	[34069-54-0]	(**27**)	1400X	90
Mogroside V	[88901-36-4]	(**28**)	256X	91
Monatin	[146142-94-1]	(**29**)	2000X	92
Monellin[a,b]	[9062-83-3]		2500X	93
Osladin	[33650-66-7]	(**30**)	3000X	83
P-4000	[553-79-7]	(**31**)	4000X	94
Pentadin	[61391-05-7]		500X	93
Perillartine	[30950-27-7]	(**32**)	2000X	95
Phyllodulcin	[55555-33-4]	(**33**)	400X	96
SRI oxime V	[59691-20-2]	(**34**)	450X	95
Suosan	[140-46-5]	(**35**)	700X	87

[a]Materials is a protein.
[b]The mabinlins, another group of proteins, are also sweet.