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Highly Sweet Compounds of Plant Origin†

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The demand for new alternative "low calorie" sweeteners for dietetic and diabetic purposes has increased worldwide. Although the currently developed and commercially used highly sweet sucrose substitutes are mostly synthetic compounds, the search for such compounds from natural sources is continuing. As of mid-2002, over 100 plant-derived sweet compounds of 20 major structural types had been reported, and were isolated from more than 25 different families of green plants. Several of these highly sweet natural products are marketed as sweeteners or flavoring agents in some countries as pure compounds, compound mixtures, or refined extracts. These highly sweet natural substances are reviewed herein.

Key words: Low-Calorie Natural Sweeteners, Plants, Glycyrrhizin, Mogroside V, Rebaudioside A, Stevioside, Thaumatin, Terpenoids, Steroids, Flavonoids, Proteins

INTRODUCTION

The consumption of sucrose as a sweetener has been associated with several nutritional and medical problems, with dental caries being the most widely described (Grenby, 1991). Therefore, there has been a great demand for new highly sweet, non-caloric and non-cariogenic sucrose substitutes for the diabetic and dietetic market. Synthetic or naturally occurring sucrose substitutes are required to exhibit a sucrose-like taste quality with properties such as demonstrated non-toxicity, non-cariogenicity, lack of any offensive odor, and should exhibit satisfactory water solubility and hydrolytic and thermal stability. The so-called "high potency" or "low calorie" sweeteners are at least 50-100 times more highly sweet than sucrose (DuBois, 1982). Such compounds are also referred to as "intense sweeteners" and may be placed in a separate sweetener category than the less sweet "bulk" or "reduced calorie" sweeteners represented by certain monosaccharides, disaccharides, and polyols, which are approximately equal to sucrose in their sweetness potency (Duffy and Anderson, 1998; O'Brien Nabors, 2001).

Most of the currently available potently sweet, low calorie sucrose substitutes in the world market are synthetic compounds, inclusive of acesulfame-K, alitame, aspartame, cyclamate, saccharin, and sucralose (Duffy and Anderson, 1998). These synthetic sweeteners are used as sucrose substitutes in most western countries but the regulations for each sweetener vary from country to country (Auerbach *et al.*, 2001; Bopp and Price, 2001; Butchko *et al.*, 2001; Goldsmith and Merkel, 2001; Pearson, 2001; von Rymon Lipinski and Hanger, 2001). At present, in the United States, five synthetic sweeteners are now permitted, namely, acesulfame-K, aspartame, neotame, saccharin, and sucralose (Duffy and Anderson, 1998; Anonymous, 2002). Neotame, approved only in 2002, is an *N*-alkylated aspartame derivative, and has a sweetness potency of 10,000 times that of sucrose (Walters *et al.*, 2000; Stargel *et al.*, 2001; Anonymous, 2002).

In the United States, the artificial sweeteners are estimated to account for an approximately $720 million market by 2003 (Seewald, 2000). However, problems have been perceived with some of these compounds in terms of their safety, stability, cost, and/or quality of taste. For example, the general-purpose sweetener aspartame may not be consumed by persons with phenylketonuria because of the formation of a major metabolite, phenylalanine (Butchko *et al.*, 2001). Saccharin has been used as a sweetener for many years, but is now permitted only on an interim basis, owing to an association with bladder cancer in laboratory

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† Dedicated to Professor Kazuo Yamasaki, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan, on the occasion of his retirement.

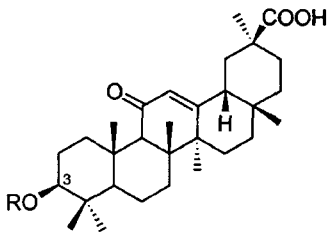
animals in work conducted some 30 years ago (Pearson, 2001). While containers of products that include saccharin must have a cancer warning and state the amount of this sweetener (Duffy and Anderson, 1998), evidence that saccharin is a carcinogen looks increasingly equivocal as time passes (O'Brien Nabors, 2001). Cyclamate is still used as a sucrose substitute in about 50 countries inclusive of those in the European Union, although not the United States, where it has not been used for over 30 years. A major metabolite of cyclamate is cyclohexylamine, which is somewhat toxic in causing testicular atrophy and untoward cardiovascular effects at high doses (Bopp and Price, 2001).

Besides the synthetic compounds mentioned above, there are a number of highly sweet plant-derived compounds known, which are mostly terpenoids, flavonoids, and proteins (Kurihara, 1992; Kinghorn *et al.*, 1995; Tanaka, 1997; Kitagawa, 2002). Several of these sweet substances are used commercially as sucrose substitutes (low-calorie sweeteners), as will be described in the next section. In addition, a number of plant substituents are known to mediate the sweet-taste response, either by inducing or inhibiting the perception of sweetness (Suttisri *et al.*, 1995). Thus far, all of the known natural product sweet-tasting substances and sweetness modifiers have been obtained from green plants, as opposed to lower plants, microbial, or marine sources (Kurihara, 1992; Kinghorn *et al.*, 1995; Suttisri *et al.*, 1995). In the sections of this review presented below, plant-derived sweet compounds with commercial use will be described, followed by individual descriptions of potent sweeteners in the categories terpenoids and steroids, phenylpropanoids, dihydroisocoumarins, flavonoids, proanthocyanidins, benzo[*b*]indeno[1,2-*d*]pyrans, amino acids, and proteins. The literature has been surveyed for this article until the middle of 2002. The plant-derived sweetness modifiers will not be considered further in this review.

