3.10 Natural Products as Sweeteners and Sweetness Modifiers

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3.10.1 Introduction

The most widely used sweetener in the world is sucrose (table sugar), a disaccharide (α-D-glucopyranosyl-(1→2)-β-fructofuranoside), which is produced from sugarcane and sugar beet.[1] However, a high daily intake of sucrose has been reported to be involved in the development of several health problems, most notably dental caries.[2] Accordingly, there has been an increasing demand for new highly sweet, noncaloric, and noncariogenic sucrose substitutes in the market. For example, the sweetener market is generally recognized as accounting currently for approximately $1 billion in sales in the United States alone. Sweet-tasting sucrose substitutes, which may be of either synthetic or natural origin, need to possess at least equal sensory properties to sucrose. Such compounds can be categorized into 'intense' or 'low-calorie sweeteners', which are 50–100 to several thousand times more

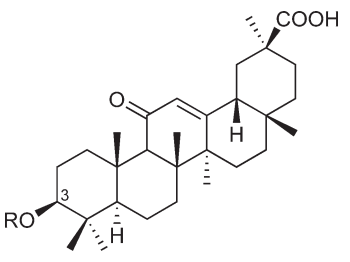
intensely sweet than sucrose,[3–5] and 'bulk' or 'reduced-calorie' sweeteners, such as certain monosaccharides, disaccharides, and polyols, which are approximately equal to sucrose in sweetness intensity.[6,7]

Synthetic sweeteners including acesulfame-K, alitame, aspartame, cyclamate, neotame, saccharin, and sucralose are currently available as potently sweet substitutes of sucrose in most western countries, but the regulations for each sweetener vary from country to country.[3,8–14] Five synthetic sweeteners, acesulfame-K, aspartame, neotame, saccharin, and sucralose, are presently approved for use in the United States, with cyclamate no longer utilized, owing to concerns about its safety.[7,11,15]

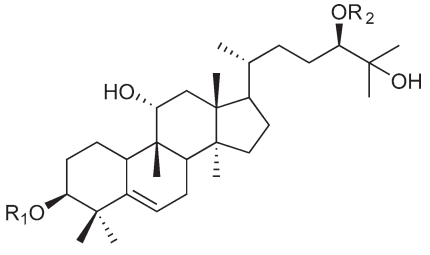
In addition to the synthetic sweeteners mentioned above, a number of highly sweet natural compounds are known to exist, which are mostly terpenoids, flavonoids (Chapter 3.16), and proteins (Chapters 5.01–5.21), and this area has been subjected to previous review.[16–24] So far, all of the known natural product sweet-tasting substances and sweetness modifiers have been discovered from green plants, as opposed to other types of organisms, such as lower plants, microbes, and marine fauna. Some of these plant-derived substances have been launched commercially in the market and are used as low-calorie sucrose substitutes, as will be mentioned in the next section. Besides these naturally occurring sweet-tasting compounds, a number of naturally occurring sweetness modifiers, either inducers or inhibitors of sweetness perception, are known to influence the sweet taste response.[23,25] In the following parts of this chapter, after sequential sections on naturally occurring sweet compounds with commercial use and how such compounds may be discovered, sweet substances in the terpenoid and steroid, phenylpropanoid, dihydroisocoumarin, flavonoid, proanthocyanidin, benzo[*b*]indeno[1,2-*d*]pyran, amino acid, and protein categories will be described. Next, the structural classes of naturally occurring sweetness inducers and sweetness inhibitors will be discussed in turn, prior to some concluding remarks. The literature for this chapter has been surveyed until the middle of 2008.

3.10.2 Commercially Used Highly Sweet Natural Products

Only a relatively few sweet-tasting plant-derived natural products have been launched commercially as sucrose substitutes to date. These natural products are used in one or more countries either in the pure form or as refined extracts, and include glycyrrhizin (**1**), mogroside V (**2**), phyllodulcin (**3**), rebaudioside A (**4**), stevioside (**5**), and thaumatin (**6**). Many of these compounds have served as lead compounds for extensive structural modification, in attempts to produce analogues that either possess better hedonic attributes or are more potently sweet tasting. A number of naturally occurring 'bulk' or 'reduced-calorie' sweeteners are commercially available as either foods or food additives. These substances include the monosaccharides fructose and D-tagatose; the disaccharides isomaltulose and trehalose; the monosaccharide polyols erythritol, mannitol, sorbitol, and xylitol; and the disaccharide polyols lactitol and maltitol. As reduced-calorie sweeteners and their hydrogenated derivatives have been dealt with in depth recently,[4–6] they will not be further described in this chapter.

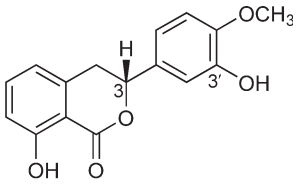


1 R = β-glcA^2-β-glcA
7 R = β-xyl
8 R = β-glcA



	R_1	R_2
2	β-glc^6-β-glc	β-glc^2-β-glc (6: β-glc)

glc: D-glucopyranosyl
xyl: D-xylopyranosyl



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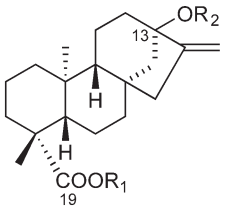


glcA: D-glucuronopyranosyl

	R_1	R_2
4	β-glc	β-glc^2-β-glc ^{3}| β-glc
5	β-glc	β-glc^2-β-glc
9	β-gal	β-glc^2-β-glc^2-β-glc^4-α-glc
10	$(CH_2)_3SO_3Na$	β-glc^2-β-glc ^{3}| β-glc

Ala-Thr-Phe-Glu-Ile-Val-Asn-Arg-Cys-Ser-Tyr-Thr-Val-Trp-Ala-Ala-Ala-Ser-Lys-Gly-
1 5 10 15 20
Asp-Ala-Ala-Leu-Asp-Ala-Gly-Gly-Arg-Gln-Leu-Asn-Ser-Gly-Glu-Ser-Trp-Thr-Ile-Asn-
21 25 30 31 40
Val-Glu-Pro-Gly-Thr-Asn-Gly-Gly-Lys-Ile-Trp-Ala-Arg-Thr-Asp-Cys-Tyr-Phe-Asp-Asp-
41 45 50 55 60
Ser-Gly-Ser-Gly-Ile-Cys-Lys-Thr-Gly-Asp-Cys-Gly-Gly-Leu-Leu-Arg-Cys-Lys-Arg-Phe-
61 65 70 75 80
Gly-Arg-Pro-Pro-Thr-Thr-Leu-Ala-Glu-Phe-Ser-Leu-Asn-Gln-Tyr-Gly-Lys-Asp-Tyr-Ile-
81 85 90 95 100
Asp-Ile-Ser-Asn-Ile-Lys-Gly-Phe-Asn-Val-Pro-Met-Asn-Phe-Ser-Pro-Thr-Thr-Arg-Gly-
101 105 110 115 120
Cys-Arg-Gly-Val-Arg-Cys-Ala-Ala-Asp-Ile-Val-Gly-Gln-Cys-Pro-Ala-Lys-Leu-Lys-Ala-
121 125 130 135 140
Pro-Gly-Gly-Gly-Cys-Asn-Asp-Ala-Cys-Thr-Val-Phe-Gln-Thr-Ser-Glu-Tyr-Cys-Cys-Thr-
141 145 150 155 160
Thr-Gly-Lys-Cys-Gly-Pro-Thr-Glu-Tyr-Ser-Arg-Phe-Phe-Lys-Arg-Leu-Cys-Pro-Asp-Ala-
161 165 170 175 180
Phe-Ser-Tyr-Val-Leu-Asp-Lys-Pro-Thr-Thr-Val-Thr-Cys-Pro-Gly-Ser-Ser-Asn-Tyr-Arg-
181 185 190 195 200
Val-Thr-Phe-Cys-Pro-Thr-Ala
201 207

6

Glycyrrhizin (**1**), also known as glycyrrhizic acid, is an oleanane-type triterpenoid diglycoside isolated from the roots of *Glycyrrhiza glabra* L. (licorice root; Leguminosae) and other species of the genus *Glycyrrhiza*.[26–28] The compound was first isolated in crystalline form about a century ago by Tschirch and Cederberg,[29] with the structure finalized several years later and involving more than one research group, as reviewed by Hodge and Inglett.[30] Glycyrrhizin (**1**) has been reported to be 93–170 times sweeter than sucrose, depending on concentration.[28] In Japan, extracts containing >90% w/w pure glycyrrhizin from *G. glabra* roots are used to sweeten foods and other products, such as cosmetics and medicines.[7,27,28] The ammonium salt of glycyrrhizin has generally recognized as safe (GRAS) status in the United States and is used primarily as a flavor enhancer.[7,28] Several attempts have been made to use various glycosylation methods in order to enhance the sweetness intensity of glycyrrhizin (**1**). The group of the late Professor Osama Tanaka[31] at Hiroshima University in Japan conducted the glycosylation of the aglycone glycyrrhetic acid to afford various glycyrrhizin monoglycoside analogues employing a chemical and enzymatic glycosylation procedure. A coupling reaction using mercury(II) cyanide ($Hg(CN)_2$) for chemical glycosylation was effective, leading to a significant enhancement of sweetness in the analogues obtained, especially 3-*O*-β-D-xylopyranoside (**7**) and 3-*O*-β-D-glucuronide (glycyrrhetic acid monoglucuronide (MGGR), **8**), with sweetness intensities rated as 544 and 941 times sweeter than sucrose, respectively. Such chemically modified products of glycyrrhizin were also found to have improved hedonic taste qualities.[20] MGGR (**8**), being more than five times sweeter than glycyrrhizin (**1**), as well as being readily soluble in water, is now used commercially as a sweetening agent in Japan for certain dairy products and soft drinks.[28,32]

Mogroside V (**2**) is a cucurbitane-type triterpenoid glycoside isolated from the fruits of *Siraitia grosvenorii* (Swingle) C. Jeffrey ex A.M. Lu & Zhi Y. Zhang (Cucurbitaceae), and was isolated initially in 1983 by Takemoto *et al.*[33] This plant is of Chinese origin and is known as 'lo han guo'. It has certain traditional uses such as to treat colds, sore throats, and minor gastrointestinal complaints.[28] Previous Latin binomials found in the phytochemical literature for this species are *Momordica grosvenorii* Swingle and *Thladiantha grosvenorii* (Swingle) C. Jeffrey. An extract of the dried fruits of *S. grosvenorii*, containing mogroside V (**2**) as the major sweet principle, is used in Japan as a sweetener in certain foods and beverages. The sweetness intensity of mogroside V has been rated as 250–425 times sweeter than sucrose, depending on concentration.[28] In a recent study, mogroside V (**2**) was confirmed as being the major constituent of the sweet-tasting ripe fruits of *S. grosvenorii*, whereas other cucurbitane glucosides are prevalent in unripe fruits and have a bitter taste.[34] The transglucosylation of mogroside V has been conducted, using cyclodextrin glucanotransferases and starch as donor substrate, and products showing sugar chain elongation were found to be less intensely sweet than the starting glycoside.[35] There is now a substantial body of literature on potential food and beverage applications of *S. grosvenorii*, particularly by Chinese authors.

Phyllodulcin (**3**), a dihydroisocoumarin-type sweetener, occurs in glycosidic form in the leaves of *Hydrangea macrophylla* Seringe var. *thunbergii* (Siebold) Makino (Saxifragaceae) ('Amacha') and other species of the genus *Hydrangea*. This compound was first isolated in 1916 by Asahina and Ueno,[36] with the structure determined in the following decade by Asahina and Juntaro, and the absolute configuration finally established as 3*R* in 1959.[37] Crushing or fermenting the leaves induces enzymatic hydrolysis of the native glycosides present to produce the sweet aglycone phyllodulcin (**3**; 400 times sweeter than 2% sucrose).[28] The fermented leaves of *H. macrophylla* var. *thunbergii* are used to prepare a sweet ceremonial tea in Japan, especially at 'Hamatsuri', a Buddhist religious festival.[28]

Rebaudioside A (**4**) and stevioside (**5**) are *ent*-kaurane-type diterpene (steviol) glycosides based on the aglycone steviol isolated from the leaves of the Paraguayan plant *Stevia rebaudiana* (Bertoni) Bertoni (Asteraceae),[20,38,39] with stevioside being the most abundant sweet compound in this plant part. Stevioside (**5**) was initially isolated in 1900 by the Paraguayan chemist Rebaudi, as reported by Bertoni,[40] but its structure was finalized only in 1963.[41] Rebaudioside A (**4**) was isolated and structurally determined in 1976 by Tanaka and co-workers[42] at Hiroshima University in Japan. The sweetness intensity of stevioside (**5**) has been estimated as 210 times sweeter than sucrose, although this value varies with concentration. However, rebaudioside A (**4**) (the second most abundant *S. rebaudiana* steviol glycoside with a sweetness intensity rated as about 240 times sweeter than sucrose) is considerably more pleasant tasting and more highly water soluble than stevioside (**5**), and thus better suited for use in food and beverages. Extracts of *S. rebaudiana* containing stevioside and/or purified stevioside are permitted as food additives in Japan, South Korea, Brazil, Argentina, and Paraguay, and are used as botanical dietary supplements elsewhere, in particular in the United States.[39]

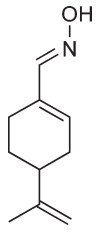
In Japan, the largest market for the *S. rebaudiana* sweeteners to date, three different forms of stevia sweetener products are commercially available, namely 'stevia extract', 'sugar-transferred stevia extract' (also known as 'enzymatically modified stevia extract' and 'glucosyl stevia'), and 'rebaudioside A-enriched stevia extract'.[43] 'Stevia extract' is a powder or granule made by several industrial steps and standardized so as to contain more than 80% of steviol glycosides, inclusive of dulcoside A (3–5%), rebaudioside A (20–25%), rebaudioside C (**20**) (5–10%), and stevioside (50–55%).[43] 'Sugar-transferred stevia extract', a complex mixture of compounds, is made by transglycosylation of steviol glycosides present in commercially available 'stevia extract' with a cyclomaltodextringlucanotransferase (CGTase)-starch system prepared from *Bacillus macerans*, followed by treatment with β-amylase.[20,43,44] Over the years, there have been many attempts to improve the taste qualities of the major *S. rebaudiana* sweet steviol glycoside, stevioside (**5**), because of its sensory limitations.[20,45–49] Several systematic studies on the structure–sweetness relationship of steviol glycosides have been conducted.[20,43,50] For example, the sweetness and pleasantness of stevioside (**5**) may be increased by treating stevioside-galactosyl ester (Sgal), prepared by removal of the 19-*O*-glucosyl group of stevioside, and replacing it with a β-galactosyl group. Transglucosylation of the intermediate with soluble starch using CGTase prepared from *B. macerans* then affords a mixture of mono-, di-, tri-, and tetra-α-glycosylated compounds. The product with four glucosyl units attached at the C-13 position showed an enhanced sweetness (**9**, Sgal-2).[48] A rebaudioside A analogue (**10**) with a (sodiosulfo)propyl group at C-19 in place of a β-glucosyl moiety showed improved sweetness qualities over the parent compound.[46] Stevioside (**5**) has been converted synthetically to rebaudioside A (**4**) by removal

of the terminal glucose unit at C-13 using amylase and then reintroducing synthetically two glucose units at different linkage positions to the remaining glucose moiety.[51] 'Rebaudioside A-enriched extract' is made from improved varieties of *S. rebaudiana*, which produce more rebaudioside A (**4**) than the native Paraguayan species.[52] Products incorporating *S. rebaudiana* sweeteners are used in more than 100 different food applications in Japan, in particular for salted foods such as Japanese-style pickles and dried seafoods, but also for beverages, yoghurt, ice cream, and sherbet.[43] In Korea, pure stevioside has become an important sucrose substitute and is used principally to sweeten 'soju' (a traditional distilled liquor made from sweet potatoes), soy sauce, pickles, and medicines.[53]

Currently, efforts are being made to introduce the sweet *S. rebaudiana ent*-kaurane (steviol) glycosides for use as sucrose substitutes in the United States and Europe. In the United States, rebaudioside A (**4**) was accorded GRAS status in late 2008 to sweeten foods and soft drinks and as a tabletop sweetener.[54] The existing literature has been surveyed and some additional studies have been performed for rebaudioside A (**4**) and, in some cases, stevioside (**5**), with regard to compound stability,[55] microbial hydrolysis,[56] genetic toxicity,[57] subchronic toxicity,[58] reproductive toxicity,[59] and toxicokinetics and metabolism in rats.[60] In humans, the pharmacokinetics after oral absorption[61] and also potential effects on adults with type 2 diabetes mellitus[62] and on healthy adults with normal and low-normal blood pressure[63] have been investigated. When taken together, these studies have led to the conclusion that rebaudioside A (**4**) (now also known as 'rebiana') seems to be appropriate for the sweetening of foods and beverages when purified to food-grade specifications.[64] In 2008, an acceptable daily intake (ADI) was established for 'steviol glycosides' at 0–4 mg kg^{-1} body weight for adults based on steviol, by the Food and Agriculture Organization of the United Nations/World Health Organization Joint Expert Committee on Food Additives (JECFA).[65] According to Renwick,[66] the estimated intake of rebaudioside A through normal use would not exceed a daily amount of steviol of 2 mg kg^{-1} body weight. In a further toxicological investigation to have appeared in the literature very recently, in a 90-day subchronic study, dietary supplements of high-dose levels of rebaudioside A (**4**) to Sprague–Dawley rats were not associated with any toxicity signs.[67]

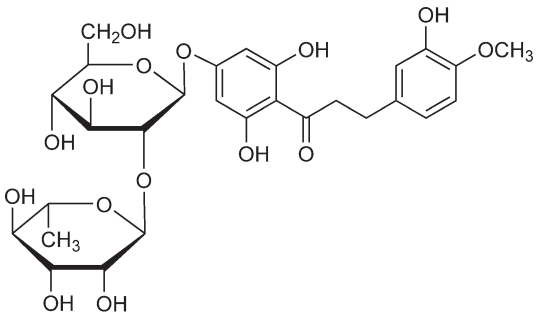
Thaumatin (**6**) is a protein sweetener isolated from the fruits of *Thaumatococcus danielli* Benth. (Marantaceae), and has been in use for several years as a sweetener and flavoring agent.[18,28,68–70] Five different thaumatin analogues (thaumatins I, II, III, a, and b) are now known, and thaumatins I and II are the major forms with both having 207-amino-acid residues.[18] The molecular weights of thaumatins I and II are 22 209 and 22 293 Da, respectively.[70] The three-dimensional (3D) structure of thaumatin I, based on X-ray analysis, has been reported.[71,72] The sweetness of thaumatin I has been rated between 1600 and 3000 times in comparison with sucrose on a weight basis, making this one of the most sweet natural substances so far discovered. Talin protein, the trade name of the commercial form of thaumatin protein as an aluminum ion adduct, was first approved as a food additive in Japan in 1979, and is an approved sweetener in Australia and, when used in limited levels, in countries of the European Union.[7] Talin protein has GRAS status as a flavor enhancer for use in chewing gum in the United States[28] and is used extensively worldwide as a flavoring ingredient.[7]

Perillartine (**11**) is a semisynthetic compound utilized on a limited basis in Japan, mainly as a replacement for maple syrup or licorice for the flavoring of tobacco.[16,28] Perillartine is an *α-syn*-oxime and can be synthesized from perillaldehyde, a monoterpenoid constituent of the volatile oil of *Perilla frutescens* (L.) Britton (Lamiaceae). This compound has a limited solubility in water and possesses a concomitant bitter taste along with sweetness.[16,28]



11

Neohesperidin dihydrochalcone (NHDC; **12**) is another semisynthetic compound and is a dihydrochalcone glycoside prepared from a flavanone constituent of *Citrus aurantium* L. (Rutaceae) (Seville orange).[73] It is permitted for use as a sweetener in a wide range of foodstuffs in countries of the European Union, as well as in Turkey and Switzerland, and has GRAS status as a flavor ingredient in the United States.[7,73]



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It is necessary for low-calorie food ingredients to undergo rigorous testing in order to receive official sanction for marketing as a low-calorie sweetener in a western country, with considerations such as safety (acute and chronic toxicity; reproductive toxicology; carcinogenicity; mutagenicity), metabolism, stability, and other attributes such as the establishment of an ADI. Kemp[7] has provided an excellent chapter that describes the regulatory processes for new sweeteners in North America and Europe and summarizes current knowledge on 11 low-calorie sweeteners used in various countries around the world.

3.10.3 Discovery of Natural Sweeteners

The general approaches to the discovery of new sweetening agents from plant sources used by the group of the senior author of this review when at the University of Illinois at Chicago have been described previously.[17,21,74–76] This work led to the discovery of several new intensely sweet compounds of the terpenoid and flavonoid types, as mentioned in Section 3.10.4. A key aspect of our work was the accession of candidate sweet-tasting plants, and for this purpose three basic strategies were used, comprising scrutiny of scientific and popular texts, collecting plants in the field after making inquiries in market places, and performing organoleptic evaluations. For the first of these, the book *Index Kewensis* may be mentioned in particular. This is a listing of plant Latin binomials, with words such as 'dulcificum', 'dulcis', 'glycyrrhiza', 'mellosa', and 'saccharum' all implying either a sweet taste or a sweet smell for a particular species.[75–77] Although fieldwork for sweet-tasting plant has paid dividends in the search for new candidate sweet-tasting plants, ethnobotanical investigators must now arrange for approved 'prior informed consent' in order to make inquiries with members of indigenous populations who may be knowledgeable about the sensory and other properties of local plants. This is as a consequence of the 1992 United Nations Convention on Biological Diversity held in Rio de Janeiro, also known as the Rio Convention.[78] Another aspect of the passage of this convention is that source countries have been recognized as having a sovereign right over their own genetic resources, so that prior to any plant collections ever taking place, it is necessary for the investigator to develop detailed agreements pointing to an equitable sharing of benefits.[75–78] Although indiscriminate organoleptic testing of plants for the presence or absence of a sweet taste cannot be recommended, this approach has led to interesting results in the past. For example, when Soejarto *et al.*[79] carefully tasted 110 dried herbarium species of the genus *Stevia* (Asteraceae), collected previously from North and South America, several of these were found to be somewhat sweet tasting, including a 62-year-old specimen of *S. rebaudiana* (Bertoni) Bertoni collected in Paraguay. In a phytochemical study of these same samples, stevioside (**5**) was detected in both a *S. rebaudiana* sample and a Mexican species, *Stevia*

phlebophylla A. Gray, where it occurred in only trace amounts. Steviol (*ent*-kaurane) glycosides were absent in the other 108 *Stevia* species analyzed.[80]

The laboratory stage of a sweetener discovery protocol requires the use of a preliminary plant extraction protocol, producing extracts of various polarities. These should not be tasted for sweetness until negative results in both a mouse acute toxicity and a bacterial mutagenicity assay are demonstrated. It was found in our previous work that it is very rare indeed for a plant part to be sweet owing to its content of one or more highly sweet compounds. It is more usual for any inherent sweetness to be a result of high levels of sugars and polyols[81,82] or of phenylpropanoids such as *trans*-anethole[83] and *trans*-cinnamaldehyde.[84] In fact, as an empirical observation, if the combined amount of saccharides and polyols exceeds 5% w/w in a given plant part, the resultant sweetness can generally be considered as being due to the presence of these 'bulk' sweeteners. A suitable dereplication procedure using gas chromatography–mass spectrometry (GC–MS) has been developed for this purpose to rule out the sweetness contribution from saccharides and polyols in candidate sweet-tasting plants.[82]

For plant materials found to contain considerable amounts of sugars and polyols, these common sweet substances may be removed before assessing the residual material for the presence or absence of sweetness. A rapid, effective screening protocol utilizing a solid-phase extraction (SPE) technique permits the facile removal of sugars and polyols. A suitable SPE cartridge that may be employed is reversed-phase octadecyl silica gel (C_{18}) eluted initially with water, followed by 30, 50, 70, and 100% MeOH. The free sugars will be eluted with water together with some types of amino acids, small organic acids, and other materials. A ^{1}H NMR spectroscopic measurement of the water eluant can readily reveal if there are any interesting, highly polar molecules coeluted in this fraction. Together with the water eluant, the MeOH-containing fractions can be lyophilized after removal of the organic solvents before tasting. If sweetness is detected in any of these fractions, the polarity of the elution solvents may serve as an indicator of the type of compounds present. For example, sweet-tasting glycosides (e.g., saponins, diterpene glycosides, and flavonoid glycosides) would be found in the 30, 50, or 70% MeOH eluants, depending on the nature of the aglycones and the numbers of sugar units in the molecules. The above SPE procedure has the ability to partially purify complex plant extracts into several well-defined fractions based on the polarity of the compounds in a short period of time. Additionally, such a procedure will facilitate subsequent sensory evaluation as it will separate any bitter-tasting molecules coexisting in the plant material from other interesting tastants. If sweetness is detected in any of the nonsugar fractions, a scale-up isolation procedure is warranted. Sequential solvent partition using hexane/petroleum ether, ethyl acetate, and *n*-butanol may be carried out on the positive leads obtained. Subsequently, sensory-guided fractionation will be conducted using a combination of chromatographic techniques, inclusive of passage over reversed-phase macroresins, such as Diaion HP-20, as well as Sephadex gels and silica gel-based sorbents, until pure sweet-tasting molecules are obtained. The loading capacity of HP-20 is much higher than that of a C_{18} cartridge, so this procedure can be easily scaled up to generate samples for taste evaluation and subsequent fine chromatographic purification.

In our sweetener discovery work, purified plant secondary metabolites were subjected to mouse acute toxicity testing and mutagenicity evaluation prior to being tasted for sweetness and then evaluated for sweetness potency in comparison with sucrose.[74–76] This approach will require approval of both the relevant Animal Care Committee and the Institutional Review Board responsible for human subjects. Moreover, a minimum of 50–100 mg of each pure sweet compounds is required for safety testing, a quantity that is not always readily obtainable from the plant material on hand.[74–76]

Efforts have been made to circumvent the use of human subjects in the screening of samples of natural products for sweetness. For example, a combination of electrophysiological and behavioral assays on the Mongolian gerbil has been used to predict the sweetness of plant extracts of varying polarities with reasonable accuracy.[85] However, this is a somewhat time-consuming method, using specialized equipment, and the Mongolian gerbil does not respond to natural product sweeteners in the same manner as humans.[86] It is now possible to screen pure compound libraries for sweetness and other tastes in a less time-consuming fashion, using receptor-binding procedures (see Section 3.10.7).[87,88] Future screening of natural products should not necessarily be focused on only green plants, and such compounds may well occur also in microorganisms, insects, and marine organisms. In addition, more primitive plants may also afford new sweet substances. For instance,

Asakawa[89] has indicated that the moss *Fissidens japonicus* Dozy & Molk. (Fissidentaceae) is sweet tasting and contains nonsugar constituents that are so far structurally uncharacterized.

3.10.4 Structural Types of Highly Sweet Natural Products

In this section, the presently known highly sweet substances of natural origin are described. Sweet-tasting compounds of natural origin are listed in **Table 1**, and the same type of arrangement used in earlier reviews and book chapters on natural noncaloric sweeteners has been expounded upon.[19,23,24] Many of the sweet compounds obtained from plants are glycosides.[22] A few semisynthetic compounds that have exhibited a significant improvement in sweetness potency or pleasantness relative to the relevant natural product prototype sweet molecule are included in **Table 1**. Values of sweetness intensity relative to sucrose on a weight basis (sucrose = 1) are provided for the compounds listed, where such data are

Table 1 Highly sweet compounds from plants

Compound type/name[a]	*Plant name*	*Sweetness potency*[b]	*Reference(s)*
Monoterpenoids			
Perillartine (**11**)[c]	*Perilla frutescens* (L.) Britton (Lamiaceae)	370	90, 91
Sesquiterpenoids			
Acyclic glycoside			
Mukurozioside IIb (**13**)	*Sapindus rarak* DC. (Sapindaceae)	~1	82, 92
Bisabolanes			
(+)-Hernandulcin (**14**)	*Lippa dulcis* Trevir. (Verbenaceae)	1500	93, 94
4β-Hydroxyhernandulcin (**15**)	*L. dulcis*	NS[d]	101
Diterpenoids			
Diterpene acid			
4β,10α-Dimethyl-1,2,3,4,5,10-hexahydrofluorene-4α,6α-dicarboxylic acid (**16**)[e]	Pine tree[f]	1300–1800[g]	103
***ent*-Kaurene glycosides**			
Cussoracoside C (**17**)	*Cussonia racemosa* Baker (Araliaceae)	NS[d]	111
Dulcoside A (**18**)	*Stevia rebaudiana* (Bertoni) Bertoni (Asteraceae)	30	106
Rebaudioside A (**4**)	*S. rebaudiana*	242	42
Rebaudioside B (**19**)	*S. rebaudiana*	150	42
Rebaudioside C (**20**)	*S. rebaudiana*	30	104
Rebaudioside D (**21**)	*S. rebaudiana*	221	105
Rebaudioside E (**22**)	*S. rebaudiana*	174	105
Rebaudioside F (**23**)	*S. rebaudiana*	NS[d]	108
Rubusoside (**24**)	*Rubus suavissimus* S.K. Lee (Rosaceae)	115	109
Steviolbioside (**25**)	*S. rebaudiana*	90	42
Steviol 13-*O*-β-D-glucoside (**26**)	*R. suavissimus*	NS[d]	109, 110
Stevioside (**5**)	*S. rebaudiana*	210	40, 41
Suavioside A (**27**)	*R. suavissimus*	NS[d]	109
Suavioside B (**28**)	*R. suavissimus*	NS[d]	109
Suavioside G (**29**)	*R. suavissimus*	NS[d]	109
Suavioside H (**30**)	*R. suavissimus*	NS[d]	109
Suavioside I (**31**)	*R. suavissimus*	NS[d]	109
Suavioside J (**32**)	*R. suavissimus*	NS[d]	109
Labdane glycosides			
Baiyunoside (**33**)	*Phlomis betonicoides* Diels (Lamiaceae); *Phlomis medicinalis* Diels (Lamiaceae)	500	112, 113
Phlomisoside I (**34**)	*P. betonicoides*; *P. medicinalis*; *Phlomis younghushbandii* Mukerjee (Lamiaceae)	NS[d]	112, 113

(*Continued*)

Table 1 (Continued)

Compound type/name[a]	*Plant name*	*Sweetness potency*[b]	*Reference(s)*
Gaudichaudioside A (**35**)	*Baccharis gaudichaudiana* DC. (Asteraceae)	55	117
Triterpenoids			
Cucurbitane glycosides			
Bryodulcoside[h]	*Bryonia dioica* Jacq. (Cucurbitaceae)	NS[d]	119
Bryoside (**36**)	*B. dioica*	NS[d]	119
Bryonoside (**37**)	*B. dioica*	NS[d]	119
Carnosifloside V (**38**)	*Hemsleya carnosiflora* C. Y. Wu et Z. L. Chen (Cucurbitaceae)	51	121
Carnosifloside VI (**39**)	*H. carnosiflora*	77	120
Isomogroside V (**40**)	*Siraitia grosvenorii*[i] (Swingle) C. Jeffrey ex A. M. Lu & Zhi Y. Zhang (Cucurbitaceae)		125
Mogroside IV (**41**)	*S. grosvenorii*	233–392[g]	124
Mogroside V (**2**)	*S. grosvenorii*	250–425[g]	33, 124
11-Oxomogroside V (**42**)	*Siraitia siamensis* (Craib) C. Jeffrey ex S. Q. Zhong & D. Fang (Cucurbitaceae)	NS[d]	123, 124
Scandenoside R6 (**43**)	*Hemsleya panacis-scandens* C.Y. Wu et Z. L. Chen (Cucurbitaceae)	54	121
Scandenoside R11 (**44**)	*H. panacis-scandens*	NS[d]	122
Siamenoside I (**45**)	*S. grosvenorii*; *S. siamensis*	563	123, 124
Cycloartane glycosides			
Abrusoside A (**46**)	*Abrus precatorius* L.; *A. fruticulosus* Wall. (Fabaceae)	30	126, 129
Abrusoside B (**47**)	*A. precatorius*; *A. fruticulosus*	100	126, 129
Abrusoside C (**48**)	*A. precatorius*; *A. fruticulosus*	50	126, 129
Abrusoside D (**49**)	*A. precatorius*; *A. fruticulosus*	75	126, 129
Abrusoside E (**50**)	*A. precatorius*	NS[d]	128, 130
Abrusoside E methyl ester (**51**)[c]	*A. precatorius*	150	130
Dammarane glycosides			
Cyclocarioside A (**52**)	*Cyclocarya paliurus* (Batal.) Iljinsk. (Juglandaceae)	200	132
Cyclocaryoside I (**53**)	*C. paliurus*	250	133
Gypenoside XX[j] (**54**)	*Gynostemma pentaphyllum* (Thunb.) Makino (Cucurbitaceae)	NS[d]	134
Oleanane glycosides			
Albiziasaponin A (**55**)	*Albizia myriophylla* Benth. (Fabaceae)	5	135
Albiziasaponin B (**56**)	*A. myriophylla*	600	135
Albiziasaponin C (**57**)	*A. myriophylla*	NS[d]	135
Albiziasaponin D (**58**)	*A. myriophylla*	NS[d]	135
Albiziasaponin E (**59**)	*A. myriophylla*	NS[d]	135
Apioglycyrrhizin (**60**)	*Glycyrrhiza inflata* Batalin (Fabaceae)	300	136
Araboglycyrrhizin (**61**)	*G. inflata*	150	136
Glycyrrhizin (**1**)	*Glycyrrhiza glabra* L. (Fabaceae)	93–170[g]	136
Periandrin I (**62**)	*Periandra dulcis* Mart. ex Benth.; *P. mediterranea* (Vell.) Taub. (Fabaceae)	90	139
Periandrin II (**63**)	*P. dulcis*; *P. mediterranea*	95	137
Periandrin III (**64**)	*P. dulcis*; *P. mediterranea*	92	138
Periandrin IV (**65**)	*P. dulcis*; *P. mediterranea*	85	137
Periandrin V (**66**)	*P. dulcis*	220	140
Secodammarane glycosides			
Pterocaryoside A (**67**)	*Pterocarya paliurus* Batalin (Juglandaceae)	50	141
Pterocaryoside B (**68**)	*P. paliurus*	100	141
Steroidal saponins			
Osladin (**69**)	*Polypodium vulgare* L. (Polypodiaceae)	500	142–145
Polypodoside A (**70**)	*Polypodium glycyrrhiza* Eat. (Polypodiaceae)	600	146, 148
Polypodoside B (**71**)	*P. glycyrrhiza*	NS[d]	147

(*Continued*)

Table 1 (Continued)