Commercially used highly sweet natural products

While many isolated natural compounds from plants have a sweet taste (Kinghorn and Soejarto, 1986, 1989; Kinghorn *et al.*, 1995; Kinghorn *et al.*, 1999), only a few of these have been developed commercially. Natural product highly sweet compounds and compound mixtures with some commercial use include glycyrrhizin (**1**), mogroside V (**2**), phyllodulcin (**3**), rebaudioside A (**4**), stevioside (**5**), "sugar-transferred" stevia extract, and thaumatin, which are used as sucrose substitutes in one or more countries (Kinghorn and Kennelly, 1995; Kinghorn, 2002). Some of these compounds have been modified chemically or biochemically to produce analogs that are more desirable as sweeteners, in being more highly sweet and/or more pleasant tasting. Although a number of commercially available "bulk" or "reduced calorie" sweeteners are naturally occurring, which exhibit approximately the same sweetness potency as sucrose, these compounds will not be considered in any detail in this chapter. Examples include the monosaccharide, fructose; the monosaccharide polyols, erythritol, mannitol, sorbitol, and xylitol; and the disaccharide polyols, lactitol and maltitol (Duffy and Anderson, 1998). Several reduced calorie sweeteners have been covered in depth in a recent volume on sweeteners (O'Brien Nabors, 2001).

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 in the genus *Glycyrrhiza* (Fenwick *et al.*, 1990; Kinghorn and Compadre, 2001; Dalton, 2002; Kitagawa, 2002). Glycyrrhizin (**1**) has been reported to be 93-170 times sweeter than sucrose, depending on concentration. In Japan, root extracts of *G. glabra* (which contain >90% w/w pure glycyrrhizin) are used to sweeten foods and other products, such as cosmetics and medicines (Kitagawa, 2002). The ammonium salt of glycyrrhizin has Generally Recognized As Safe (GRAS) status in the United States and is used primarily as a flavor enhancer (Kinghorn and Compadre, 2001). There have been several attempts using various glycosylation methods to increase the sweetness potency of glycyrrhizin (**1**). The group of the late Professor Osama Tanaka at Hiroshima University in Japan glycosylated glycyrrhetinic acid to afford various glycyrrhetic acid monoglycoside analogs employing a chemical and enzymatic glycosylation procedure (Mizutani *et al.*, 1994). A coupling reaction using mercury(II) cyanide [$Hg(CN)_2$] for chemical glycosylation was effected, resulting in a significant enhancement of sweetness in the analogs obtained, especially the 3-*O*-β-D-xylopyranoside (**6**) and the 3-*O*-β-D-glucuronide (MGGR, **7**). The sweetness intensities of compounds **6** and **7** were rated as 544 and 941 times sweeter than sucrose, respectively. Such chemically modified products of glycyrrhizin also showed improved taste qualities (Tanaka, 1997). MGGR (**7**), in being more than five times sweeter than glycyrrhizin (**1**), as well as being readily soluble in water, is now used commer-



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

cially as a sweetening agent in Japan (Mizutani *et al.*, 1998).

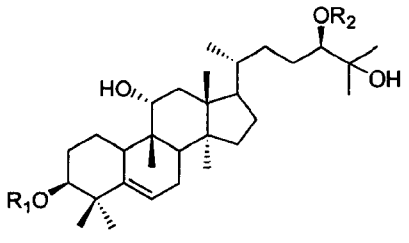
Mogroside V (**2**) is a cucurbitane-type triterpenoid glycoside isolated from the fruits of *Siraitia grosvenorii* (Swingle) C. Jeffrey (Cucurbitaceae) (Takemoto *et al.*, 1983a). An extract of the dried fruits of *S. grosvenorii*, containing mogroside V (**2**) as the major sweet principle, is used in Japan as a sweetener in certain foods and beverages. The sweetness intensity of mogroside V (**2**) has been rated as 250-425 times sweeter than sucrose, depending on concentration (Kinghorn and Compadre, 2001). Recently, a major corporation in the United States has filed a patent concerning the use of extracts of *S. grosvenorii* and other *Siraitia* species as sweet juices (Fischer *et al.*, 1994).

A dihydroisocoumarin-type sweetener, phyllodulcin (**3**) occurs in glycosidic form in the leaves of *Hydrangea macrophylla* Seringe var. *thunbergii* (Siebold) Makino (Saxifragaceae) ("Amacha") and other species in this genus. After the fermentation of the leaves or by crushing, the native glycosides are enzymatically hydrolyzed, and the sweet phyllodulcin (**3**, $\times$400 sweeter than 2% sucrose) is produced. The fermented leaves of *H. macrophylla* are used to prepare a sweet ceremonial tea in Japan, especially at "Hamatsuri", a Buddhist religious festival (Kinghorn and Compadre, 2001).

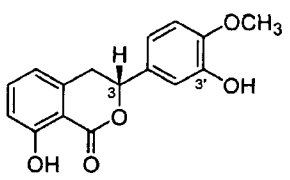
Rebaudioside A (**4**) and stevioside (**5**) are *ent*-kaurene-type diterpene glycosides based on the aglycone steviol isolated from the leaves of the Paraguayan plant, *Stevia rebaudiana* (Bertoni) Bertoni (Compositae) (Kohda *et al.*, 1976 Tanaka, 1997; Kinghorn *et al.*, 2001), with stevioside (**5**) being the most abundant sweet compound in this plant part. The sweetness intensity of stevioside (**5**) has been rated as 210 times sweeter than sucrose, although this value varies with concentration. However, rebaudioside A (**4**) (the second most abundant *S. rebaudiana ent*-kaurene 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 herbal dietary supplements elsewhere, in particular the United States (Kinghorn *et al.*, 2001).

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 (Kamiya *et al.*, 1979; DuBois *et al.*, 1984; Esaki *et al.*, 1984; Mizutani *et al.*, 1989; Ishikawa *et al.*, 1990; Tanaka, 1997). Several systematic studies on the structure-sweetness relationship of steviol glycosides have been conducted (Fukunaga *et al.*, 1989; Mizutani *et al.*, 1989; Ohtani and Yamasaki, 2002). For example, the sweetness-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 (**8**, Sgal-2) (Mizutani *et al.*, 1989). A rebaudioside A analog (**9**) with a (sodiosulfo)propyl group at C-19 in place of a β-glucosyl moiety showed improved sweetness qualities (DuBois *et al.*, 1984). Stevioside (**5**) has been converted synthetically to rebaudioside A (**4**), by removing a glucose unit from stevioside (**5**) at the C-13 position using amylase and then reintroducing synthetically two glucose units of different linkage to the remaining glucose unit at the C-13 position (Kaneda *et al.*, 1977).

In Japan, the largest market for the *S. rebaudiana*



	R_1	R_2
2	β-glc^6-β-glc	β-glc^2-β-glc |^6 β-glc



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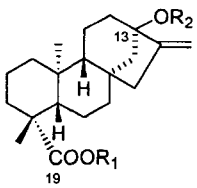

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	R_1	R_2
4	β-glc	β-glc^2-β-glc |^3 β-glc
5	β-glc	β-glc^2-β-glc
8	β-gal	β-glc^2-β-glc^2-β-glc^4-α-glc
9	$(CH_2)_3SO_3Na$	β-glc^2-β-glc |^3 β-glc

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" (Mizutani and Tanaka, 2002). "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 (5-10%), and stevioside (50-55%) (Shibasato, 1995; Mizutani and Tanaka, 2002). "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 (Tanaka, 1997; Mizutani and Tanaka, 2002; Ohtani and Yamasaki, 2002). "Rebaudioside A-enriched extract" is made from improved varieties of *S. rebaudiana*, which produce more rebaudioside A (**4**) than the native Paraguayan species (Shibasato, 1995). Products incorporating *Stevia rebaudiana* sweeteners are used in over 100 different food applications in Japan, in particular for salted foods such as Japanese-style pickles and dried seafoods, but also beverages, yoghurt, ice cream, and sherbet (Mizutani and Tanaka, 2002). In Korea, 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 (Kim, J. *et al.*, 2002).

Thaumatin is a protein sweetener isolated from the fruits of *Thaumatococcus daniellii* (Bennett) Benth. (Marantaceae) (Van der Wel and Loeve, 1972). Five different thaumatin analogs are now known (thaumatins I, II, III, a, and b), and thaumatins I and II are the major forms with both having 207 amino acid residues (Kurihara, 1992). The molecular weights of thaumatins I and II are 22,209 daltons and 22,293 daltons, respectively (Gibbs *et al.*, 1996). The three-dimensional structure of thaumatin I, based on X-ray analysis has been reported (Ogata *et al.*, 1992; Ko *et al.*, 1994). The sweetness of thaumatin I is rated between 1,600 and 3,000 times in comparison to sucrose on a weight basis. Talin® protein, the trade name of the commercial form of thaumatin protein as an aluminum ion adduct, is approved as a sweetener in Australia, the European Union, as well as some other countries, and was first permitted for use as a food additive in Japan in 1979. Talin® protein has GRAS status as a flavor enhancer for use in chewing gum in the United States (Kinghorn and Compadre, 2001).

Perillartine is a natural product derived semisynthetic compound utilized on a limited basis in Japan (Kinghorn and Compadre, 2001). Perillartine is an α-*syn*-oxime and synthesized from perillaldehyde, a monoterpenoid constituent of the volatile oil of *Perilla frutescens* (L.) Britton (Labiatae), and used as a replacement for maple syrup or licorice for the sweetening of tobacco. The compound has a limited solubility in water and an appreciably bitter taste (Kinghorn and Soejarto, 1986; Kinghorn and Compadre, 2001). Neohesperidin dihydrochalcone is another semisynthetic compound, which is a dihydrochalcone glycoside prepared from a flavanone constituent of *Citrus aurantium* L. (Rutaceae), which is permitted for use as a sweetener in a wide range of foodstuffs in countries of the European Union, as well as the Czech Republic, Turkey, and Switzerland (Borrego and Montijano, 2001).

Discovery of natural sweeteners

The general approach to the discovery of new sweetening agents of natural origin used at the University of Illinois at Chicago has been described previously (Kinghorn and Soejarto, 1989; Kinghorn and Kennelly, 1995; Kinghorn *et al.*, 1998; Kinghorn and Soejarto, 2002). Searching for novel high-potency sweeteners from plants requires an initial dereplication stage for the presence of saccharides and polyols, which, as indicated earlier, exhibit sweetness potencies close to that of sucrose. If the combined amount of those saccharides and polyols exceeds 5% w/w in a given plant part, the resultant sweetness can 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, polyols, and sweet-tasting phenylpropanoids in candidate sweet-tasting plants (Hussain *et al.*, 1990a, 1990b; Chung *et al.*, 1997).

HIGHLY SWEET NATURAL PRODUCTS

In this section, the presently known highly sweet substances of natural origin are described. Sweet-tasting compounds are listed in Table I, with information published subsequent to an earlier chapter (Kinghorn *et al.*, 1995) generally discussed in greater detail. The structures of the compounds mentioned will be interspersed in the text, with the following abbreviations used to designate the sugar units of glycosides: api = D-apiofuranosyl; ara = L-arabinopyranosyl; alm = 6-deoxy-3-*O*-methyl-D-allose; cym = D-cymarose; dig = D-digitoxose; glc = D-glucopyranosyl; glcA = D-glucuronopyranosyl; ole = D-oleandrose; qui = D-quinovosyl, rha = L-rhamnopyranosyl; tal = L-talosyl; the = D-thevetose; xyl = D-xylopyranosyl. A number of semisynthetic compounds are included in Table I, in those cases where they represent a significant improvement in sweetness potency relative to the natural product prototype sweet molecule. Compounds have been rated for sweetness intensity relative to sucrose on a weight basis (sucrose