Compound type/name[a]	*Plant name*	*Sweetness potency*[b]	*Reference(s)*
Telosmoside A_8 (**72**)	*Telosma procumbens* Merr. (Asclepiadaceae)	NS[d]	149
Telosmoside A_9 (**73**)	*T. procumbens*	NS[d]	149
Telosmoside A_{10} (**74**)	*T. procumbens*	NS[d]	149
Telosmoside A_{11} (**75**)	*T. procumbens*	NS[d]	149
Telosmoside A_{12} (**76**)	*T. procumbens*	NS[d]	149
Telosmoside A_{13} (**77**)	*T. procumbens*	NS[d]	149
Telosmoside A_{14} (**78**)	*T. procumbens*	NS[d]	149
Telosmoside A_{15} (**79**)	*T. procumbens*	1000	149
Telosmoside A_{16} (**80**)	*T. procumbens*	NS[d]	149
Telosmoside A_{17} (**81**)	*T. procumbens*	NS[d]	149
Telosmoside A_{18} (**82**)	*T. procumbens*	NS[d]	149
Phenylpropanoids			
trans-Anethole[k] (**83**)	*Foeniculum vulgare* Mill. (Apiaceae) *Illicium verum* Hook f. (Illiciaceae) *Myrrhis odorata* Scop. (Apiaceae) *Osmorhiza longistylis* DC. (Apiaceae) *Piper marginatum* Jacq. (Piperaceae) *Tagetes filicifolia* Lag. (Asteraceae)	13	83
trans-Cinnamaldehyde (**84**)	*Cinnamomum osmophloeum* Kaneh. (Lauraceae)	50	84
Dihydroisocoumarin			
Phyllodulcin[l] (**3**)	*Hydrangea macrophylla* Seringe var. *thunbergii* (Siebold) Makino (Saxifragaceae)	400	36, 37, 150
Flavonoids			
Dihydrochalcone glycosides			
Glycyphyllin (**85**)	*Smilax glycyphylla* Hassk. (Liliaceae)	NS[d]	152, 153, 156
Naringin dihydrochalcone[c] (**86**)	*Citrus paradisi* Macfad. (Rutaceae)	300	73, 156
Neohesperidin dihydrochalcone[c] (**12**)	*Citrus aurantium* L. (Rutaceae)	1000	73, 156
Phlorizin (**87**)	*Lithocarpus litseifolius* Chun (Fagaceae); *Symplocos lancifolia* Siebold et Zucc. (Symplocaceae)	NS[d]	154
Trilobatin (**88**)	*L. litseifolius*; *Symplocos microcalyx* Hayata (Symplocaceae)	NS[d]	154
Dihydroflavonols and dihydroflavonol glycosides			
3-Acetoxy-5,7-dihydroxy-4′-methoxyflavanone (**89**)	*Aframomum hanburyi* K. Schum.; *Aframomum pruinosum* Gagnep. (Zingiberaceae)	NS[d]	157, 158
2*R*,3*R*-(+)-3-Acetoxy-5,7,4′-trihydroxyflavanone (**90**)	*A. hanburyi*	NS[d]	157
(2*R*,3*R*)-Dihydroquercetin 3-*O*-acetate (**91**)	*T. dodoneifolia* (Hook. & Arn.) Cabrera (Asteraceae); *Hymenoxys turneri* K.F. Parker (Asteraceae)	80	159, 162
Dihydroquercetin 3-*O*-acetate 4′-methyl ether[e] (**92**)	*T. dodoneifolia*	400	159
(2*R*,3*R*)-2,3-Dihydro-5,7,3′,4′-tetrahydroxy-6-methoxy-3-*O*-acetylflavonol (**93**)	*H. turneri*	25	162
(2*R*,3*R*)-2,3-Dihydro-5,7,3′,4′-tetrahydroxy-6-methoxyflavonol (**94**)	*H. turneri*	15	162
(2*R*,3*R*)-2,3-Dihydro-5,7,4′-trihydroxy-6-methoxy-3-*O*-acetylflavonol (**95**)	*H. turneri*	20	162
Huangqioside E (**96**)	*Engelhardtia chrysolepis* Hance (Juglandaceae)	NS[d]	161
Neoastilbin (**97**)	*E. chrysolepis*	NS[d]	160

(*Continued*)

Table 1 (Continued)

Compound type/name[a]	*Plant name*	*Sweetness potency*[b]	*Reference(s)*
Proanthocyanidins			
Cinnamtannin B-1 (**98**)	*Cinnamomum sieboldii* Meisn. (Lauraceae)	NS[d]	163
Cinnamtannin D-1 (**99**)	*C. sieboldii*	NS[d]	163
Selligueain A (**100**)	*Selliguea feei* Bory (Polypodiaceae); *Polypodium decumanum* Willd. (Polypodiaceae); *Polypodium triseriale* Sw. (Polypodiaceae)	35	164, 167
Unnamed (**101**)	*Arachniodes sporadosora* (Kuntze) Nakaike; *A. exilis* Ching (Aspidiaceae)	NS[d]	164
Unnamed (**102**)	*A. sporadosora*; *A. exilis*	NS[d]	164
Benzo[*b*]indeno[1,2-*d*]pyran			
Hematoxylin (**103**)	*Haematoxylum campechianum* L. (Fabaceae)	120	169
Amino acid			
Monatin (**104**)	*Sclerochiton ilicifolius* A. Meeuse (Acanthaceae)	1200–1400[g]	171
Proteins			
Brazzein (**105**)	*Pentadiplandra brazzeana* Baill. (Capparaceae)	2000	175
Curculin (**106**)	*Curculigo latifolia* Dryand. (Hypoxidaceae)	550	178
Mabinlin[m] (**107**)	*Capparis masakai* Levl. (Capparaceae)	NS[d]	179, 180
Monellin (**108**)	*Dioscoreophyllum cumminsii* Diels (Menispermaceae)	3000	181
Neoculin (**109**)	*Curculigo latifolia* Dryand. (Hypoxidaceae)	4000	183
Pentadin[n]	*Pentadiplandra brazzeana* Baillon (Capparaceae)	500	184
Thaumatin[o] (**6**)	*Thaumatococcus danielli* Benth. (Marantaceae)	1600	68, 185

[a] The structures of the compounds are shown in the text (**1**–**6**, **11**–**109**).
[b] Values of relative sweetness are on a weight comparison basis to sucrose (=1.0), and are taken from either the original literature report of the sweet compound concerned or from later reports, and represent consensus figures.
[c] Semisynthetic derivative of the natural product.
[d] NS = sweetness potency not given.
[e] Synthetic sweetener based on the natural product lead compound.
[f] Plant Latin binomial not given in the original reference.
[g] Relative sweetness varied with the concentration of sucrose.
[h] Complete structure and stereochemistry not determined.
[i] Formerly named *Momordica grosvenorii* Swingle and *Thladiantha grosvenorii* (Swingle) C. Jeffrey.
[j] Although a known compound, the sweet taste becomes evident only after the initial compound isolation.[22]
[k] Identified as a sweet-tasting constituent of these six species. However, this compound has a wider distribution in the plant kingdom.
[l] The plant of origin may be crushed or fermented in order to generate phyllodulcin (**3**).
[m] The structure of mabinlin II is shown in the text.
[n] The amino acid sequence of pentadin has not yet been determined.
[o] The structure of thaumatin I is shown in the text.

available. However, it is to be noted that sweetness intensity values for a given sweet molecule vary with concentration as well as the organoleptic method used. A more detailed discussion of sensory testing methods is provided in Section 3.10.7.

It may be seen from **Table 1** that the principal groups of highly sweet-tasting compounds of plant origin are terpenoids, flavonoids, and proteins, although compounds of other chemical classes have also been found to be highly sweet, inclusive of an amino acid, a benzo[*b*]indeno[1,2-*d*]pyran, a dihydroisocoumarin, phenylpropanoids, proanthocyanidins (Chapter 6.18), and steroidal saponins (Chapter 4.16). Within the terpenoid and flavonoid categories, a number of subgroups are represented. Among the terpenoids, there are several subclasses of diterpenoids (Chapter 1.17) and triterpenoids (Chapter 1.18), whereas both the dihydrochalcones and the dihydroflavonols are known to be sweet among the flavonoids. Accordingly, 20 major structural types of plant-derived sweeteners have been found to date. Altogether, about 100 structurally characterized natural products and 6 semisynthetic or synthetic compounds are included in **Table 1**, and these were obtained from species representative of more than 25 separate plant families. The distribution of plant families containing sweet-tasting compounds, according to a Dahlgren's

superorder organizational scheme, has been found to be random.[17] However, certain plant families biosynthesize natural sweeteners of more than one structural class, as exemplified by the family Asteraceae, which produces such compounds of both the *ent*-kaurane diterpenoid and the dihydroflavonol types.[17] It may be seen from **Table 1** that species of the same genus occasionally biosynthesize the same sweet-tasting constituent. Also, all three structural variants known to date of the oleanane-type glycosides (viz., the albiziasaponins, glycyrrhizin derivatives, and the periandrins) are all biosynthesized from plants of the family Fabaceae.

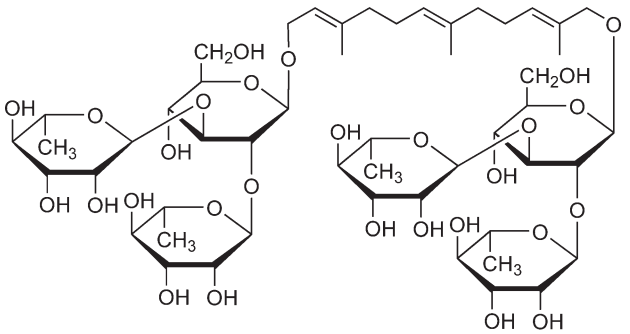
3.10.4.1 Terpenoids and Steroids

3.10.4.1.1 Monoterpenoids

As mentioned earlier, perillartine (**11**) has been known for many years as a highly sweet semisynthetic analogue prepared from the naturally occurring monoterpenoid (Chapter 1.15) perillaldehyde, a constituent of the volatile oil of *P. frutescens* (L.) Britton (Lamiaceae).[90,91] Although this compound is the only member of the monoterpenoid group of compounds so far known to be potently sweet, its poor solubility and sweetness qualities have precluded any significant commercial development.[16,28] However, owing to its inherent sweetness, perillartine remains of current interest in the literature, both for its potential applications and as a standard substance in sweetener research.

3.10.4.1.2 Sesquiterpenoids

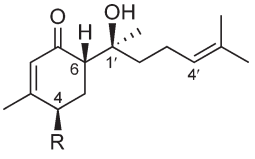
3.10.4.1.2(i) Acyclic Mukuroziosides IIb (**13**) is an acyclic sesquiterpene glycoside isolated and characterized initially from the pericarps of *Sapindus mukorossi* Gaertn. (Sapindaceae).[92] As a result of work performed at the University of Illinois at Chicago, this compound was isolated from the fruits of *Sapindus rarak* DC. (Sapindaceae) collected in Indonesia, where it was found to occur in a high yield (6.8% w/w). This is the first identification of an acyclic sesquiterpene glycoside with a sweet taste from a plant source, and it possesses a sweetness potency approximately equal to that of sucrose.[82]



13

3.10.4.1.2(ii) Bisabolane (+)-Hernandulcin (**14**) is a highly sweet bisabolane-type sesquiterpenoid, (Chapter 1.16), which was first purified and characterized at the University of Illinois at Chicago from a sweet-tasting herb collected in Mexico, *Lippia dulcis* Trevir. (Verbenaceae), a plant known to the Aztecs.[93,94] The sweetness potency of this substance was rated as 1500 times sweeter than 0.25 mol l^{-1} sucrose on a weight basis, but this compound was also found to possess some bitterness and a somewhat unpleasant aftertaste.[93] Of the four possible diastereomers for the structure of this compound, it was found after total synthesis that only the 6*S*,1′*S* configuration of hernandulcin shows intense sweetness.[95] Three primary structural units involved in the mediation of the sweet taste of this rather simple molecule have been resolved (i.e., the C-1′ hydroxyl group, the C-6 carbonyl, and the C-4′, C-5′ double bond).[96] Souto Bachiller *et al.*[97] have demonstrated that there are at least two different

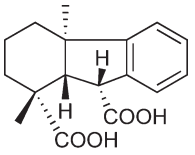
chemotypes of *L. dulcis*, with the Puerto Rican type containing (+)-hernandulcin as the major component (33% w/w) of its volatile oil and the Mexican type containing only trace amounts of this sesquiterpenoid. Hernandulcin has been produced both by total synthesis[21,98,99] and from both shoot and hairy root cultures of *L. dulcis*[21] and subjected to microbial biotransformation.[100] A second sesquiterpene-type analogue in this series, namely 4β-hydroxyhernandulcin (**15**), was isolated in the laboratory of the senior author of this chapter from a sample of *L. dulcis* collected in Panama. However, the sweetness potency of this compound relative to sucrose was not evaluated because of the paucity of availability of **15**.[101] Recently, six further bisabolane analogues of hernandulcin have been isolated and characterized by Japanese workers from the aerial parts of *L. dulcis*, although these were not evaluated for the presence or absence of a sweet taste.[102] Now that nearly 25 years have elapsed since hernandulcin (**14**) was first discovered, this structurally simple highly sweet substance remains of interest as a tool for sweetener research, although it is probably too unstable and unpleasant tasting for commercial development.



14 R = H
15 R = OH

3.10.4.1.3 Diterpenoids

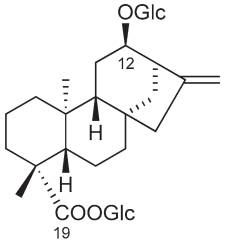
3.10.4.1.3(i) Diterpene acid In 1971, Tahara *et al.*[103] described four stereoisomers of 4β,10α-dimethyl-1,2,3,4,5,10-hexahydrofluorene-4α,6-dicarboxylic acid derived from pine tree resin. One of these compounds, **16**, was found to be highly sweet, but also bitter tasting. There has been very little follow-up to this initial literature report on this sweet-tasting diterpene acid.



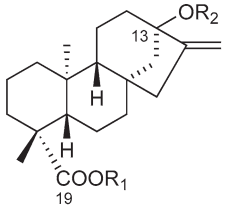
16

3.10.4.1.3(ii) *ent*-Kaurane As mentioned earlier in this chapter, two steviol glycosides, rebaudioside A (**4**) and stevioside (**5**), have commercial applications in various forms, and there is considerable interest in extending these uses further.[39,43,53,54] Several additional sweet diterpene glycosides of the *ent*-kaurane type were isolated from two plant species, *S. rebaudiana*[42,104–106] and *Rubus suavissimus* S. K. Lee (Rosaceae),[107] in the 1970s and 1980s. Dulcoside A (**18**) and rebaudioside C (**20**) are the major constituents of the leaves of *S. rebaudiana*, but occur in somewhat lower yields (0.4–0.7 and 1–2% w/w, respectively) when compared with stevioside (**5**) and rebaudioside A (**4**).[104–106] Other less abundant sweet principles of *S. rebaudiana* leaves are rebaudioside B (**19**),[42] rebaudioside D (**21**),[105] rebaudioside E (**22**),[105] and steviolbioside (**25**).[42] It is possible that rebaudioside B and steviolbioside are actually artifacts of extraction as opposed to being actual natural products. More recently, a ninth sweet-tasting principle has been obtained from *S. rebaudiana* leaves, namely rebaudioside F (**23**), which contains a β-xylose unit as part of the C-13 saccharide substituent.[108] Rubusoside (=desglucosylstevioside) (**24**) is the main *ent*-kaurene glycoside from *R. suavissimus* leaves (a sweet-tasting species originally published in the literature as *Rubus chingii* Hu[107]) and its sweetness potency was rated as 115 times sweeter than sucrose, but also with the perception of some bitterness and an unpleasant aftertaste.[109] Additional *ent*-kaurene-type diterpene glycosides were isolated as minor constituents of

R. suavissimus leaves, namely suaviosides A, B, G, H, I, and J (**27**–**32**) and steviol 13-*O*-β-D-glucoside (steviol monoside) (**26**).[109,110] However, their sweetness intensities have not been determined. No other species of the genus *Stevia* or *Rubus* appears to biosynthesize sweet-tasting *ent*-kaurene glycosides to any significant degree.[21] Like stevioside (**5**), rubusoside (**24**) was subjected to extensive structural modification by the group of the late Professor Osamu Tanaka at Hiroshima University in order to improve on its quality of taste.[20,44,48,49] Several *ent*-kaurene glycosides were isolated in 2002 by Yamasaki *et al.*[111] from the Madagascan plant *Cussonia racemosa* Baker (Araliaceae), and one of these compounds, cussoracoside C (**17**), bearing a β-glucose unit at C-12, was stated to be sweet tasting, although its relative potency compared with sucrose was not documented.

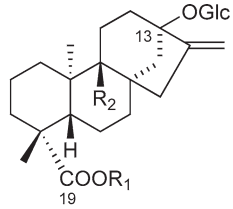


17

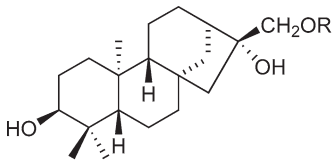


	R_1	R_2
18	β-glc	β-glc^2-α-rha
19	H	β-glc^2-β-glc
		$\vert^3$
		β-glc
20	β-glc	β-glc^2-α-rha
		$\vert^3$
		β-glc
21	β-glc^2-β-glc	β-glc^2-β-glc
		$\vert^3$
		β-glc
22	β-glc^2-β-glc	β-glc^2-β-glc
23	β-glc	β-glc^2-β-xyl
		$\vert^3$
		β-glc
25	H	β-glc^2-β-glc

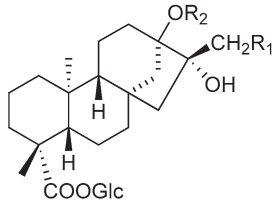
rha: L-rhamnopyranosyl



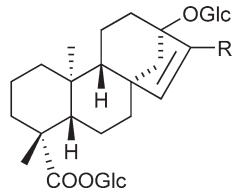
	R_1	R_2
24	β-glc	H
26	H	H
28	β-glc	OH



27 R = β-glc



	R_1	R_2
29	H	β-glc
31	OH	H



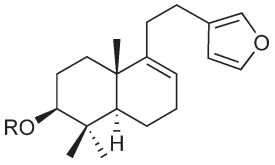
30 R = CHO
32 R = CH_2OH

Rebaudioside A (**4**) has a branched trisaccharide unit at C-13 and is sweeter and more pleasant tasting than stevioside (**5**), with a C-13 sophorosyl disaccharide moiety. Removal of the C-19 sugar unit of rebaudioside A, so as to produce rebaudioside B (**19**), results in a less potently sweet-tasting compound. Rebaudioside C (**20**),

having a terminal glucose unit at C-13 replaced by rhamnose, is not only less sweet than rebaudioside A (**4**), but is somewhat bitter. Sauvioside A (**27**) is unusual among the *ent*-kaurane sweet glycosides in that it contains no C-16, C-17-exomethylene group. Sauvioside B (**28**), which differs from rubusoside (**24**) only in the presence of a C-9 hydroxy group, has only half of the resultant sweetness potency (**Table 1**).[19,109]

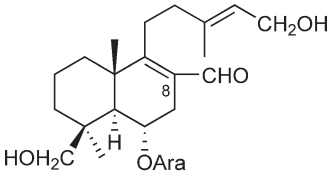
There is now a very large technical and patent literature on *S. rebaudiana* and its sweet steviol glycoside constituents. This information refers principally to methods for the purification of these substances, procedures for taste improvement, and biological test results.

3.10.4.1.3(iii) Labdane Two furanolabdane-type diterpene glycosides, baiyunoside (**33**) and phlomisoside I (**34**), were isolated as sweet constituents from the roots of a Chinese plant, *Phlomis betonicoides* Diels (Lamiaceae).[112,113] Baiyunoside (**33**) was rated about 500 times sweeter than sucrose, whereas the sweetness intensity of phlomisoside I (**34**) was not determined. Both **33** and **34** were also isolated from a second species, *Phlomis medicinalis* Diels (roots), whereas phlomisoside I (**34**) occurred in the roots of *Phlomis younghushbandii* Mukerjee. The specimens of *P. medicinalis* and *P. younghushbandii* investigated were collected in Tibet.[114] The sweet-tasting compound phlomisoside I (**34**) has a C-3 neohesperidyl group, whereas when this sugar unit is replaced by a sophorosyl group moiety as in phlomisoside II, the compound is bitter tasting.[112,113] In Japan, Nishizawa *et al.*[115,116] at Tokushima Bunri University have prepared a large number of synthetic analogues of baiyunoside (**33**), with some of these found to be sweeter than the natural product.