Table 1. Highly sweet compounds from plants

Compound type/name[a]	Plant name	Sweetness potency[b]	Reference(s)
MONOTERPENE			
Perillartine (**10**)[c]	*Perilla frutescens* (L.) Britton (Labiatae)	370	Kinghorn and Soejarto, 1986
SESQUITERPENES			
Bisabolanes			
(+)-Hernandulcin (**11**)	*Lippia dulcis* Trev. (Verbenaceae)	1,500	Kinghorn and Soejarto, 1986
4β-Hydroxyhernandulcin (**12**)	*L. dulcis*	N.S.[d]	Kaneda *et al.*, 1992
Acyclic glycoside			
Mukurozioside IIb (**13**)	*Sapindus rarak* DC. (Sapindaceae)	*ca.* 1	Kasai *et al.*, 1986; Chung *et al.*, 1997
DITERPENES			
Diterpene acid			
4β,10α-Dimethyl-1,2,3,4,5,10-hexahydrofluorene-4α,6α-dicarboxylic acid (**14**)[e]	Pine tree[f]	1,300-1,800[g]	Kinghorn and Soejarto, 1986
***ent*-Kaurene glycosides**			
Dulcoside A (**15**)	*Stevia rebaudiana* (Bertoni) Bertoni (Compositae)	30	Kinghorn and Soejarto, 1986
Rebaudioside A (**4**)	*S. rebaudiana*	242	Kinghorn and Soejarto, 1986
Rebaudioside B (**16**)	*S. rebaudiana*	150	Kinghorn and Soejarto, 1986
Rebaudioside C (**17**)	*S. rebaudiana*	30	Kinghorn and Soejarto, 1986
Rebaudioside D (**18**)	*S. rebaudiana*	221	Kinghorn and Soejarto, 1986
Rebaudioside E (**19**)	*S. rebaudiana*	174	Kinghorn and Soejarto, 1986
Rebaudioside F (**20**)	*S. rebaudiana*	N.S.[d]	Starratt *et al.*, 2002
Rubusoside (**21**)	*Rubus suavissimus* S. Lee (Rosaceae)	115	Kasai *et al.*, 1986
Steviolbioside (**22**)	*S. rebaudiana*	90	Kinghorn and Soejarto, 1986
Stevio 13-*O*-β-D-glucoside (**23**)	*R. suavissimus*	N.S.[d]	Hirono *et al.*, 1990; Ohtani *et al.*, 1992
Stevioside (**5**)	*S. rebaudiana*	210	Kinghorn and Soejarto, 1986
Suavioside A (**24**)	*R. suavissimus*	N.S.[d]	Ohtani *et al.*, 1992
Suavioside B (**25**)	*R. suavissimus*	N.S.[d]	Ohtani *et al.*, 1992
Suavioside G (**26**)	*R. suavissimus*	N.S.[d]	Ohtani *et al.*, 1992
Suavioside H (**27**)	*R. suavissimus*	N.S.[d]	Ohtani *et al.*, 1992
Suavioside I (**28**)	*R. suavissimus*	N.S.[d]	Ohtani *et al.*, 1992
Suavioside J (**29**)	*R. suavissimus*	N.S.[d]	Ohtani *et al.*, 1992
Labdane glycosides			
Baiyunoside (**30**)	*Phlomis betonicoides* Diels (Labiatae)	500	Kinghorn and Soejarto, 1986
Phlomisoside I (**31**)	*P. betonicoides*	N.S.[d]	Kinghorn and Soejarto, 1989
Gaudichaudioside A (**32**)	*Baccharis gaudichaudiana* DC. (Compositae)	55	Fullas *et al.*, 1991
TRITERPENES			
Cucurbitane glycosides			
Bryodulcoside[h]	*Bryonia dioica* Jacq. (Cucurbitaceae)	N.S.[d]	Kinghorn and Soejarto, 1986

[a] Structures of the non-protein compounds are shown in the text. [b] Values of relative sweetness on a weight comparison basis to sucrose (= 1.0) are taken from the relevant literature source or from a review article/book chapter. [c] Semisynthetic derivative of natural product. [d] N.S. = Sweetness potency not given. [e] Semisynthetic sweetener. [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 (Kinghorn and Kennelly, 1995). [j] Although a known compound, the sweet taste only become evident recently (Kinghorn *et al.*, 1999). [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**).

Table I. Highly sweet compounds from plants (continued)

Compound type/name[a]	Plant name	Sweetness potency[b]	Reference(s)
Cucurbitane glycosides (continued)			
Bryoside (**33**)	*B. dioica*	N.S.[d]	Oobayashi *et al.*, 1992
Bryonoside (**34**)	*B. dioica*	N.S.[d]	Oobayashi *et al.*, 1992
Carnosifloside V (**35**)	*Hemsleya carnosiflora* C.Y. Wu et Z.L. Chen (Cucurbitaceae)	51	Kasai *et al.*, 1988b
Carnosifloside VI (**36**)	*H. carnosiflora*	77	Kinghorn and Soejarto, 1989
Mogroside IV (**37**)	*Siraitia grosvenorii* (Swingle) Lu & Zhang[i] (Cucurbitaceae)	233-392[g]	Matsumoto *et al.*, 1990
Mogroside V (**2**)	*S. grosvenorii*	250-425[g]	Kinghorn and Soejarto, 1986
11-Oxomogroside V (**38**)	*Siraitia siamensis* Craib (Cucurbitaceae)	N.S.[d]	Kasai *et al.*, 1989
Scandenoside R6 (**39**)	*Hemsleya panacis-scandens* C.Y. Wu et Z.L. Chen (Cucurbitaceae)	54	Kasai *et al.*, 1988; Matsumoto *et al.*, 1990
Scandenoside R11 (**40**)	*H. panacis-scandens*	N.S.[d]	Kubo *et al.*, 1996
Siamenoside I (**41**)	*Siraitia grosvenorii*; *S. siamensis*	563	Kasai *et al.*, 1989; Matsumoto *et al.*, 1990
Cycloartane glycosides			
Abrusoside A (**42**)	*Abrus precatorius* L.; *A. fruticulosus* Wall et W.& A. (Leguminosae)	30	Choi *et al.*, 1989; Choi *et al.*, 1989; 1990
Abrusoside B (**43**)	*A. precatorius*; *A. fruticulosus*	100	Choi *et al.*, 1989; Fullas *et al.*, 1990
Abrusoside C (**44**)	*A. precatorius*; *A. fruticulosus*	50	Choi *et al.*, 1989; Fullas *et al.*, 1990
Abrusoside D (**45**)	*A. precatorius*; *A. fruticulosus*	75	Choi *et al.*, 1989; Fullas *et al.*, 1990
Abrusoside E (**46**)	*A. precatorius*	N.S.[d]	Kennelly *et al.*, 1996
Dammarane glycosides			
Cyclocarioside A (**47**)	*Cyclocarya paliurus* (Batal.) Iljinsk (Juglandaceae)	200	Yang *et al.*, 1992
Cyclocaryoside I (**48**)	*C. paliurus*	250	Shu *et al.*, 1995
Gypenoside XX[j] (**49**)	*Gynostemma pentaphyllum* Makino (Cucurbitaceae)	N.S.[d]	Takemoto *et al.*, 1983
Oleanane glycosides			
Albiziasaponin A (**50**)	*Albizia myriophylla* Benth. (Leguminosae)	5[d]	Yoshikawa *et al.*, 2002
Albiziasaponin B (**51**)	*A. myriophylla*	600	Yoshikawa *et al.*, 2002
Albiziasaponin C (**52**)	*A. myriophylla*	N.S.[d]	Yoshikawa *et al.*, 2002
Albiziasaponin D (**53**)	*A. myriophylla*	N.S.[d]	Yoshikawa *et al.*, 2002
Albiziasaponin E (**54**)	*A. myriophylla*	N.S.[d]	Yoshikawa *et al.*, 2002
Apioglycyrrhizin (**55**)	*Glycyrrhiza inflata* Batal. (Leguminosae)	300	Kitagawa *et al.*, 1989
Araboglycyrrhizin (**56**)	*G. inflata*	150	Kitagawa *et al.*, 1989
Glycyrrhizin (**1**)	*Glycyrrhiza glabra* L. (Leguminosae)	93-170[g]	Kinghorn and Soejarto, 1986; Kitagawa, 2002
Periandrin I (**57**)	*Periandra dulcis* Mart.; *P. mediterranea* (Vell.) Taub. (Leguminosae)	90	Kinghorn and Soejarto, 1986
Periandrin II (**58**)	*P. dulcis*; *P. mediterranea*	95	Kinghorn and Soejarto, 1986
Periandrin III (**59**)	*P. dulcis*; *P. mediterranea*	92	Kinghorn and Soejarto, 1986
Periandrin IV (**60**)	*P. dulcis*; *P. mediterranea*	85	Kinghorn and Soejarto, 1986
Periandrin V (**61**)	*P. dulcis*	220	Suttisri *et al.*, 1993
Secodammarane glycosides			
Pterocaryoside A (**62**)	*Pterocarya paliurus* Batal. (Juglandaceae)	50	Kennelly *et al.*, 1995
Pterocaryoside B (**63**)	*P. paliurus*	100	Kennelly *et al.*, 1995

Table . H ghly sweet compounds from plants (continued)

Compc unc type/name[a]	Plant name	Sweetness potency[b]	Reference(s)
STERC IDAL SAPONINS			
Osladir (64)	*Polypodium vulgare* L. (Polypodiaceae)	500	Nishizawa and Yamada, 1996
Polypo los de A (**65**)	*Polypodium glycyrrhiza* DC. Eaton (Polypodiaceae)	600	Kim *et al.*, 1988, Kinghorn and Kim, 1993, Nishizawa *et al.*, 1994
Polypo los de B (**66**)	*P. glycyrrhiza*	N.S.[d]	Kinghorn and Kim, 1993, Kim and Kinghorn, 1989
Telosm osicle A_8 (**67**)	*Telosma procumbens* (Hence) Merr. (Asclepiadaceae)	N.S.[d]	Huan *et al.*, 2001
Telosm osicle A_9 (**68**)	*T. procumbens*	N.S.[d]	Huan *et al.*, 2001
Telosm osicle A_{10} (**69**)	*T. procumbens*	N.S.[d]	Huan *et al.*, 2001
Telosm osicle A_{11} (**70**)	*T. procumbens*	N.S.[d]	Huan *et al.*, 2001
Telosm osicle A_{12} (**71**)	*T. procumbens*	N.S.[d]	Huan *et al.*, 2001
Telosm osicle A_{13} (**72**)	*T. procumbens*	N.S.[d]	Huan *et al.*, 2001
Telosm osicle A_{14} (**73**)	*T. procumbens*	N.S.[d]	Huan *et al.*, 2001
Telosm osicle A_{15} (**74**)	*T. procumbens*	1,000	Huan *et al.*, 2001
Telosm osicle A_{16} (**75**)	*T. procumbens*	N.S.[d]	Huan *et al.*, 2001
Telosm osicle A_{17} (**76**)	*T. procumbens*	N.S.[d]	Huan *et al.*, 2001
Telosm osicle A_{18} (**77**)	*T. procumbens*	N.S.[d]	Huan *et al.*, 2001
PHEN' 'LPROPANOIDS			
trans-A net ole[k] (**78**)	*Foeniculum vulgare* Mill. (Umbelliferae)	13	Hussain *et al.*, 1990
	Illicium verum Hook f. (Illiciaceae)		
	Myrrhis odorata Scop. (Umbelliferae)		
	Osmorhiza longistylis DC. (Umbelliferae)		
	Piper marginatum Jacq. (Piperaceae)		
	Tagetes filicifolia Lag. (Compositae)		
trans-C innamaldehyde (**79**)	*Cinnamomum osmophloeum* Kanehira (Lauraceae)	50	Kinghorn and Soejarto, 1989
DIHYL ROISOCOUMARIN			
Phyllo ulc n[l] (**3**)	*Hydrangea macrophylla* Seringe var. *thunbergii* (Siebold) Makino (Saxifragaceae)	400	Kinghorn and Soejarto, 1986
FLAV( )NC IDS			
Dihyd ochalcone glycosides			
Glycyp nyll n (**80**)	*Smilax glycyphylla* Sm. (Liliaceae)	N.S.[d]	Kinghorn and Soejarto, 1986
Narin n d hydrochalcone[c] (**81**)	*Citrus paradisi* Macfad. (Rutaceae)	300	Kinghorn and Soejarto, 1986
Neohe spe idin dihydrochalcone[c] (**82**)	*Citrus aurantium* L.	1,000	Kinghorn and Soejarto, 1986
Phlor zin (83)	*Symplocos lancifolia* Sieb. et Zucc. (Symplocaceae)	N.S.[d]	Kinghorn and Soejarto, 1986
Trilob in (**84**)	*Symplocos microcalyx* Hayata	N.S.[d]	Kinghorn and Soejarto, 1986
Dihyd oflavonols and Dihyd oflavonol glycosides			
3-Acet oxy-5,7-dihydroxy-4′-metho yflavanone (**85**)	*Aframomum hanburyi* K. Schum. (Zingiberaceae)	N.S.[d]	Tsopmo *et al.*, 1996
2*R*,3*R* (+)-3-Acetoxy-5,7,4′-trihydr oxyl avanone (**86**)	*A. hanburyi*	N.S.[d]	Tsopmo *et al.*, 1996
Dihydr oquercetin 3-*O*-acetate 4′-methyl ether[c] (**87**)	*Tessaria dodoneifolia* (Hook. & Arn.) Cabrera (Compositae)	400	Kinghorn and Soejarto, 1989
(2*R*,3*R*)-D hydroquercetin 3-*O*-acetate (**88**)	*T. dodoneifolia*; *Hymenoxys turneri* K. Parker (Compositae)	80	Kinghorn and Soejarto, 1989