33 R = β-glc^2-β-xyl
34 R = α-rha^2-β-glc

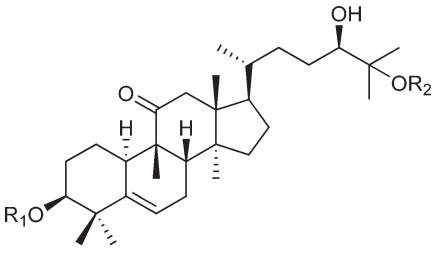
Another labdane-type diterpene glycoside, namely gaudichaudioside A (**35**), was isolated from the aerial parts of a species collected in Paraguay, *Baccharis gaudichaudiana* DC. (Asteraceae) (local name 'chilca melosa'), in work carried out at the University of Illinois at Chicago.[117] It was found that gaudichaudioside A was 55 times sweeter than 2% w/w sucrose solution and gave only a very low perception of bitterness.[117] Several closely related compounds with the same carbon skeleton as gaudichaudioside A were isolated but were not highly sweet. Instead, these derivatives exhibited other taste properties (sweet-bitter, bitter, and neutral tasting).[117] For example, when the C-8 aldehyde group of gaudichaudioside A (**35**) was replaced with a $-CH_2OH$ group, as in gaudichaudioside B, a fleeting sensation of sweetness lasting only a few seconds occurred when tasted, followed by prolonged bitterness.[117] *Baccharis* species are somewhat bitter tasting, so the occurrence of a sweet-tasting labdane glycoside, such as compound **35** in *B. gaudichaudiana*, seems to be an anomaly.



35
Ara: L-arabinopyransyl

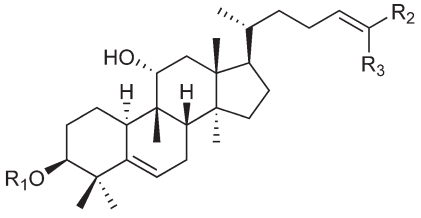
3.10.4.1.4 Triterpenoids

3.10.4.1.4(i) Cucurbitane Many cucurbitane-type triterpenoid glycosides have been isolated as sweet principles from several plants of the family Cucurbitaceae, and this is now one of the largest groups of natural highly sweet compounds. Two cucurbitane-type glycosides, bryoside (**36**) and bryonoside (**37**), have been reported from the roots of *Bryonia dioica* Jacq. as sweet principles, although their sweetness intensities relative to sucrose were not reported.[118,119] The structure of bryonoside (**37**) was revised by Arihara and co-workers[119] in 1992. The structure of a third sweet compound from *B. dioica*, bryodulcoside, has not yet been resolved.[119]

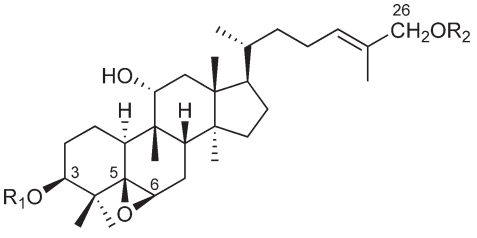


	R_1	R_2
36	β-glc^2-α-rha	β-glc
37	β-glc^2-α-rha	β-glc^6-β-glc

Two species of the genus *Hemsleya*, namely *H. carnosiflora* C.Y. Wu et Z.L. Chen and *H. panacis-scandens* C.Y. Wu and Z.L. Chen, have afforded between them three sweet cucurbitane-type triterpene glycosides, carnosifllosides V (**38**) and VI (**39**), and scandenoside R6 (**43**).[120,121] In addition, several other cucurbitane-type triterpenoid glycosides, scandenosides R8–R11, were isolated from *H. panacis-scandens*.[122] Of these, only scandenoside R11 (**44**) was reported to be sweet tasting, but its sweetness potency was not stated.[122]

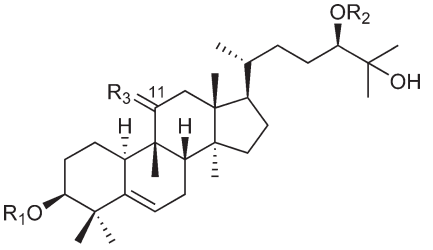


	R_1	R_2	R_3
38	β-glc	CH_2O-β-glc^2-β-glc	CH_3
39	β-glc	CH_2O-β-glc^6-β-glc	CH_3
43	β-glc	CH_3	CH_2O-β-glc^2-β-glc



	R_1	R_2
44	β-glc	β-glc^6-β-glc

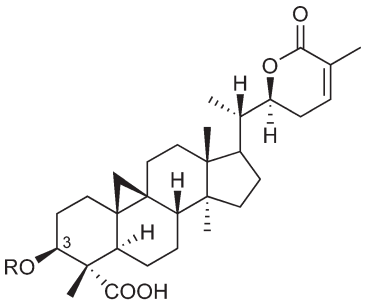
Several highly sweet cucurbitane-type triterpene glycosides have been isolated from the dried fruits of the Chinese medicinal plant *S. grosvenorii* (Swingle) C. Jeffrey ex A.M. Li & Zhi Y. Zhang, a plant mentioned already in this chapter (Section 3.10.2).[33–35,123,124] Mogrosides IV (**41**) and V (**2**) and siamenoside I (**45**) are the major sweet principles of this plant species and their sweetness intensities were rated as 233–392, 250–425, and 563 times sweeter than sucrose, respectively.[124] Siamenoside I (**45**) was also isolated as a minor constituent from another species of the genus *Siraitia*, *S. siamensis* (Craib) C. Jeffrey ex S.Q. Zhong & D. Fang, together with 11-oxomogroside V (**42**), with the sweetness intensity of the latter compound unreported.[123,124] Recently, Jia and Yang[125] have described a further sweet-tasting glycoside from *S. grosvenorii*, namely isomogroside V (**40**).



	R_1	R_2	R_3
40	β-glc^4-β-glc	β-glc^2-β-glc $\vert^6$ β-glc	α-OH, β-H
41	β-glc^6-β-glc	β-glc^2-β-glc	α-OH, β-H
42	β-glc^6-β-glc	β-glc^2-β-glc $\vert^6$ β-glc	=O
45	β-glc	β-glc^2-β-glc $\vert^6$ β-glc	α-OH, β-H

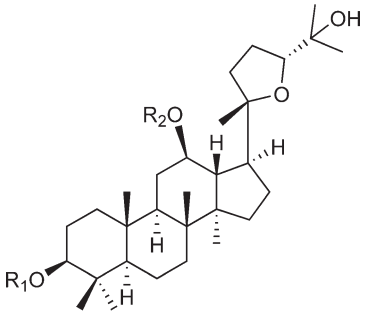
Analysis of many cucurbitane glycosides has indicated that at least three sugar units need to be present in the molecule for the exhibition of sweetness, with glycosides of aglycones containing 11α-hydroxy, 11β-hydroxy, and 11-keto functionalities being highly sweet, neutral tasting, and less highly sweet or bitter, respectively.[19,121,124]

3.10.4.1.4(ii) Cycloartane Abrusosides A–E (**46–50**) are prototype triterpenoid sweeteners of the cycloartane type and were isolated at the University of Illinois at Chicago from a sample of the leaves of *Abrus precatorius* L. (Fabaceae) collected in Florida.[126–128] Of these, compounds **46–49** were isolated from a second species of the genus, *A. fruticulosus* Wall. from Thailand.[129] The aglycone of these compounds, namely abrusogenin, was identified as having a novel carbon skeleton, as confirmed by single-crystal X-ray crystallography of abrusogenin methyl ester.[127] Abrusosides A–E differ structurally from one another in the type of saccharide unit affixed to the C-3 position. The sweetness intensities of the ammonium salts of abrusosides A–D were evaluated as 30, 100, 50, and 75 times sweeter than 2% w/w sucrose solution, respectively.[126] The sweetness intensity of abrusoside E per se was not determined, whereas the semisynthetic monomethyl ester (the 6″-methyl-β-D-glucuronopyranosyl-(1→2)-β-D-glucopyranosyl derivative) of abrusoside E (**51**) was found to exhibit about 150 times the sweetness potency of 2% sucrose, making it the sweetest compound in this series.[130] When the aglycone carboxylic acid group was methylated, as in abrusoside E dimethyl ester, no sweetness was perceived.[130] Abrusogenin methyl ester has been synthesized in our laboratories.[131] Thus far, the abrusosides seem to be the only sweet constituents from the genus *Abrus*.



46 R = β-glc
47 R = β-glcA-6-CH_3^2-β-glc
48 R = β-glc^2-β-glc
49 R = β-glcA^2-β-glc
50 R = β-glc^2-β-glcA
51 R = β-glc^2-β-glcA-6-CH_3

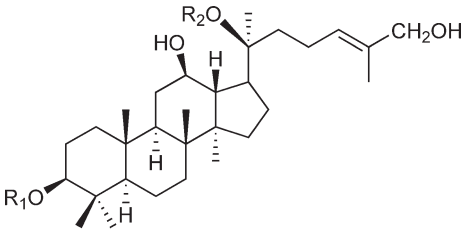
3.10.4.1.4(iii) Dammarane Cyclocarioside A (**52**), a dammarane-type triterpenoid glycoside sweet principle from the leaves of *Cyclocarya paliurus* (Batal.) Iljinsk. (Juglandaceae), was isolated and characterized from a plant used in the People's Republic of China as a treatment for diabetes.[132] Later, another sweet-tasting principle, cyclocarioside I (**53**), was isolated from the same plant along with two other compounds with the same dammarane-type triterpenoid aglycone structure.[133] Cyclocarioside I was shown to exhibit about 250 times the sweetness potency of sucrose.[133]



	R_1	R_2
52	α-araf-5-Ac	α-rha
53	α-araf	β-qui

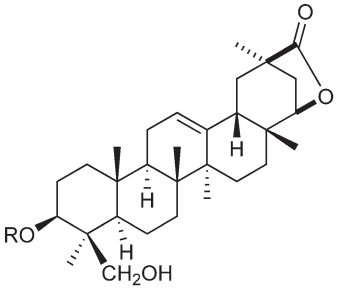
qui: D-quinovosyl
araf: D-arabinofuranosyl

From the crude extract of the vine of *Gynostemma pentaphylum* (Thunb.) Makino (Cucurbitaceae), a plant used to make a sweet tea ('Amachazuru') in Japan, gypenoside XX (**54**) was isolated by Takemoto *et al.*[134] in Tokushima. Although the sweetness of this compound was not reported when it was first characterized, it was later stated to be sweet.[22] The relative sweetness potency of gypenoside XX (**54**) to sucrose has not appeared in the literature.



	R_1	R_2
54	β-glc^2-β-glc | 6 α-rha	β-glc^6-β-glc

3.10.4.1.4(iv) Oleanane Five oleanane-type triterpene saponins, namely albiziasaponins A–E (**55**–**59**), have been reported by Yoshikawa and co-workers from Kyoto Pharmaceutical University as sweet principles of stems of *Albizia myriophylla* Benth. (Fabaceae), a traditional medicinal plant collected in Thailand, used as a substitute for Glycyrrhizae Radix (licorice root) as a sweetening agent. A lactone ring was attached to the C-20,22 positions in ring E of the aglycone portion of albiziasaponins A and C–E (**55**, **57**–**59**). Albiziasaponin B (**56**), which has a C-29 carboxyl group instead, was rated as about 600 times sweeter than sucrose.[135]

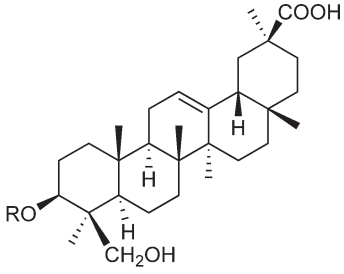


55 R = β-glcA^2-β-glcA^2-α-rha

57 R = β-glcA^2-β-glc^2-α-rha
|
3
β-glc

58 R = β-glcA^2-β-glc^2-α-rha
|
3
β-xyl

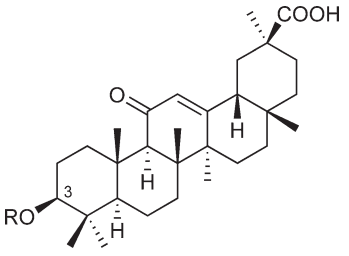
59 R = β-glcA^2-β-glcA^2-α-rha
|
3
β-xyl



56 R = β-glcA^2-β-glcA^2-α-rha

As mentioned earlier, glycyrrhizin (**1**) and its ammonium salts are available commercially for sweetening and flavoring purposes, and glycyrrhetic acid 3-*O*-D-glucuronide (MGGR, **7**) is a promising new intense sweetener.[27,28,32] Apioglycyrrhizin (**60**) and araboglycyrrhizin (**61**) have been isolated from the roots of *Glycyrrhiza inflata* Batalin (Fabaceae) by Kitagawa and colleagues.[136] Glycyrrhizin has a C-3-affixed diglucuronate unit, whereas apioglycyrrhizin (**60**) has a β-D-apiofuranosyl-(1→2)-β-D-glucuronopyranosyl group and araboglycyrrhizin (**61**) an α-L-arabinopyranosyl-(1→2)-β-D-glucuronopyranosyl group at the C-3 position of the aglycone glycyrrhetic acid. The sweetness intensities of apioglycyrrhizin (**60**) and araboglycyrrhizin (**61**) were rated as 300 and 150 times sweeter than sucrose, respectively.[136] In a published review of 13 glucuronide

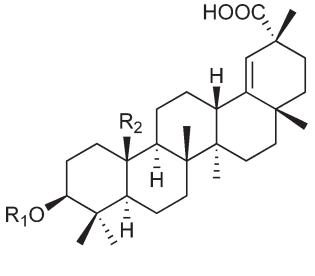
saponins from licorice, it was pointed out that 11-deoxoglycyrrhizin is bitter, thereby showing the requirement for the presence of the C-11 carbonyl group for the mediation of sweetness in glycyrrhizin (**1**) and its sweet derivatives.[27]



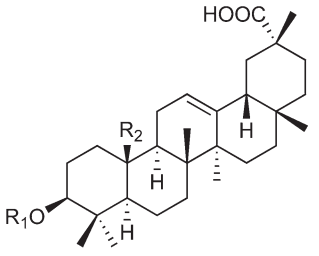
60 R = β-glcA^2-β-api
61 R = β-glcA^2-α-ara

api: D-apiofuranosyl

Periandrins I–IV (**62–65**) were characterized in the 1980s as oleanane-type triterpenoid glycoside sweeteners from the roots of *Periandra dulcis* Mart. ex Benth. (Fabaceae) (Brazilian licorice) by Hashimoto *et al.*[137–139] at Kobe Pharmaceutical University in Japan, and the sweetness potency was determined as about 90 times sweeter than sucrose for each compound. Previously, the sweet principle of Brazilian licorice roots was thought to be glycyrrhizin (**1**).[16] Periandrins I–IV (**62–65**) were also found in another species, *Periandra mediterranea* (Vell.) Taub.[137–139] A fifth compound in this series, periandrin V (**66**), was isolated from the roots of *P. dulcis* at the University of Illinois at Chicago, and was found to be based on the same aglycone as periandrin I (**62**). The terminal D-glucuronic acid residue of periandrin I was substituted by a D-xylose moiety in periandrin V. Periandrin V (**66**) exhibited 220 times the sweetness of 2% sucrose and was accordingly ranked as the sweetest substance obtained so far in the periandrin series.[140]



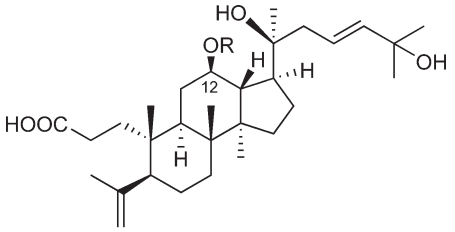
	R_1	R_2
62	β-glcA^2-β-glcA	CHO
64	β-glcA^2-β-glcA	CH_2OH
66	β-glcA^2-β-xyl	CHO



	R_1	R_2
63	β-glcA^2-β-glcA	CHO
65	β-glcA^2-β-glcA	CH_2OH

3.10.4.1.4(v) Secodammarane Two new sweet secodammarane glycosides, pterocaryosides A (**67**) and B (**68**), were isolated and structurally determined from the leaves and stems of *Pterocarya paliurus* Batalin (Juglandaceae), at the University of Illinois at Chicago.[141] *Pterocarya paliurus* Batal. is a preferred taxonomic name for *C. paliurus* (Batal.) Iljinsk (see Section 3.10.4.1.4(iii)). The leaves of *P. paliurus* are used by local

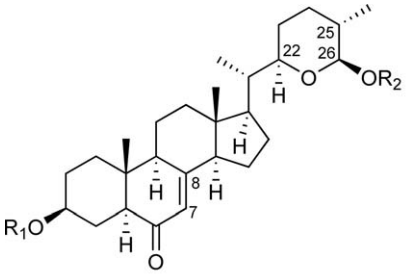
populations in Hubei Province of the People's Republic of China to sweeten cooked foods. Pterocaryoside A (**67**), which has a β-quinovose unit attached to the C-12 position, is 50 times sweeter than sucrose, whereas pterocaryoside B (**68**), with an α-arabinose unit at C-12, is 100 times sweeter than sucrose.[141] These are the first highly sweet secodammarane glycosides to have been isolated and structurally characterized, and represent interesting lead compounds for potential synthetic optimization.