Table I. Highly sweet compounds from plants (continued)

Compound type/name[a]	Plant name	Sweetness potency[b]	Reference(s)
(2*R*,3*R*)-2,3-Dihydro-5,7,3′,4′-tetrahydroxy-6-methoxy-3-*O*-acetylflavonol (**89**)	*H. turneri*	25	Gao *et al.*, 1990
(2*R*,3*R*)-2,3-Dihydro-5,7,3′,4′-tetrahydroxy-6-methoxyflavonol (**90**)	*H. turneri*	15	Gao *et al.*, 1990
(2*R*,3*R*)-2,3-Dihydro-5,7,4′-trihydroxy-6-methoxy-3-*O*-acetylflavonol (**91**)	*H. turneri*	20	Gao *et al.*, 1990
Huangqioside E (**92**)	*Engelhardtia chrysolepis* Hance (Juglandaceae)	N.S.[d]	Kasai *et al.*, 1991
Neoastilbin (**93**)	*E. chrysolepis*	N.S.[d]	Kasai *et al.*, 1988a
PROANTHOCYANIDINS			
Cinnamtannin B-1 (**94**)	*Cinnamomum sieboldii* Meisner (Lauraceae)	N.S.[d]	Morimoto *et al.*, 1985
Cinnamtannin D-1 (**95**)	*C. sieboldii*	N.S.[d]	Morimoto *et al.*, 1985
Selligueain A (**96**)	*Selliguea feei* Bory (Polypodiaceae)	35	Baek *et al.*, 1993
Unnamed (**97**)	*Arachniodes sporadosora* Nakaike; *A. exilis* Ching (Aspidiaceae)	N.S.[d]	Tanaka *et al.*, 1991
Unnamed (**98**)	*A. sporadosora*; *A. exilis*	N.S.[d]	Tanaka *et al.*, 1991
BENZO[*b*]INDENO[*1,2-d*]PYRAN			
Hematoxylin (**99**)	*Haematoxylon campechianum* L. (Leguminosae)	120	Masuda *et al.*, 1991
AMINO ACID			
Monatin (**100**)	*Schlerochiton ilicifolius* A. Meeuse (Acanthaceae)	1,200-1,400[g]	Vleggaar *et al.*, 1992
PROTEINS			
Brazzein	*Pentadiplandra brazzeana* Baillon (Pentadiplandraceae)	2,000	Ming and Hellekant, 1994
Curculin	*Curculigo latifolia* Dryand. (Hypoxidaceae)	550	Yamashita *et al.*, 1990
Mabinlin	*Capparis masaikai* Levl. (Capparidaceae)	N.S.[d]	Hu and He, 1991; Kohmura and Ariyoshi, 1998
Monellin	*Dioscoreophyllum cumminsii* (Stapf) Diels. (Menispermaceae)	3,000	Van der Wel, 1972
Pentadin	*Pentadiplandra brazzeana* Bailon (Pentadiplandraceae)	500	Van der Wel *et al.*, 1989
Thaumatin	*Thaumatococcus daniellii* (Bennett) Benth. (Marantaceae)	1,600	Van der Wel and Loeve, 1972; Kurihara, 1992

= 1). 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. We have previously described the sensory method used at the University of Illinois at Chicago with a small taste panel (Kinghorn *et al.*, 1995; Kinghorn and Kennelly, 1995; Kinghorn *et al.*, 1998; Kinghorn and Soejarto, 2002).

In Table I, it may be seen 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, and steroidal saponins. Within the terpenoid and flavonoid categories several subgroups are represented. Thus for the terpenoids, there are one, two, three, and five subclasses of mono-, sesqui-, di-, and triterpenoids, respectively, while two subclasses of sweet flavonoids, the dihydrochalcones and the dihydroflavonols, are known. Accordingly, 20 major structural types of plant-derived sweetener have been found to date. Altogether, 98 natural products and five semisynthetic compounds are included in Table I, and were obtained from species representative of over 25 separate plant families. In a previous contribution, the distribution of highly sweet-tasting compounds from monocotyledons and dicotyledons arranged according to Dahlgren's superorders indicated their random distribution (Kinghorn and Soejarto, 1989). It may be seen from Table I that certain plant families biosynthesize more than one structural class of natural sweetener.

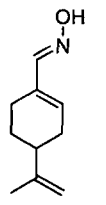
Terpenoids and steroids

As mentioned earlier in this chapter, the α-*syn*-oxime, perillartine (**10**) is a semisynthetic compound prepared from the naturally occurring monoterpenoid, perillaldehyde, isolated from *Perilla frutescens* (L.) Britton (Labiatae). Although it is used commercially in Japan, its poor solubility and sweetness qualities have hindered its further development (Kinghorn and Soejarto, 1986, 1989).