67 R = β-qui
68 R = α-ara

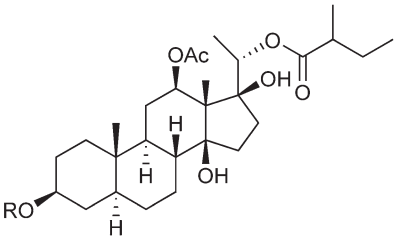
3.10.4.1.5 Steroidal saponins

The steroidal saponin osladin (**69**) was isolated as a sweet principle from the fern *Polypodium vulgare* L. (Polypodiaceae) nearly 40 years ago by Czech workers.[142] However, the original structure proposed was later revised because when this compound was synthesized by Nishizawa and Hamada[143–145] it was not sweet at all. The correct structure of osladin (**69**) was characterized by single-crystal X-ray crystallography and the stereochemistry of osladin was reassigned as 22*R*, 25*S*, and 26*R*. The actual sweetness potency of osladin was revised to 500 times, rather than 3000 times, sweeter than sucrose, as originally published.[143–145] Polypodosides A (**70**) and B (**71**) were isolated at the University of Illinois at Chicago from the rhizomes of the North American fern *Polypodium glycyrrhiza* Eat. (Polypodiaceae) as additional highly sweet steroidal glycosides.[146,147] The aglycone on which these compounds are based, polypodogenin, is the $\Delta^{7,8}$-derivative of the aglycone of osladin. The structure of polypodoside A (**70**) was also revised as 22*R*, 25*S*, 26*R*, by a chemical interconversion procedure, in collaboration with Nishizawa of Tokushima Bunri University.[148] Polypodoside A (**70**) shows a high sweetness potency and was rated as 600 times sweeter than sucrose.[146] In order to exhibit sweetness, steroidal saponins of this type must be bidesmosidic, with saccharide substitution at both C-3 and C-26.[19] Polypodoside C, a third compound in the polypodoside series, has an L-acofriopyranosyl (3-*O*-methylrhamnosyl) unit attached at C-26, in place of the L-rhamnosyl moiety of polypodoside B (**71**), and is devoid of sweetness.[19,147]



	R_1	R_2	Other
69	β-glc^2-α-rha	α-rha	7,8-dihydro
70	β-glc^2-α-rha	α-rha	-
71	β-glc	α-rha	-

Telosmosides A_8–A_{18} (**72–82**), pregnane-type steroidal saponins, were isolated by Yamasaki and co-workers[149] at Hiroshima University as sweet principles of the stems of *Telosma procumbens* Merr. (Asclepiadaceae). This plant has been used as a traditional medicinal plant in certain Asian countries and employed as a licorice substitute in Vietnam. Several unusual sugars such as D-cymarose, D-oleandrose, D-digitoxose, D-thevetose, and 6-deoxy-3-*O*-methyl-D-allose were found in the saccharide moieties attached at the C-3 position of the common aglycon of these compounds. Telosmoside A_{15} (**79**) was reported to exhibit a sweetness intensity 1000 times greater than that of sucrose.[149]

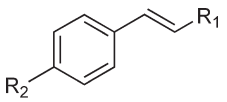


72 R = β-dig^4-β-cym^4-β-ole^4-β-glc
73 R = β-dig^4-β-ole^4-β-the^4-β-glc
74 R = β-dig^4-β-cym^4-β-ole^4-β-ole^4-β-ole
75 R = β-dig^4-β-cym^4-β-ole^4-β-ole^4-β-the
76 R = β-dig^4-β-cym^4-β-ole^4-β-ole^4-β-glc
77 R = β-dig^4-β-dig^4-β-ole^4-β-ole^4-β-the
78 R = β-dig^4-β-cym^4-β-ole^4-β-ole^4-β-ole^4-β-glc
79 R = β-dig^4-β-cym^4-β-ole^4-β-ole^4-β-the^4-β-glc
80 R = β-dig^4-β-cym^4-β-ole^4-β-ole^4-β-glc^4-β-glc
81 R = β-dig^4-β-cym^4-β-ole^4-β-ole^4-β-alm^4-β-glc
82 R = β-dig^4-β-cym^4-β-ole^4-β-the^4-β-glc^4-β-glc

dig: D-digitoxosyl
alm: 6-deoxy-3-*O*-methyl-D-allosyl
cym: D-cymarosyl
the: D-thevetosyl
ole: D-oleandrosyl

3.10.4.2 Phenylpropanoids

The phenylpropanoids *trans*-anethole (**83**) and *trans*-cinnamaldehyde (**84**) are used as flavoring agents in foods in the United States and many other countries.[16] In work performed at the University of Illinois at Chicago, *trans*-cinnamaldehyde (**84**) was isolated from *Cinnamomum osmophloeum* Kaneh. (Lauraceae) as a sweet principle,[84] whereas *trans*-anethole (**83**) was isolated as the volatile oil constituent responsible for the sweet taste of several plant species, as listed in **Table 1**.[83] These two compounds occur widely in the plant kingdom. As previously indicated, it is necessary to rule out their presence in any candidate sweet plant when searching for new natural product sweeteners, by preliminary analysis using GC–MS.[83,84]



	R_1	R_2
83	CH_3	OCH_3
84	CHO	H

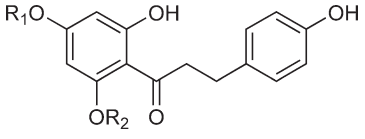
3.10.4.3 Dihydroisocoumarins

The leaves of *H. macrophylla* var. *thunbergii*, containing the dihydroisocoumarin 3*R*-phyllodulcin (**3**), were mentioned earlier in the chapter as having a limited use in Japan.[28,36,37] It has been demonstrated that 3*R*-phyllodulcin occurs naturally in unprocessed leaves of its plant of origin as a 5:1 enantiomer with the previously undescribed compound 3*S*-phyllodulcin.[150] Also reported were several new 3*R*- and 3*S*-phyllodulcin 3′-*O*-glycosides, although the presence or absence of a sweet taste in these three new phyllodulcin analogues was not disclosed.[150] Much work has been performed on the synthesis of dihydroisocoumarin sweeteners, using phyllodulcin (**3**) as a lead compound. For example, Merlini *et al.*[151] have recently summarized their research data on the effects of the structural modification of this compound on sweetness, wherein 120 compounds containing an isovanillyl unit were produced.

3.10.4.4 Flavonoids

3.10.4.4.1 Dihydrochalcones

Glycyphyllin (**85**), phlorizin (**87**), and trilobatin (**88**) are dihydrochalcone glycosides reputed to be sweet and were isolated from *Smilax glycyphylla* Hassk. (Smilacaceae),[16,152,153] *Symplocos lancifolia* Siebold et Zucc.,[154] and *Symplocos microcalyx* Hayata (Symplocaceae),[154] respectively. Trilobatin (**88**) was isolated as a major sweet compound along with phlorizin (**87**) from the leaves of *Lithocarpus litseifolius* Chun (Fagaceae).[155] According to Horowitz and Gentili,[156] glycyphyllin is bittersweet, with the bitterness predominating. Naringin dihydrochalcone (**86**) and neohesperidin dihydrochalcone (**12**) are semisynthetic dihydrochalcone glycosides and can be obtained as by-products of the citrus industry.[73,156] Neohesperidin dihydrochalcone (NHDC; **12**; 250–1800 times sweeter than sucrose, depending on concentration) is sweeter than compound **86**, and has acceptable hedonic properties, and is used in a wide variety of foodstuffs as a sweetener and flavor ingredient, as mentioned earlier.[71,73,156] There have been several attempts to synthesize improved sweet-tasting dihydrochalcones, with such compounds requiring 3-hydroxy-4-alkoxy substitution in ring B.[73,156]

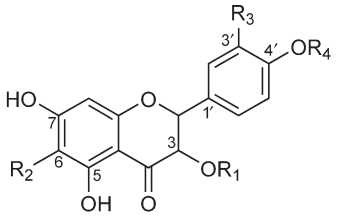


	R_1	R_2
85	H	α-rha
86	β-glc^2-α-rha	H
87	H	β-glc
88	β-glc	H

3.10.4.4.2 Dihydroflavonols

The seeds of *Aframomum hanburyi* K. Schum. (Zingiberaceae) are used as an antidote and ingredient in certain medicinal preparations in Cameroon. From an acetone extract of the seeds of this plant, two sweet dihydroflavonols, 3-acetoxy-5,7-dihydroxy-4′-methoxyflavanone (**89**) and 2*R*,3*R*-(+)-3-acetoxy-5,7,4′-trihydroxyflavanone (**90**), were isolated.[157] 3-Acetoxy-5,7-dihydroxy-4′-methoxyflavanone (**89**) was previously isolated from a different species, *Aframomum pruinosum* Gagnep.[158] However, the sweetness intensities of these compounds were not indicated.[157,158] The previously known (2*R*,3*R*)-dihydroquercetin 3-*O*-acetate (**91**), which was rated as 80 times sweeter than sucrose, was isolated at the University of Illinois at Chicago as a sweet principle from the young leaves of *Tessaria dodoneifolia* (Hook. & Arn.) Cabrera (Asteraceae), collected in Paraguay.[159] The sweetness of this compound was increased to 400 times that of sucrose by methylation at the

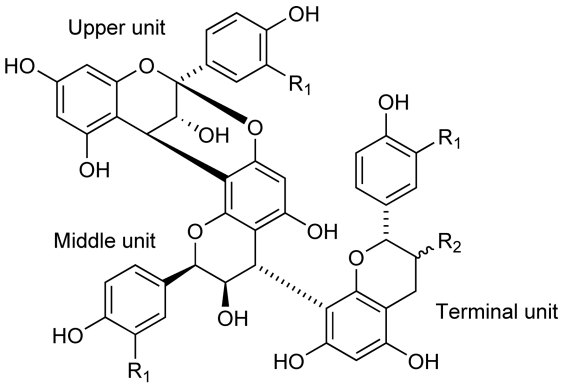
C-4′ hydroxyl to form a synthetic isovanillyl derivative (**92**).[159] Two dihydroflavonols, huangqioside E (**96**) and neoastilbin (**97**), were purified from *Engelhardtia chrysolepis* Hance (Juglandaceae).[160,161] However, their sweetness intensities were not evaluated. Compound **91** and three additional sweet dihydroflavonols (**93–95**) with a C-6 methoxy group were isolated from the leaves of *Hymenoxys turneri* K.F. Parker (Asteraceae), collected in Texas.[162] Compound **93**, the 6-methoxylated analogue of compound **91**, showed less than 50% of its sweetness potency.[19,162]



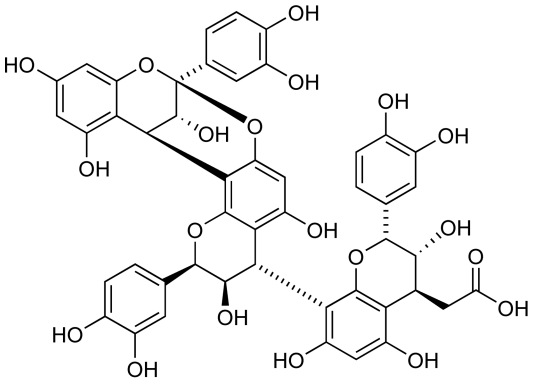
	R_1	R_2	R_3	R_4	Other
89	Ac	H	H	CH_3	2*R*,3*R*
90	Ac	H	H	H	2*R*,3*R*
91	Ac	H	OH	H	2*R*,3*R*
92	Ac	H	OH	CH_3	-
93	Ac	CH_3O	OH	H	2*R*,3*R*
94	H	CH_3O	OH	H	2*R*,3*R*
95	Ac	CH_3O	H	H	2*R*,3*R*
96	α-rha^3-β-glc	H	OH	H	2*R*,3*R*
97	α-rha	H	OH	H	2*S*,3*S*

3.10.4.5 Proanthocyanidins

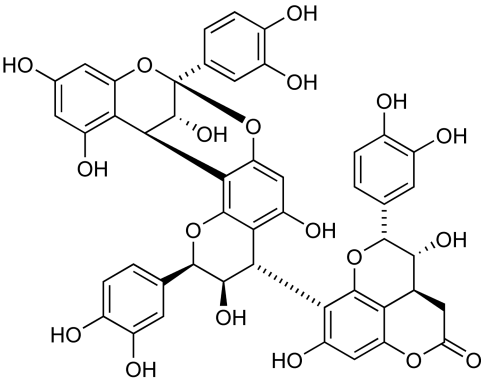
Several doubly linked ring-A proanthocyanidins are known to be sweet tasting. For example, two proanthocyanidins, cinnamtannin B-1 (**98**) and cinnamtannin D-1 (**99**), isolated from the roots of *Cinnamomum sieboldii* Meisn. (Lauraceae) showed sweet properties.[163] Other sweet-tasting proanthocyanidins with carboxylic acid (**101**) and lactone (**102**) functionalities were isolated from the ferns *Arachniodes sporadosora* (Kuntze) Nakaike and *Arachniodes exilis* Ching (Aspidiaceae).[164] However, none of these proanthocyanidins was ever quantitatively rated for its sweetness intensity relative to sucrose. A sweet-tasting proanthocyanidin, selligueain A (**100**), was isolated at the University of Illinois at Chicago from the rhizomes of the fern *Selliguea feei* Bory (Polypodiaceae), collected in Indonesia.[165] Selligueain A may be distinguished from previously known sweet-tasting doubly linked ring-A trimeric proanthocyanidins **98** and **99**, as it has an afzelechin residue rather than an epicatechin moiety as the lower terminal unit of the molecule. When evaluated by a small human taste panel, selligueain A (**100**) showed 35 times the sweetness of a 2% sucrose solution and was not perceived as astringent when in solution.[165] A further doubly linked ring-A proanthocyanidin, selligueain B, was also isolated from the rhizomes of *S. feei*, but was not perceived as sweet tasting.[166] As a result of the investigation of selligueain A (**100**) and related compounds, stringent structural requirements seem to be necessary for proanthocyanidins of this type to exhibit a sweet taste. In this connection, it is notable that an epimer of selligueain A (epiafzelechin-(4β→8,2β→*O*→7)-epiafzelechin-(4β→8)-epiafzelechin) was astringent without any hint of sweetness.[165,166] Bohlin and co-workers[167] have demonstrated that selligueain A (**100**) is present in low yields in two *Polypodium* species collected in Honduras, and that this sweet-tasting compound is also an elastase inhibitor in human neutrophils. Moreover, Subarnas and Wagner[168] have reported the analgesic and antiinflammatory activities of selligueain A (**100**) in two *in vivo* models.



	R_1	R_2
98	OH	α-OH
99	OH	β-OH
100	H	β-OH



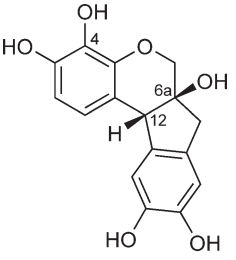
101



102

3.10.4.6 Benzo[*b*]indeno[1,2-*d*]pyrans

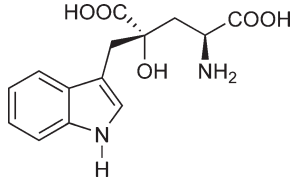
From the extract of the heartwood of *Haematoxylum campechianum* L. (Fabaceae), a sweet principle was isolated, namely (+)-hematoxylin (**103**). This compound has been used for a long time as a microscopic staining reagent, but the sweetness of this compound was not recognized previously. Also, in the same study, brazilin, the 4-deoxy derivative of (+)-hematoxylin and a constituent of *Caesalpinia echinata* Lam. (Fabaceae), was found not to be sweet.[169] It was concluded that requirements for sweetness of compound **103** include the C-4 hydroxy group and the *cis* junction of the cyclopentene and pyran rings.[19,169] In a follow-up study, (+)-hematoxylin (**103**) was rated as 120 times sweeter than 3% sucrose, whereas its synthetic (−)-enantiomer was only 50 times sweeter.[169,170]



103

3.10.4.7 Amino Acids

A highly sweet amino acid, (−)-monatin (**104**), was isolated from an African plant, *Sclerochiton ilicifolius* A. Meeuse (Acanthaceae).[171] Monatin (**104**) was rated as being comparable in sweetness to the synthetic amino acid 6-chloro-D-tryptophan, which showed a sweetness intensity 1300 times that of sucrose. Monatin (**104**) appears to be the only native plant amino acid with a highly sweet taste to have been discovered. This compound has been synthesized in chiral form.[172,173] A structure–sweet-tasting activity relationship study on synthetic analogues of monatin has been carried out in the laboratory of Merlini at the University of Milan. The 2*R*,4*R* isomer, rather than the natural 2*S*,4*S* isomer, is the sweetest of three of the four stereoisomers of monatin found to be sweet tasting.[174]



104

3.10.4.8 Proteins

Several plant-derived proteins, including brazzein (**105**),[175–177] curculin (**106**),[18,178] mabinlin (**107**),[179,180] monellin (**108**),[181,182] neoculin (**109**),[183] pentadin,[184] and thaumatin (**6**),[18,28,68–70,185] have been reported as sweeteners, with thaumatin mentioned earlier in this chapter as having commercial use as a sweetener and a flavor enhancer. The amino acid sequence of at least one form of each of these proteins is provided in this chapter, and information on their species of origin is given in **Table 1**. In a book chapter, Crammer[186] has summarized the recent literature for the plant proteins, including their subtypes, so this information is not repeated here. The genes for the production of curculin, mabinlin, monellin, and thaumatin have been expressed in microorganisms and solid-phase synthesis has been used to produce mabinlin and monellin.[182] The two most recently discovered sweet-tasting plant proteins are brazzein and neoculin, and these will be briefly described in turn. Brazzein (**105**), isolated from the fruits of a West African climbing vine, *Pentadiplandra brazzeana* Baill. (Capparaceae), by Ming and Hellekant at the University of Wisconsin, has 54-amino-acid residues and a molecular weight of 6473 Da, making it a relatively small protein compared to other sweet proteins such as curculin (12 491 Da), mabinlin (12 441 Da), monellin (11 086 Da), and thaumatin (22 209 Da).[175,177] Brazzein has four disulfide bridges and promising thermostability, as its sweetness was not destroyed even after 4 h exposure at 80 °C.[176] Most of the other protein sweeteners are unstable to heat and inappropriate for use at high temperature. The sweetness potency of brazzein (**105**) was rated as 2000 times greater than that of 2% sucrose, so this protein offers considerable potential as a new naturally occurring sweetener, and there are plans for its commercialization.[187] Markley and co-workers[187] have designed a new protocol for the production of brazzein by *Escherichia coli* as a fusion protein, and the potential mode of interaction of this sweet protein with the sweet taste receptor has been investigated by computer homology modeling.[188] Neoculin (**109**), a heterodimer of an acidic, glycosylated subunit of 113-amino-acid residues and a basic subunit that is the monomeric curculin itself, was isolated from the fruit of *Curculigo latifolia* Dryand. (Hypoxidaceae).[183] This protein tastes sweeter (40 000 times) than sucrose on a molar basis and converts sourness to sweetness. Interestingly, neoculin exhibits its potent sweetness at a weakly acidic pH and interacts with the hT1R3 human sweet taste receptor.[189,190]

PyrGlu-Asp-Lys-Cys-Lys-Lys-Val-Tyr-Glu-Asn-Tyr-Pro-Val-Ser-Lys-Cys-Gln-Leu-Ala-Asn-
1 5 10 15 20
Gln-Cys-Asn-Tyr-Asp-Cys-Lys-Leu-Asp-Lys-His-Ala-Arg-Ser-Gly-Glu-Cys-Phe-Tyr-Asp-
21 25 30 35 40
Glu-Lys-Arg-Asn-Leu-Gln-Cys-Ile-Cys-Asp-Tyr-Cys-Glu-Tyr
41 45 50 54

105

Asp-Asn-Val-Leu-Leu-Ser-Gly-Gln-Thr-Leu-His-Ala-Asp-His-Ser-Leu-Gln-Ala-Gly-Ala-
1 5 10 15 20
Tyr-Thr-Leu-Thr-Ile-Gln-Asn-Asn-Cys-Asn-Leu-Val-Lys-Tyr-Gln-Asn-Gly-Arg-Gln-Ile-
21 25 30 35 40
Trp-Ala-Ser-Asn-Thr-Asp-Arg-Arg-Gly-Ser-Gly-Cys-Arg-Leu-Thr-Leu-Leu-Ser-Asp-Gly-
41 45 50 55 60
Asn-Leu-Val-Ile-Tyr-Asp-His-Asn-Asn-Asn-Asp-Val-Asn-Gly-Ser-Ala-Cys-Cys-Gly-Asp-
61 65 70 75 80
Ala-Gly-Lys-Tyr-Ala-Leu-Val-Leu-Gln-Lys-Asp-Gly-Arg-Phe-Val-Ile-Tyr-Gly-Pro-Val-
81 85 90 95 100
Leu-Trp-Ser-Leu-Gly-Pro-Asn-Gly-Cys-Arg-Arg-Val-Asn-Gly
101 105 110 114

106

Glu-Leu-Trp-Arg-Cys-Gln-Arg-Gln-Phe-Leu-Gln-His-Gln-Arg-Leu-Arg-Ala-Cys-Gln-Arg-
1 5 10 15 20
Phe-Ile-His-Arg-Arg-Ala-Gln-Phe-Gly-Gly-Gln-Pro-Asp
21 25 30 33

A chain

Glu-Pro-Arg-Arg-Pro-Ala-Leu-Arg-Gln-Cys-Cys-Asn-Gln-Leu-Arg-Gln-Val-Asp-Arg-Pro-
1 5 10 15 20
Cys-Val-Cys-Pro-Val-Leu-Arg-Gln-Ala-Ala-Gln-Gln-Val-Leu-Gln-Arg-Gln-Ile-Ile-Gln-
21 25 30 35 40
Gly-Pro-Gln-Gln-Leu-Arg-Arg-Leu-Phe-Asp-Ala-Ala-Arg-Asn-Leu-Pro-Asn-Ile-Cys-Asn-
41 45 50 55 60
Ile-Pro-Asn-Ile-Gly-Ala-Cys-Pro-Phe-Arg-Ala-Trp
61 65 70 72

B chain

107

Arg-Glu-Ile-Lys-Gly-Tyr-Glu-Tyr-Gln-Leu-Tyr-Val-Tyr-Ala-Ser-Asp-Lys-Leu-Phe-Arg-
1 5 10 15 20
Ala-Asp-Ile-Ser-Glu-Asp-Tyr-Lys-Thr-Arg-Gly-Arg-Lys-Leu-Leu-Arg-Phe-Asn-Gly-Pro-
21 25 30 35 40
Val-Pro-Pro-Pro
41 44

A chain

(Thr)-Gly-Glu-Trp-Glu-Ile-Ile-Asp-Ile-Gly-Pro-Phe-Thr-Gln-Asn-Leu-Gly-Lys-Phe-Ala-Val-
1 5 10 15 20
Asp-Glu-Glu-Asn-Lys-Ile-Gly-Gln-Tyr-Gly-Arg-Leu-Thr-Phe-Asn-Lys-Val-Ile-Arg-Pro-
21 25 30 35 40
Cys-Met-Lys-Lys-Thr-Ile-Tyr-Glu-Glu-Asn
41 45 50

B chain

108

```
Asp-Ser-Val-Leu-Leu-Ser-Gly-Gln-Thr-Leu-Tyr-Ala-Gly-His-Ser-Leu-Thr-Ser-Gly-Ser-
1                5                  10                  15                  20
Tyr-Thr-Leu-Thr-Ile-Gln-Asn-Asn-Cys-Asn-Leu-Val-Lys-Tyr-Gln-His-Gly-Arg-Gln-Ile-
21               25                  30                  35                  40
Trp-Ala-Ser-Asp-Thr-Asp-Gly-Gln-Gly-Ser-Gln-Cys-Arg-Leu-Thr-Leu-Arg-Ser-Asp-Gly-
41               45                  50                  55                  60
Asn-Leu-Ile-Ile-Tyr-Asp-Asp-Asn-Asn-Met-Val-Val-Trp-Gly-Ser-Asp-Cys-Trp-Gly-Asn-
61               65                  70                  75                  80
X-Gly-Thr-Tyr-Ala-Leu-Val-Leu-Gln-Gln-Asp-Gly-Leu-Phe-Val-Ile-Tyr-Gly-Pro-Val-
80               85                  90                  95                 100
Leu-Trp-Pro-Leu-Gly-Leu-Asn-Gly-Cys-Arg-Ser-Leu-Asn
111              115                 110         113
```

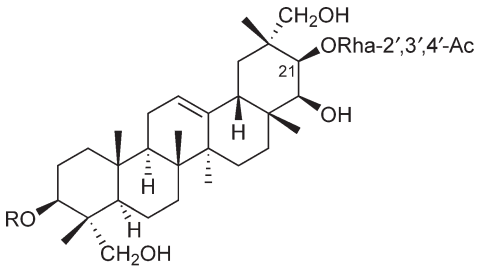
Acidic subunit (NAS) of neoculin

109

3.10.5 Naturally Occurring Sweetness Inducers

3.10.5.1 Triterpenoids

Five oleanane-type triterpenoid glycosides, strogins 1–5, were isolated from the leaves of the Malaysian plant *Staurogyne merguensis* Kuntze (Acanthaceae) by Kurihara and co-workers.[191] Strogins 1, 2, and 4 (**110–112**) show a persistent sweetness-inducing activity, in response to tasting cold water, which lasts for at least an hour.[192] In its country of origin, *S. merguensis* grows wild and local populations have used the leaves to sweeten rice during cooking.[191] The sweetness-inducing activities of strogins 1–5 were measured by a psychometric method.[191–193] Thus, the compounds were held in the mouth by a small taste panel for 3 min at a concentration of 1 mmol l^{-1} and then expectorated. The subjects then tasted water and the induced sweetness activity was determined by comparison with 0.05–0.4 mol l^{-1} standard sucrose solutions. Strogins 1, 2, and 4 also showed a sweet taste, lasting less than a minute, with strogin 1 (**110**) tasting sweeter than strogin 2 (**111**) or 4 (**112**). In contrast, strogins 3 and 5 were neither sweet tasting nor sweetness enhancing.[191,192] The sweetness-inducing activity of strogin 1 (**110**) reduced the antisweet activity of gymnemic acid (see Section 3.10.6), and was not reduced by the presence of Ca^{2+} and Mg^{2+} cations, unlike miraculin (**115**) (see Section 3.10.5.3).[192]

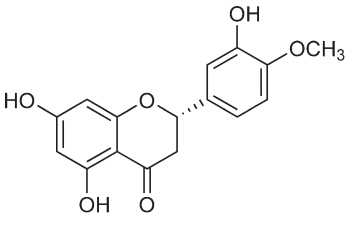


110 R = β-glcA^2-β-xyl
111 R = β-glcA
112 R = β-glcA^2-β-glc

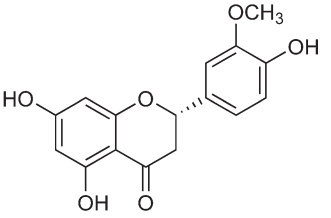
3.10.5.2 Flavonoids

Recently, several flavonoids have been reported to enhance sweetness or to improve taste in the patent literature. For example, the flavanone hesperetin (**113**), the aglycone of hesperidin, a glycoside found in citrus fruits, has been demonstrated as a sweetness-enhancing agent.[194] Homoeriodictyol (**114**), a naturally occurring

structurally related substance to compound **113**, was found to exhibit a 6% sweetness-enhancing activity when present at 100 ppm and evaluated with a 5% w/v sucrose solution.[195] When dissolved in water at 100 ppm, compound **114** exhibited a sweet, vanillin-like, phenolic taste.[195] Both hesperetin (**113**) and homoeriodictyol (**114**) occur in *Eriodictyon californicum* Decne. (Hydrophyllaceae) ('Herba Santa').[196]



113



114

3.10.5.3 Proteins

Miraculin (**115**) is a protein isolated from the fruits of the West African plant *Richardella dulcifica* (Schumacher & Thonn.) Baehni (Sapotaceae) (miracle fruit)[18,186,197,198] and has the property of making sour or acidic materials taste sweet. Miraculin is a homodimer of two glycosylated 191-amino-acid polypeptides linked by disulfide bonds, having a molecular weight of about 24 000 Da, with the monomeric form shown (**115**).[199] It was found that at acidic pH this protein converts a sour taste to a sweet taste, by an unknown molecular mechanism, whereas at neutral pH it tastes flat. The compound has no sweet taste per se. Miracle fruit concentrate was formerly on the market in the United States, but was removed because prior FDA approval for the scientific claims made had not been realized.[28] Although miraculin so far has not been expressed by *E. coli*,[186] this protein has been produced in transgenic lettuce[200] and tomatoes.[201]

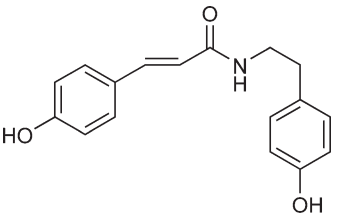
Asp-Ser-Ala-Pro-Asn-Pro-Val-Leu-Asp-Ile-Asp-Gly-Glu-Lys-Leu-Arg-Thr-Gly-Thr-Asn-
1 5 10 15 20
Tyr-Tyr-Ile-Val-Pro-Val-Leu-Arg-Asp-His-Gly-Gly-Gly-Leu-Thr-Val-Ser-Ala-Thr-Thr-
21 25 30 31 40
Pro-Asn-Gly-Thr-Phe-Val-Cys-Pro-Pro-Arg-Val-Val-Gln-Thr-Arg-Lys-Glu-Val-Asp-His-
41 45 50 55 60
Asp-Arg-Pro-Leu-Ala-Phe-Phe-Pro-Glu-Asn-Pro-Lys-Glu-Asp-Val-Val-Arg-Ala-Ser-Thr-
61 65 70 75 80
Asp-Leu-Asn-Ile-Asn-Phe-Ser-Ala-Phe-Met-Pro-Cys-Arg-Trp-Thr-Ser-Ser-Thr-Val-Ser-
81 85 90 95 100
Arg-Leu-Asp-Lys-Tyr-Asp-Glu-Ser-Thr-Gly-Gln-Tyr-Phe-Val-Thr-Ile-Gly-Gly-Val-Lys-
101 105 110 115 120
Gly-Asn-Pro-Gly-Pro-Glu-Thr-Ile-Ser-Ser-Trp-Phe-Lys-Ile-Glu-Glu-Phe-Cys-Gly-Ser-
121 125 130 135 140
Gly-Phe-Tyr-Lys-Leu-Val-Phe-Cys-Pro-Thr-Val-Cys-Gly-Ser-Cys-Lys-Val-Lys-Cys-Gly-
141 145 150 155 160
Asp-Val-Gly-Ile-Tyr-Ile-Asp-Gln-Lys-Gly-Arg-Arg-Arg-Leu-Ala-Leu-Ser-Asp-Lys-Pro-
161 165 170 175 180
Phe-Ala-Phe-Glu-Phe-Asn-Lys-Thr-Val-Tyr-Phe
181 185 190

115

Curculin (**106**)[18,178] and neoculin (**109**),[18,183,189,190] proteins isolated from the fruits of *C. latifolia* (see Section 3.10.4.8), also have sweetness-inducing activity. These proteins have a sweet taste that dissipates before the sweetness-inducing effect on water becomes evident.

3.10.5.4 Miscellaneous Compounds

The plant constituent *N-trans*-coumaroyltyramine (**116**), found in various plants inclusive of *Berberis vulgaris* L. (Berberidaceae),[202] has also been found to be a sweetness-inducing agent.[195] This compound was rated as being sweet when evaluated at a concentration of 100 ppm by a taste panel, and demonstrated a 6% sweetness-enhancing activity when evaluated in the same manner as compound **114** described above.[195] The effects of the caffeic acid conjugates cynarin and chlorogenic acid in turning water sweet have been documented.[25,203]



116

As relatively small percentage increases in sweetness enhancement by a given ingredient of foods and beverages may be important, it can be expected that additional naturally occurring compounds of this type will be discovered in the near future, especially now that screening via receptor binding is possible.[87,88]

3.10.6 Naturally Occurring Triterpenoid Sweetness Inhibitors

It has been known for some years that a number of synthetic compounds and certain enzymes suppress the sweet taste in humans and animals.[28,204–211] In addition, three plant species in particular, *Gymnema sylvestre* (Retz.) Schult. (Asclepiadaceae), *Hovenia dulcis* Thunb. (Rhamnaceae), and *Ziziphus jujuba* Mill. (Rhamnaceae), have been studied extensively for their sweetness-inhibitory (antisweet) constituents.[25] In recent years, additional sweetness-inhibiting agents have been isolated from *G. sylvestre* and *H. dulcis*, as well as three other plant species, *Gymnema alterniflorum* (Lour.) Merr. (Asclepiadaceae), *Stephanotis lutchuensis* Koidz. var. *japonica* (Asclepiadaceae), and *Styrax japonica* Sieb. et Zucc. (Styracaceae). The presently known oleanane- and dammarane-type triterpenoid sweetness-inhibitory agents from these species are reported in **Table 2**. In addition to antisweet triterpenoids, a 35-amino-acid peptide called gurmarin has been isolated from the leaves of *G. sylvestre* and has also been found to exhibit a sweetness-inhibitory effect.[210,211]

The sweetness-inhibitory activity of plant triterpenoids has been evaluated by placing 5 ml of a 50% or 1 mmol l^{-1} solution of the compound under consideration in the mouth for 2–3 min. On expectorating, the mouth is washed with distilled water. Subsequently, different concentrations of sucrose (0.1–1 mmol l^{-1}) are tasted. The maximum concentration of sucrose at which complete suppression of sweetness is perceived is then recorded for each tastant.[23,25,212] In practice, antisweet compounds of plant origin have been ranked in terms of sweetness-inhibitory potency by comparison with gymnemic acid I (**120**).[23]

Since the initial reports of sweetness-inhibitory oleanane-type gymnemic acids from the leaves of *Gymnema sylvestre*, plant species of the family Asclepiadaceae have served as the sources of several sweetness-inhibitory compounds. The initial isolation and structural characterization of these compounds was very challenging, and these early investigations have been reviewed.[23,25] In 1989, gymnemic acids I–VI (**120–125**) were isolated, with a common gymnemagenin (**191**) oleanane-type aglycone structure and a glucuronic acid moiety.[213–215] Gymnemic acid I (**120**) is the compound with which all other 'antisweet' compounds are compared (**Table 2**). This compound is structurally β-D-glucopyranosiduronic acid, (3β,4α,16β,21β,22α)-28-(acetyloxy)-16,22,23-trihydroxy-21-[(2*S*)-2-methyl-1-oxobutoxy]olean-12-en-3-yl. A different series of antisweet compounds, namely gymnemasaponins III–V (**117–119**), were then isolated.[212] These nonacylated compounds

Table 2 Sweetness inhibitors from plants

Compound name[a]	*Plant name*	*Sweetness-inhibitory potency*[b]	*Reference(s)*
Gymnemasaponin III (**117**)	*Gymnema sylvestre* (Retz.) Schult. (Asclepiadaceae)	0.125	212
Gymnemasaponin IV (**118**)		0.125	212
Gymnemasaponin V (**119**)		0.125	212
Gymnemic acid I (**120**)		1	213
Gymnemic acid II (**121**)		1	213
Gymnemic acid III (**122**)		0.5	213
Gymnemic acid IV (**123**)		0.25	214
		0.5	213
Gymnemic acid V (**124**)		0.5	215
Gymnemic acid VI (**125**)		0.5	215
Gymnemic acid VIII (**126**)		NS[c]	216
Gymnemic acid IX (**127**)		NS[c]	216
Gymnemic acid X (**128**)		0.5	217
Gymnemic acid XI (**129**)		1	217
Gymnemic acid XII (**130**)		1	217
Gymnemic acid XIII (**131**)		0.5	217
Gymnemic acid XIV (**132**)		0.5	217
Gymnemic acid XV (**133**)		1	218
Gymnemic acid XVI (**134**)		1	218
Gymnemic acid XVII (**135**)		1	218
Gymnemic acid XVIII (**136**)		1	218
21β-*O*-Benzoylsitakisogenin-3-*O*-β-D-glucopyranosyl (1→3)-β-D-glucuronopyranoside (**137**)		1	219
Alternoside I (**138**)	*Gymnema alterniflorum* (Lour.) Merr. (Asclepiadaceae)	0.25	222
Alternoside II (**139**)		0.25	222
Alternoside III (**140**)		0.25	222
Alternoside IV (**141**)		0.25	222
Alternoside V (**142**)		0.25	222
Alternoside XI (**143**)		0.25	223
Alternoside XII (**144**)		0.25	223
Alternoside XIII (**145**)	*Gymnema alterniflorum* (Asclepiadaceae)	0.25	223
Alternoside XIV (**146**)		0.25	223
Alternoside XV (**147**)		0.25	223
Alternoside XVI (**148**)		0.25	223
Alternoside XVII (**149**)		0.25	223
Jujuboside B (**150**)	*Hovenia dulcis* Thunb. var. *tomentella* Makino (Rhamnaceae)	0.25	225
Hoduloside I (**151**)		0.25	225
Hoduloside II (**152**)		0.125	225
Hoduloside III (**153**)		0.125	225
Hoduloside IV (**154**)		0.125	225
Hoduloside V (**155**)		0.125	225
Hoduloside VII (**156**)		0.25	226
Hoduloside VIII (**157**)		0.25	226
Hoduloside IX (**158**)		0.25	226
Hoduloside X (**159**)		NS[c]	226
Hovenoside I (**160**)		0.125	225
Saponin C_2 (**161**)		0.125	225
Saponin E (**162**)		0.125	225
Saponin H (**163**)		0.0625	225

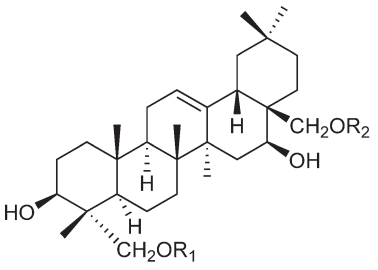
(*Continued*)

Table 2 (Continued)

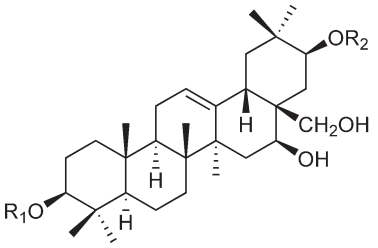
Compound name[a]	*Plant name*	*Sweetness-inhibitory potency*[b]	*Reference(s)*
Sitakisoside I (**164**)	*Stephanotis lutchuensis* Koidz. var. *japonica* (Asclepiadaceae)	0.25	227
Sitakisoside II (**165**)		0.25	227
Sitakisoside III (**166**)		0.25	227
Sitakisoside IV (**167**)		0.25	227
Sitakisoside V (**168**)		0.5	227
Sitakisoside VI (**169**)		0.25	228
Sitakisoside VII (**170**)		0.25	228
Sitakisoside VIII (**171**)		0.25	228
Sitakisoside IX (**172**)		0.25	228
Sitakisoside XI (**173**)	*Stephanotis lutchuensis* Koidz. var. *japonica* (Asclepiadaceae)	0.25	229
Sitakisoside XII (**174**)		0.25	229
Sitakisoside XIII (**175**)		0.25	229
Sitakisoside XVI (**176**)		0.25	229
Sitakisoside XVIII (**177**)		0.25	229
Jujubasaponin II (**178**)	*Ziziphus jujuba* Mill. (Rhamnaceae)	0.5	230
Jujubasaponin III (**179**)		0.5	230
Jujubasaponin IV (**180**)		0.25	230
Jujubasaponin V (**181**)		0.25	230
Jujubasaponin VI (**182**)		0.25	230
Jujuboside B (**150**)		0.25	230
Ziziphin (**183**)		0.5	230, 231
Zizyphus saponin I (**184**)		0.125	230
Zizyphus saponin II (**185**)		0.125	230
Zizyphus saponin III (**186**)		0.25	230
Jegosaponin A (**187**)	*Styrax japonicus* Siebold et Zucc. (Styracaceae)	0.25	232
Jegosaponin B (**188**)		0.25	232
Jegosaponin C (**189**)		0.25	232
Jegosaponin D (**190**)		0.25	232

[a] The structures of the compounds are shown in the text (**117**–**190**).
[b] As compared with gymnemic acid I (**120**) (×1).
[c] NS = sweetness-inhibitory potency not given.