(+)-Hernandulcin (**11**) is a highly sweet bisabolane-type sesquiterpene alcohol and was first isolated from *Lippia dulcis* Trev. (Verbenaceae) collected in Mexico (Kinghorn and Soejarto, 1986, 1989). The sweetness intensity of this compound was rated as 1,500 times sweeter than 0.25 M sucrose on a weight basis. Although the sweetness intensity is high, this compound exhibits some bitterness and has a somewhat unpleasant aftertaste. Of the four possible diastereomers, only the 6*S*,1′*S* configuration of hernandulcin shows intense sweetness (Mori and Kato, 1986; Kinghorn *et al.*, 1995). There have been a number of previous chemical syntheses of (+)-hernandulcin, which have been reviewed (Kinghorn *et al.*, 1998; Kinghorn and Soejarto 2002). In addition, a group at Chonnam National University in Korea has synthesized (+)-hernandulcin (**11**) and its non-sweet diastereomer, (-)-epihernandulcin from isopulegol, in 15% and 11% yield, respectively (Kim, J. H. *et al.*, 2002). Natural (+)-hernandulcin has been produced from both shoot and hairy root cultures of *L. dulcis*, with a 2.9% w/w yield being obtained in the shoot culture (reviewed in Kinghorn *et al.*, 1998). Another sweet sesquiterpene alcohol in this series, namely, 4β-hydroxyhernandulcin (**12**), was isolated from a sample of *L. dulcis* collected in Panama. However, it was not possible to rate the sweetness of this compound relative to sucrose because 4β-hydroxyhernandulcin (**12**) was obtained in insufficient quantity from the Panamanian collection (Kaneda *et al.*, 1992). The presence of a hydroxyl group at C-4 in 4β-hydroxyhernandulcin (**12**) provides a potential point of attachment for sugars or other polar moieties in order to generate more water-soluble analogs of hernandulcin (Kinghorn *et al.*, 1998; Kinghorn and Soejarto, 2002).

Mukurozioside IIb (**13**), an acyclic sesquiterpene glycoside, identified previously from *Sapindus mukurossi* Gaertn. (Sapindaceae) (Kasai *et al.*, 1986), was isolated from the fruits of *Sapindus rarak* DC. (Sapindaceae) collected in Indonesia (Chung *et al.*, 1997). This compound was revealed as being sweet during a dereplication procedure because of its comparatively high yield in the plant (6.8% w/w). This is the first acyclic sesquiterpene glycoside from a plant source to have been determined to have a sweet taste, albeit with only a sweetness potency of about the same as that of sucrose (Chung *et al.*, 1997).

Three types of diterpenoids from plants are known as sweet natural products including a tricyclic resin acid (**14**), and *ent*-kaurene and labdane glycosides. As mentioned earlier in this review, two steviol glycosides, rebaudioside A (**4**) and stevioside (**5**) have commercial use in various forms (Kinghorn *et al.*, 2001). Several additional sweet diterpene glycosides of the *ent*-kaurene and labdane types have been isolated from two plant species, *Stevia rebaudiana* and *Rubus suavissimus* S. Lee (Rosaceae) in the 1980s and 1990s, with rebaudioside F (**20**) being reported only recently (Starratt *et al.*, 2002). Among them, dulcoside A (**15**) and rebaudioside C (**17**) are regarded as major constituents of *S. rebaudiana*, but occur in lower yields (0.4-0.7 and 1-2% w/w, respectively) compared with stevioside (**5**) and rebaudioside A (**4**) (Kinghorn and Soejarto, 1986). Rubusoside (= desglucosylstevioside) (**21**) is the principal *ent*-kaurene glycoside from *Rubus suavissimus* and its sweetness intensity was rated as 115 times sweeter than sucrose but it has some bitterness and a perceptible aftertaste (Ohtani *et al.*, 1992). Additional *ent*-kaurene-type diterpene glycosides in this series were isolated as minor constituents of *R. suavissimus* leaves, namely, suaviosides A, B, G, H, I, and J (**24-29**) and



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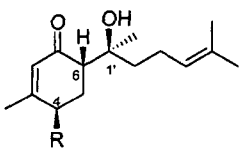
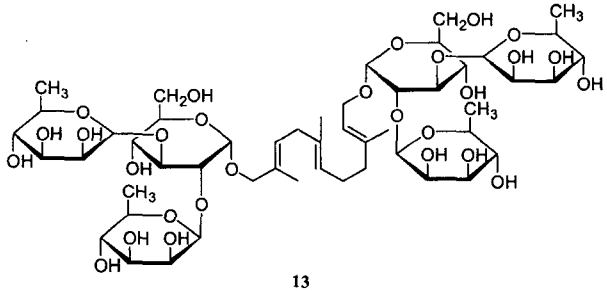


11 R = H
12 R = OH



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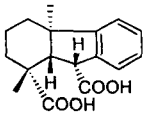

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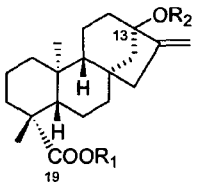
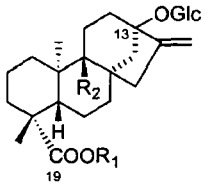
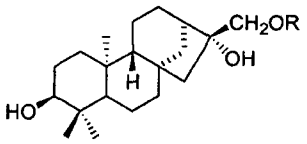


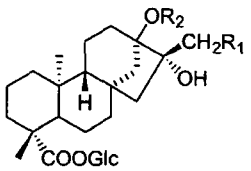
	R_1	R_2
15	β-glc	β-glc^2-α-rha
16	H	β-glc^2-β-glc |^3 β-glc
17	β-glc	β-glc^2-α-rha |^3 β-glc
18	β-glc^2-β-glc	β-glc^2-β-glc |^3 β-glc
19	β-glc^2-β-glc	β-glc^2-β-glc
20	β-glc	β-glc^2-β-xyl |^3 β-glc
22	H	β-glc^2-β-glc



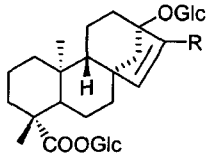
	R_1	R_2
21	β-glc	H
23	H	H
25	β-glc	OH



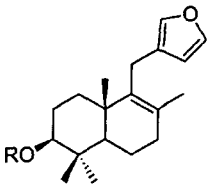
24 R = β-glc



	R_1	R_2
26	H	β-glc
28	OH	H



27 R = CHO
29 R = CH_2OH

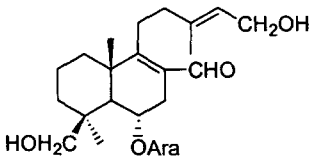


30 R = β-glc^2-β-xyl
31 R = β-glc^2-α-rha

steviol 13-O-β-D-glucoside (steviolmonoside) (**23**) (Hirono *et al.*, 1990; Ohtani *et al.*, 1992). However, their sweetness intensities have not been determined. It is of interest to note that no other species in either the genera *Stevia* or *Rubus* appear to biosynthesize sweet-tasting *ent*-kaurene glycosides in significant amounts (Kinghorn *et al.*, 1998). Like stevioside (**5**), rubusoside (**21**) has been subjected to extensive structural modification by Tanaka's group at Hiroshima University in order to improve on its hedonic limitations (Mizutani *et al.*, 1989; Ishikawa *et al.*, 1990; Tanaka, 1997; Ohtani and Yamasaki, 2002).