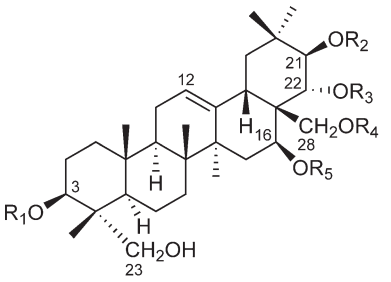
show slightly less potent sweetness-inhibitory activities compared with the previously isolated gymnemic acid I (**120**). Subsequently, the additional sweetness-inhibitory gymnemic acids VIII–XVIII (**126**–**136**) and 21β-*O*-benzoylsitakisogenin-3-*O*-β-D-glucopyranosyl (1→3)-β-D-glucuronopyranoside (**137**) have been isolated from *G. sylvestre*.[216–219] Gymnemic acids XIII (**131**) and XIV (**132**) were previously named gymnemic acids VIII and IX when they were isolated by Yoshikawa *et al.*[217] However, Liu *et al.*[216] independently isolated different compounds designated as gymnemic acids VIII (**126**) and IX (**127**) from the same plant species. Therefore, for clarification purposes, gymnemic acids VIII and IX were renamed as gymnemic acids XIII (**131**) and XIV (**132**), respectively.[218] The antisweet potencies of gymnemic acids XIII (**131**) and XIV (**132**) were rated as about half the potency of gymnemic acid I (**120**). The sweetness-inhibitory potencies of gymnemic acids XV–XVIII (**133**–**136**) and compound **137** were judged to be as about the same as that of gymnemic acid I (**120**).[218,219] There is an extensive literature on *Gymnema sylvestre* exclusive of its sweetness-inhibiting properties, such as its potential antidiabetic and antiobesity effects.[220,221] Preparations containing *G. sylvestre* leaves are sold in health food stores in the United States as a botanical dietary supplement.

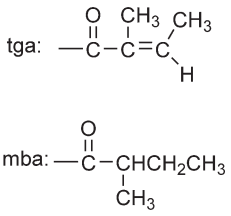


	R_1	R_2
117	β-glc	β-glc^6-β-glc
118	β-glc^6-β-glc	β-glc
119	β-glc^6-β-glc	β-glc^6-β-glc



137 $R_1 = \beta$-glcA^3-β-glc R_2 = Bz



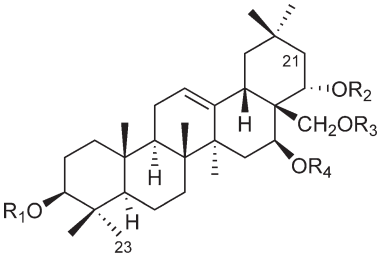


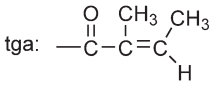
	R_1	R_2	R_3	R_4	R5
120	β-glcA	tga	H	Ac	H
121	β-glcA	mba	H	Ac	H
122	β-glcA	mba	H	H	H
123	β-glcA	tga	H	H	H
124	β-glcA	tga	tga	H	H
125	β-glcA^3-β-glc	tga	H	H	H
126	β-glcA^3-β-OG	mba	H	H	H
127	β-glcA^3-β-OG	tga	H	H	H
128	β-glcA	H	H	Ac	H
129	β-glcA	tga	H	tga	H
130	β-glcA^3-β-glc	tga	H	Ac	H
131	β-glcA	H	H	mba	H
132	β-glcA	H	H	tga	H
133	β-glcA	mba	tga	H	H
134	β-glcA	H	tga	H	tga
135	β-glcA	Bz	H	H	H
136	β-glcA	H	H	Bz	H
191	H	H	H	H	H

Gymnema alterniflorum is an evergreen tree growing in the forests of Taiwan and the southern part of mainland China. The roots of this plant have been used for detoxification purposes and for the treatment of edema and fever.[222] Several oleanane-type triterpenoid glycosides, alternosides I–V and XI–XVII (**138–149**), have been isolated as sweetness inhibitors from the roots of *G. alterniflorum*.[222,223] Complete hydrolysis of alternosides I–V (**138–142**) and XIII–XVII (**145–149**) yielded a known oleanane-type triterpenoid, chichipegenin (**192**).[223,224] There is no functional group at the C-21 and C-23 positions of the alternosides, as commonly present in the gymnemic acids. The antisweet effects of alternosides I–V and XI–XVII (**138–149**) have been evaluated using a 1 mmol l^{-1} solution of each compound, and were found to completely suppress the sensation of sweetness induced by a 0.2 mol l^{-1} sucrose solution in all cases. The sweetness-inhibitory potencies of alternosides I–V and XI–XVII (**138–149**) were rated as about half those of gymnemic acids XIII (**131**) and XIV (**132**).[217]

Subsequent to the isolation of the dammarane-type triterpenoid glycosides jujuboside B (**150**), hodulosides I–V (**151–155**), hovenoside I (**160**), and saponins C2, E, and H (**161–163**) as sweetness inhibitors from the leaves of *H. dulcis* Thunb. var. *tomentella* Makino,[225] hodulosides VII–X (**156–159**) were isolated as sweetness-inhibitory agents.[226] Hodulosides I (**151**) and II (**152**) have hovenolactone (**193**) as their aglycone, the same compound as for saponins E (**162**) and H (**163**). Hodulosides III–V and VII–X (**153–159**) are based on two different dammarane-type aglycone structures, however.[225,226] The sweetness-inhibitory potencies of hodulosides are shown in **Table 2**. The sweetness-inhibitory potency of hoduloside X (**159**) was not determined.[226]

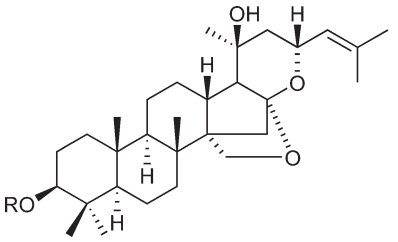
From the stems of *Stephanotis lutchuensis* var. *japonica*, an evergreen woody climber growing in forests near the warm coastal areas of Japan, several oleanane-type sweetness-inhibitory triterpenoid glycosides, namely sitakisosides I–IX, XI–XIII, XVI, and XVIII (**164–177**),[227–229] have been isolated. Some sitakisosides such as *N*-sitakisosides VI (**169**), VII (**170**), XI (**173**), XII (**174**), and XIII (**175**) afforded sitakisogenin (**194**),[228,229] whereas hydrolysis of sitakisosides II (**165**) and XVIII (**177**) yielded marsglobiferin (**195**).[227,229] In turn, hydrolysis of sitakisoside VIII (**171**) afforded 3β,16β,21β,28β-tetrahydroxyoleanan-12-en-22-one (**196**) as the aglycone.[228] Sitakisoside IX (**172**) has a gymnestrogenin-type aglycone structure (**197**).[228] The sweetness-inhibitory potencies of the sitakisosides are about 25% of that of gymnemic acid I, except for the most potent analogue sitakisoside V (**165**; 50% of the activity of gymnemic acid I (**120**)) (**Table 2**).





	R_1	R_2	R_3	R_4
138	β-glcA^3-β-glc	Ac	α-rha	H
139	β-glcA^3-β-glc	H	α-rha	Ac
140	β-glcA^3-β-glc	tga	α-rha	H
141	β-glcA	Ac	α-rha	H
142	β-glcA	H	α-rha	Ac
143	β-glcA^3-β-glc	tga	H	H
144	β-glcA^3-β-glc	H	tga	H
145	β-glcA^3-β-glc	H	H	tga
146	β-glcA^3-β-glc	tga	β-glc	H
147	β-glcA^3-β-glc	tga	β-fuc	H
148	β-glcA^3-β-glc	tga	β-xyl	H
149	β-glcA	tga	α-rha	H
192	H	H	H	H

fuc: D-fucosyl



150 R = α-ara^3-β-glc^2-β-xyl
|^2
α-rha

153 R = α-ara^2-β-qui
|^3
β-glc

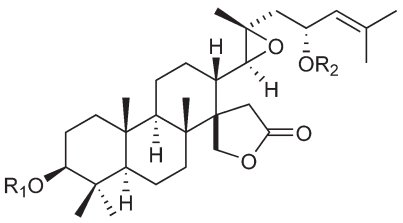
154 R = α-ara^2-β-glc
|^3
β-glc

155 R = β-glc^2-α-rha
|^3
β-glc

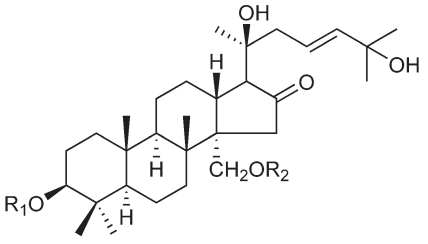
160 R = α-ara^2-β-xyl
|^3
β-glc

161 R = α-ara^2-α-rha
|^3
β-glc

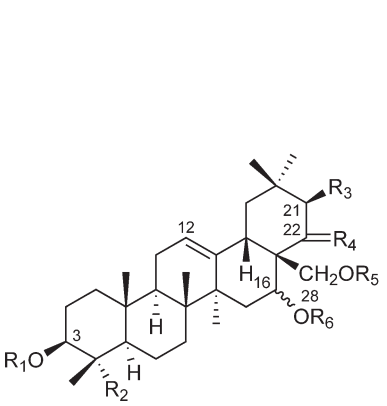
ara: L-arabinopyranosyl

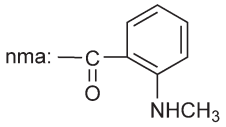


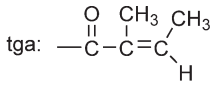
	R_1	R_2
151	β-glc^2-α-rha	β-glc
152	β-glc^2-α-rha |^3 β-glc	H
162	β-glc^2-α-rha	H
163	β-glc	H
193	H	H

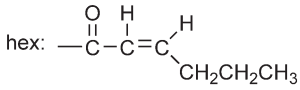


	R_1	R_2
156	α-ara^2-α-rha	β-glc
157	α-ara	β-glc^6-β-xyl
158	α-ara^2-α-rha	β-glc^6-β-xyl
159	α-ara^2-α-rha |^3 β-glc	β-glc



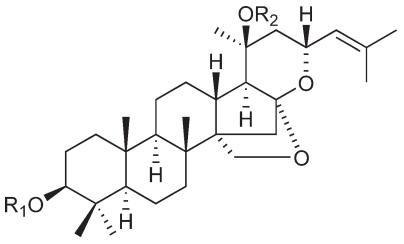






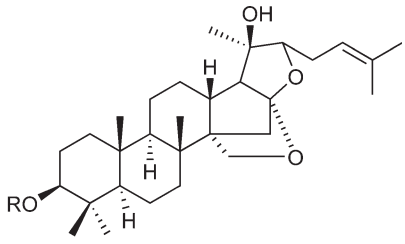
	R_1	R_2	R_3	R_4	R_5	R_6
164	β-glc^6-β-glc^6-β-xyl	CH_3	H	α-*O*-nma, β-H	H	β-OH
165	β-glc^6-β-glc^6-β-xyl	CH_3	*O*-nma	α-OH, β-H	H	β-OH
166	β-glc^6-β-glc^6-β-xyl	CH_3	H	α-OH, β-H	nma	β-OH
167	β-glc^6-β-glc^6-β-glc	CH_3	H	α-*O*-nma, β-H	H	β-OH
168	β-glc^6-β-glc^6-β-xyl	CH_3	H	α-*O*-tga, β-H	H	β-OH
169	β-glc^6-β-glc^6-β-xyl	CH_3	*O*-β-glc^6-nma	H_2	H	β-OH
170	β-glc^6-β-glc^6-β-xyl	CH_3	*O*-β-glc^4-nma	H_2	H	β-OH
171	β-glc^6-β-glc^6-β-xyl	CH_3	*O*-nma	O_2	H	β-OH
172	β-glc^6-β-glc^6-β-xyl	CH_2OH	*O*-β-glc^6-nma	H_2	H	β-OH
173	β-glc^6-β-glc^6-β-xyl	CH_3	*O*-β-glc^4-tga	H_2	H	β-OH
174	β-glc^6-β-glc^6-β-xyl	CH_3	*O*-β-glc^6-tga (4 → glc)	H_2	H	β-OH
175	β-glc^6-β-glc^6-β-xyl	CH_3	*O*-β-glc^3-nma	H_2	H	β-OH
176	β-glc^6-β-glc^6-β-xyl	CH_3	H	α-OH, β-H	tga	β-OH
177	β-glc^6-β-glc^6-β-xyl	CH_3	OH	α-OH, β-H	nma	β-OH
187	β-glcA^3-β-gal^2-α-rha (2 → β-glc)	CH_3	*O*-tga	α-OAc, β-H	H	α-OH
188	β-glcA^3-β-gal^2-α-rha (2 → β-glc)	CH_3	*O*-tga	α-OH, β-H	Ac	α-OH
189	β-glcA^3-β-gal^2-α-rha (2 → β-glc)	CH_3	OH	α-tga, β-H	Ac	α-OH
190	β-glcA^3-β-gal^2-α-rha (2 → β-glc)	CH_3	*O*-hex	α-OH, β-H	Ac	α-OH
194	H	CH_3	OH	H_2	H	β-OH
195	H	CH_3	OH	α-OH, β-H	H	β-OH
196	H	CH_3	OH	O_2	H	β-OH
197	H	CH_2OH	OH	H_2	H	β-OH
198	H	CH_3	OH	α-OH, β-H	H	α-OH

In the late 1980s, ziziphin (**183**) was isolated from the Chinese jujube tree *Ziziphus jujuba* P. Miller as the first recognized antisweet principle of this plant.[23,25] Ziziphin (**183**) has the same dammarane-type aglycone structure as hodulosides III–V (**153–155**). Yoshikawa *et al.*[230] isolated nine additional antisweet compounds, namely jujubasaponins II–VI (**178–182**), ziziphin (**183**), and zizyphus saponins I–III (**184–186**), from the leaves of *Ziziphus jujuba* (**Table 2**). Among them, three acylated compounds, ziziphin (**183**) and jujubasaponins II (**178**) and III (**179**), showed the most potent antisweet activity, equivalent to 50% of that of gymnemic acid I (**120**)[231] (**Table 2**).



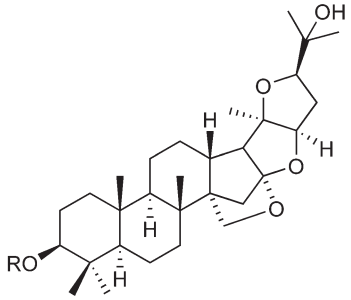
	R_1	R_2
178	α-ara^2-α-rha	α-rha^2-Ac
179	α-ara^2-α-rha	α-rha^3-Ac
183	α-ara^4-α-rha	α-rha^2-Ac $\vert^3$ Ac
184	α-ara^3-β-glc $\vert^2$ 6-deoxy-α-tal	H
185	α-ara^3-β-glc $\vert^2$ α-rha	H
186	α-ara^3-β-glc^2-β-xyl $\vert^2$ α-rha	H

tal: D-talosyl



180 R = β-gal^2-α-rha
$\vert^3$
β-glc

181 R = β-glc^2-α-rha
$\vert^3$
β-glc



182 R = β-gal^2-α-rha

Styrax japonicus Siebold et Zucc. (Styracaeae) is a deciduous tree distributed in Japan, Korea, and mainland China. Recently, jegosaponins A–D (**187–190**), four new oleanane-type saponins, were isolated from the fresh fruits of this tree as sweetness inhibitors.[232] The structures of jegosaponins A–D (**187–190**) are based on the aglycone barringtogenol C (**198**) and they all have the same tetraglycoside chain at C-3, with different acylated groups at C-21, C-22, and C-28. The antisweet activities of jegosaponins A–D (**187–190**) are about half those of gymnemic acids III (**122**), IV (**123**), and VI (**124**).[232]

3.10.7 Sensory Evaluation of Natural Products for Sweetness and Sweetness-Modifying Properties

Sensory evaluation using the human tongue as a detector is a crucial step in the discovery of natural sweeteners and sweetness modifiers. The human tasting stage can be divided into raw material screening, sensory-guided fractionation, and sensory evaluation of purified natural sweeteners. After a careful safety evaluation (see Section 3.10.3), tasting can be carried out on the samples of candidate sweet-tasting plants extracted with MeOH or MeOH–water, sometimes at an elevated temperature. Then, additional dried extracts prepared by partitioning the initial MeOH or MeOH–water extract with solvents of various polarities and thoroughly removing the residual solvent in each case may also be tasted for the presence or absence of sweetness. For relatively clean samples, that is, certain fruit extracts, the above-mentioned solvent partition steps may be omitted, thus avoiding the tedious solvent removal steps prior to human tasting.

Pure natural product compounds need to be subjected to a rigorous safety evaluation as a prerequisite to human tasting. Thus, toxicological evaluation may include acute toxicity evaluation in mice and bacterial mutagenicity testing.[74–76,84] Once approved for human tasting, pure samples are typically dissolved in water for preliminary evaluation. For some samples with poor solubility in water, samples may be solubilized with the aid

of ethanol and then diluted with distilled water before tasting. Caution should be taken to keep the quantity of ethanol to a minimum as this solvent has an inherent sweetness that may interfere with sensory evaluation. Samples that are completely devoid of sweetness, that are strongly bitter, or that have a strong off-taste will be eliminated at this stage.

Samples of further interest are evaluated as to their relative sweetness, taste profile, and temporal profile when compared to a sucrose standard. The relative sweetness is utilized to indicate the potency of the natural sweetener concerned. Many natural sweeteners are high-potency sweeteners that are at least 50–100 times sweeter than sucrose. The sweetening power of highly potent sweeteners varies due to many factors and decreases relative to that of sucrose as concentration increases.[233] Relative sweetness can be best determined using a ranking test.[234] The taste panelists involved should be prescreened for their sensitivity and trained to respond to other common tastes (bitter, sour, salty, umami, etc.). The panel size should be at least eight. The sample concentration needs to be adjusted so that the perceived sweetness would be in the proper range within that of the sucrose references. A prescreened sample is presented randomly to the panel together with a series of sucrose standards in coded cups. The panel is instructed to taste each sample and then rinse the mouth thoroughly with water. All tasting should be carried out at ambient temperature. The panel is asked to rank the samples from low to high with respect to perceived sweetness. The relative sweetness of the sample can then be determined after statistical analysis of the sensory data. In lieu of a formal sensory evaluation, relative sweetness can be estimated by bench tasting using paired comparison with a smaller panel.[117,126]

The relative sweetness of natural sweeteners may be evaluated against different concentrations of sucrose. It is not uncommon to determine the relative sweetness of natural sweeteners at or near the sucrose threshold; generally, this is around 0.5% w/v. The natural sweetener (2*R*,3*R*)-dihydroquercetin 3-*O*-acetate (**91**) isolated from the Paraguayan plant *T. dodoneifolia* was rated as being 80 times sweeter than a 2% w/v sucrose solution (**Table 1**).[159] The semisynthetic, intensely sweet NHDC (**12**) has been thoroughly studied by several groups.[235,236] At or near threshold, compound **12** was determined to be 1800 times sweeter than sucrose. At 1 and 5% sucrose levels, the sweetness potency of **12** was rated as 600 and 250 times sweeter than sucrose, respectively, indicating that the perceived sweetness intensity of the compound decreases as concentration increases.[237] Another example is telosmoside A_{15} (**79**), a natural pregnane-type sweetener isolated from the Vietnamese plant *Telosma procumbens* (**Table 1**).[149] This molecule was dissolved in 7% ethanol solution and tasted at different concentrations against a series of sucrose references ranging from 3.2 to 9.6% (w/v). Telosmoside A_{15} (**79**) at a concentration of 0.008% was iso-sweet to 8% sucrose and thus determined to be 1000 times sweeter than 8% sucrose. As indicated above, the taste and temporal profiles are also important factors associated with natural sweeteners. Compared to sucrose, which exhibits a characteristic time–intensity profile, many of the natural high-intensity sweeteners show a slow onset, a lingering aftertaste, bitterness, or a metallic off-taste. These characteristics can be indicated during sensory evaluation by an experienced panel.

There are increasing health concerns about the high intake of calorie-rich sugar-sweetened food, which can contribute to obesity, diabetes, and other chronic diseases, in addition to dental caries.[2,238] Accordingly, it has been a long-time goal of the food and beverage industry to reduce the sucrose content in their products without sacrificing food palatability. Sugar replacement to reduce the caloric consumption can be achieved via the addition of the highly potent artificial or natural sweeteners. One characteristic often associated with high-potent sweeteners is their synergy when combined with other sweeteners.[239] Synergy refers to the total sweetness intensity of a mixture when greater than the theoretical sum of the intensities of the individual components. However, many artificial and natural sweeteners have off-tastes and different taste profiles from that of sucrose.

Another alternative is to utilize sweetness enhancers to enhance the perception of the sweet taste, and thus be able to reduce the quantity of sugar content in food products. The ideal sweetness enhancer would have no intrinsic taste and aroma but would increase the sweetness of sucrose without imparting any negative effect on other flavor profiles.[240] However, most (if not all) of the sweetness enhancers reported so far have some intrinsic sweetness, for example, hesperetin (**113**)[194] and the 4-hydroxydihydrochalcones.[241] Therefore, it is important to distinguish if the enhancement of sweetness is from true synergy or merely the additive effect from the intrinsic sweetness of the ingredients. The preliminary screening of sweetness-enhancing activity for botanical extracts, chromatographic fractions, or isolated compounds can be carried out by a small, sweetness-sensitive taste panel. Samples are added to an aqueous sugar solution, for example at 2% (w/v), and then administered to

the panel along with a positive control (2% sugar) in coded beakers. The panel members are then asked to compare their sweetness. If the samples are evidently sweeter than the control, further purification and sensory evaluation are warranted. As there is the possibility that the samples of interest may have intrinsic sweetness, the formal sensory evaluation procedure needs to determine if the elevation of the sweetness is due to an additive effect or true synergy. Evidence has shown that there is a positive correlation between the sweetness-enhancing effect and the intrinsic sweetness of the test samples. However, the sample size may be too small for a definite conclusion to be made.[195]

The relative sweetness of pure samples can be determined using the ranking method discussed above. The test sample at a certain concentration (say, 100 ppm) in water is ranked versus a series of sucrose (say, 0.5, 1.0, 1.5, 2.0% w/v) references. The concentration range of the references chosen depends on the sweetness of the test samples. The sweetness-enhancing evaluation can be carried out in a 5% sucrose solution because the change in sweetness can be most easily detected at this concentration.[195] The sample sweetness in 5% sugar solution can be determined using a ranking test or a paired comparison versus 5, 6, 7, and 8% sucrose reference solutions. The difference between the actual measured sweetness of the test sample in a 5% sugar solution and the calculated sweetness of a pure 5% sucrose solution plus the measured sweetness of the sample (at 100 ppm) will reveal if the elevation of the sweetness is from additive effects or a true synergy.

The time- and material-consuming process of sensory evaluation is limited to those samples cleared for human tasting, and sometimes this is precluded by the demonstration or presumption of toxicity for a given sample under consideration. In the past few years, considerable progress has been made in research on human/mammalian taste receptors.[87,88,242,243] The sweet receptor is a G-protein-coupled receptor (GPCR) and is composed of two proteins, T1R2 and T1R3, expressed on the surface of taste bud cells.[244,245] Sweet receptor-based assay systems have been used in high-throughput screening of molecules for sweeteners and sweetness enhancers or modifiers.[88] Receptor-based assay systems have many potential advantages over the classical human tasting method owing to their speed, sensitivity, and selectivity, and thus can aid in the discovery of novel natural sweeteners and sweetness modifiers. However, human taste perception is a very complex process and sensory evaluation can give an overall characterization of the sweeteners owing to its holistic approaches. The combination of an *in vitro* assay with human panel sensory evaluation would be ideal for the discovery of novel natural sweeteners and sweetness enhancers.

3.10.8 Interactions of Natural Products at the Sweet Receptor

Before the recent discovery of the mammalian/human sweet receptor, proposals for the structure–activity relationships (SAR) of classes of sweeteners were based on the analysis of their structures and the activities of various derivatives. Many synthetic analogues of natural sweeteners have been made to study how structural variation influences their sweetness activities. Such approaches led to the identification of essential structural features (glucophores) necessary for the sweetness and potency of these molecules. Through indirect mapping, several models of the hypothetical ligand binding sites for the sweet receptor have been developed.[246] The consensus feature of these models is the presence of AH–B groups, in which the AH group is a hydrogen donor and the B group is an electronegative center.[247] According to this theory, all sweet-tasting compounds contain a hydrogen bond donor (AH) and a hydrogen bond acceptor (B), separated by a distance of 2.5–4.0 Å, that react with a complementary AH–B pair on the receptor. For example, plant-derived sweeteners such as phyllodulcin (**3**) and NHDC (**12**) owe their sweetness to the presence of the so-called isovanillyl glucophoric (3-hydroxy-4-methoxyphenyl) group. The adjacent hydrogen donor (–OH) and hydrogen acceptor (OCH_3) of the isovanillyl group satisfy the requirements of the AH–B theory. For instance, the sweet principle (2*R*,3*R*)-dihydroquercetin 3-*O*-acetate (**91**), from the young leaves of *T. dodoneifolia*, was rated as 80 times sweeter than sucrose while the sweetness of this compound was increased fivefold by methylation at the C-4′ hydroxyl to form a synthetic isovanillyl derivative (**92**).[159] Interestingly, (2*R*,3*R*)-dihydroquercetin (taxifolin) itself is not sweet but bitter.[248] These hypothetical models became generally accepted for many of the small-molecule synthetic and natural product sweeteners, but not for all of them, indicating that these sweet molecules may have different binding sites on the receptor. Additionally, such models have been unable to explain the sweetness of sweet proteins. It has been postulated that there may be more than one type of sweet receptor.[249]

At the present time, it is clear that the detection of sweet taste is mediated by a heterodimeric receptor comprised of T1R2 and T1R3 proteins.[243,244] The sweet receptor belongs to class C type of GPCRs, which also include several metabotropic glutamate receptors, the umami receptor, and the bitterness receptor. These receptors are characterized by a large clam shell-shaped extracellular N-terminal domain linked to a hydrophobic domain with the seven-transmembrane topology common to all GPCRs. This N-terminal domain is responsible for ligand binding and has a characteristic structure known as the 'Venus flytrap' module. These membrane-bound proteins are difficult to crystallize; hence, a 3D structure has not been solved so far for the sweet taste receptor, making it difficult to use structure-based methods to study the SAR and design new sweeteners. The sweet taste receptor is similar to the dimeric metabotropic glutamate receptor mGluR1 and the crystal structures of the extracellular ligand-binding region of mGluR1 have been determined.[250] Several 3D homology models of sweet receptor have been built using the known structure of the N-terminal domain of mGluR1 as a template.[245,251,252] With the new knowledge gained from molecular biology and homology modeling studies, it is evident that the human sweet receptor has multiple active sites.[245,249,253,254] The artificial sweeteners aspartame and neotame were found to interact at the N-terminal domain of human T1R2 whereas the binding site of cyclamate was localized to the human T1R3 transmembrane domain.[254,255] The well-known sweetness blocker lactisole was found to interact with the transmembrane domain of human T1R3 to inhibit the sweet taste.[254,256]

Sweet proteins may act via a mechanism different from that of low-molecular-weight sweeteners. Chimera studies have indicated that the sweet protein brazzein (**105**) interacts with the cysteine-rich domain of human T1R3.[257] A wedge model for sweet protein binding to the receptor was proposed based on extensive modeling of the human sweet receptor and docking studies of both sweet proteins and small sweet molecules.[245] The above findings also shed some light on the synergy effect between different sweeteners. If two sweeteners act via the same mechanism, then they will compete for the same binding site and behave in an additive way. It has long been known that aspartame and cyclamate are synergistic in sensory experiments.[258] Recent findings have revealed that these two sweeteners have separate orthostatic binding sites[254] and a cooperative binding effect may well explain their synergy.[259]

With the discovery of the sweet receptor, our understanding toward the SAR of sweet molecules increases significantly. Homology modeling, molecular docking studies, and molecular biology have yielded useful information regarding the binding sites of the sweet receptor. These results may be used as a guide to design new and better sweeteners. Despite these advances, there are still many unanswered questions regarding the details of the binding activities. Some of these questions may have to wait until a 3D structure is finally established for the sweet receptor.

3.10.9 Conclusions

In this chapter, information has been provided concerning the botanical source, structure, and sweetness potencies relative to sucrose of more than 100 highly sweet natural products. Also mentioned are seven known sweetness enhancers from organisms, and over 80 antisweet plant constituents. These substances are chemically quite diverse and represent the terpenoid, flavonoid, and protein classes of compounds, in particular. A number of sweet compounds described have present use or future commercial potential as sucrose substances, and these are expected to increase in the near future to meet a public demand for ingredients of natural origin in foods and beverages in western countries. The approval of natural sweet substances varies from country to country, and of paramount concern in the approval process is the need for demonstrated safety. Not all of the commercially used sweeteners are innocuous in terms of their potential toxicity. For example, glycyrrhizin (**1**) has an adrenocorticomimetic effect and may lead to abnormal fluid retention (hypokalemia) and hypertension when ingested in licorice-flavored confectionary or when used in drug formulations.[26,260,261] Therefore, it is necessary for an upper limit to be placed on the amount of glycyrrhizin (**1**) ingested daily.[28] Because almost all natural sweeteners of plant origin have hedonic limitations in their quality of taste, many efforts have been made to produce more pleasant-tasting modified analogues either synthetically or enzymatically, and several key references in this regard have been cited in the present chapter.

Although ideally low-calorie sweeteners should have no significant biological activities other than a sweet effect, the recent work by Konoshima[262] on the potential cancer chemopreventive activity of these compounds is worthy of mention. Cancer chemoprevention has been described as "a strategy of cancer control by administration of synthetic compounds to reverse or suppress the process of carcinogenesis".[263] In a model of the inhibition of Epstein–Barr virus early antigen (EBV-EA) induction, both stevioside (**5**) and mogroside V (**2**) were shown to exhibit potent activity in this assay and were more active than several other natural sweeteners. Furthermore, stevioside and mogroside V showed significant anticarcinogenic effects in a follow-up *in vivo* model of two-stage carcinogenesis in mice.[262]

The search for highly sweet substances has proven to be fascinating, and scientific reports of new substances of this type have attracted wide attention. While several groups in Japan and the United States, in particular, reported frequently on the isolation and structural characterization of new sweet principles from green plants in the last quarter of the twentieth century, such reports have recently declined in frequency. The principal reason for this seems to be the fact that many if not all of the more obvious candidate sweet plant leads have already been discovered. Indeed, it is unlikely that another organism will be found with, for example, the profound sweet taste exhibited by the leaves of the plant *S. rebaudiana*. However, it is entirely possible that additional sweet-tasting or sweetness-inducing plants are used by local populations for sweetening purposes, and are as yet undiscovered, in more remote geographical locations. The search for new sweet-tasting compounds from plants by fieldwork has become more complex than previously, as a result of the passage of the United Nations Convention on Biological Diversity in Rio de Janeiro in 1992, so it is now necessary to obtain 'prior informed consent' and to develop benefit-sharing agreements with the source country before accessing indigenous traditional knowledge and accessing plant material. Therefore, this approach now requires a great deal of preplanning and may have an uncertain outcome. Sweetener discovery from natural sources may best be done with a multidisciplinary team consisting of taxonomists, natural products chemists, and biologists.[21,74,75] The prospects of a greatly increased knowledge on the occurrence of sweet-tasting and sweetness-modifying natural products, not only from plants, but also from other terrestrial and marine organisms, may be expected in the future. This is due to the recent availability of receptor-binding assays, which can be applied to libraries of pure natural products and then be followed by sensory testing using human taste panels, as discussed in Section 3.10.7.

A question that often arises is why do plants produce low-calorie sweet-tasting compounds at all? There is no generally agreed upon answer to this question. However, it has been postulated that secondary metabolites of plants and other organisms accumulate under the pressure of natural selection to bind to specific receptors and thus help in the survival of the producing organism.[264] Therefore, one might suppose that bitter-tasting compounds would be preferred for organism survival rather than sweet-tasting compounds, in order to ward off predators, by being less palatable when chewed. If the organoleptic results obtained by Soejarto *et al.*[79] on the taste properties of the leaves of more than 100 *Stevia* species are typical, then this group of plants was found to be overwhelmingly bitter tasting, with only a few specimens somewhat sweetish, including a sample of *S. rebaudiana*. The bitterness of the vast majority of the *Stevia* species represented would be expected to be due to constituents such as sesquiterpene lactones[265] and *ent*-atisane diterpenoids[266] that are known to be biosynthesized in this genus. Accordingly, the production of such high concentration levels of sweet-tasting steviol glycosides in just one species (*S. rebaudiana*) of the group evaluated in this manner seems to be genetically illogical. However, given that two glycosidic constituents of this plant (rebaudioside A (**4**) and stevioside (**5**)) have wide use as noncaloric sucrose substitutes, this is very much to the benefit of humankind.

Abbreviations

ADI	acceptable daily intake
CGTase	cyclomaltodextringlucanotransferase
EBV-EA	Epstein–Barr virus early antigen
GC–MS	gas chromatography–mass spectrometry
GPCR	G-protein-coupled receptor
GRAS	generally recognized as safe
JECFA	Joint Expert Committee on Food Additives
MGGR	glycyrrhetic acid monoglucuronide

NHDC	neohesperidin dihydrochalcone
SAR	structure–activity relationships
SPE	solid-phase extraction

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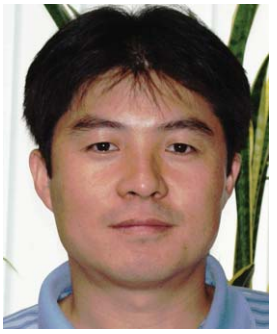

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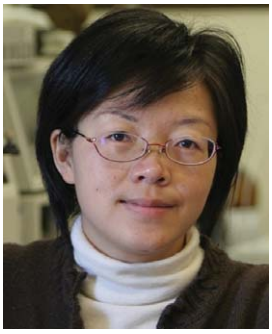

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