Two sweet labdane-type diterpene glycosides, baiyunoside (**30**) and phlomisoside I (**31**), were isolated from a Chinese plant, *Phlomis betonicoides* Diels (Labiatae) (reviewed by Kinghorn and Soejarto, 1986). While the sweetness of baiyunoside (**30**) was rated about 500 times sweeter than sucrose, the sweetness intensity of phlomisoside I (**31**) was not determined. In Japan, the Nishizawa group at Tokushima Bunri University has prepared a large number of synthetic analogs of baiyunoside (**30**), with some of these found to be sweeter than the natural product (Yamada and Nishizawa, 1992). Another labdane-type diterpene glycoside was isolated from *Baccharis gaudichaudiana* DC. (Compositae), namely, gaudichaudioside A (**32**) (Fullas *et al.*, 1991). The sweetness of gaudichaudioside A was rated as 55 times sweeter than 2% w/w sucrose solution, and gave only a very low perception of bitterness (Fullas *et al.*, 1991; Kinghorn *et al.*, 1998; Kinghorn and Soejarto, 2002). The compound has been isolated along with several closely related compounds based on the same carbon skeleton, which were not highly sweet, but exhibited other taste properties (i.e., sweet-bitter, bitter, and neutral tasting) (Fullas *et al.*, 1991; Kinghorn *et al.*, 1998; Kinghorn and Soejarto, 2002).

Many cucurbitane-type triterpenoid glycosides have been isolated as sweet principles from several plants in the Cucurbitaceae, and this is now one of the largest groups of natural highly sweet compounds. Two cucurbitane-type glycosides, bryoside (**33**) and bryonoside (**34**), have been reported from the roots of *Bryonia dioica* Jacq. as sweet principles, although their intensities relative to sucrose



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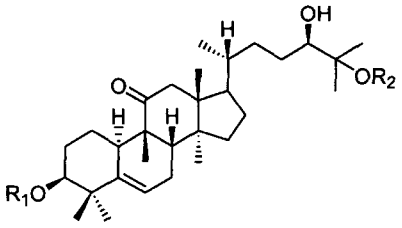
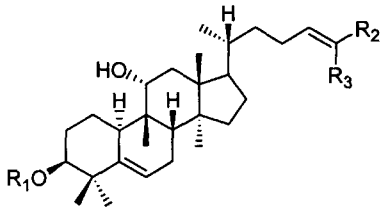
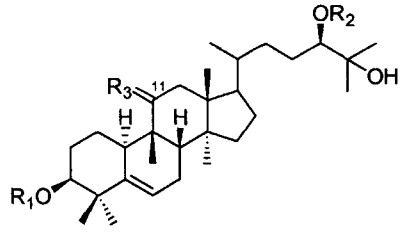


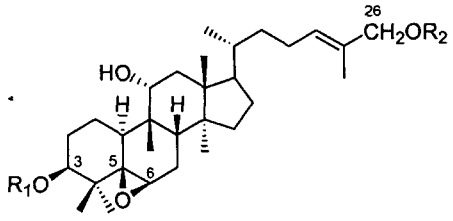
	R_1	R_2
33	β-glc^2-α-rha	β-glc
34	β-glc^2-α-rha	β-glc^2-β-glc



	R_1	R_2	R_3
35	β-glc	CH_2O-β-glc^2-β-glc	CH_3
36	β-glc	CH_2O-β-glc^6-β-glc	CH_3
39	β-glc	CH_3	CH_2O-β-glc^2-β-glc



	R_1	R_2	R_3
37	β-glc^2-β-glc	β-glc^2-β-glc	α-OH, β-H
38	β-glc^6-β-glc	β-glc^2-β-glc |^6 β-glc	=O
41	β-glc	β-glc^2-β-glc |^6 β-glc	α-OH, β-H

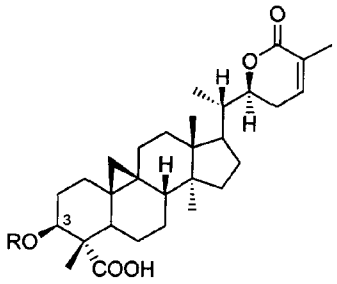


	R_1	R_2
40	β-glc	β-glc^6-β-glc

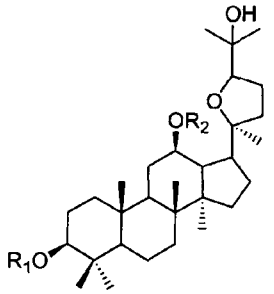
were not reported (Oobayashi *et al.*, 1992). From two species of the genus *Hemsleya*, three sweet cucurbitane-type triterpene glycosides were isolated, carnosiflosides V (**35**) and VI (**36**), and scandenoside R6 (**39**) (Kasai *et al.*, 1988b; Matsumoto *et al.*, 1990). Scandenoside R6 (**39**) was reported to show potential cancer chemopreventive activity, through the *in vitro* inhibition of Epstein-Barr early virus antigen activation, and by inhibiting mouse skin tumorigenesis *in vivo* (Konoshima and Takasaki, 2002).

More recently, several additional cucurbitane-type triterpenoid glycosides, namely, scandenosides R8-R11, have been isolated from *Hemsleya panacis-scandens* C. Y. Wu et Z. L. Chen (Kubo *et al.*, 1996). Only scandenoside R11 (**40**) was reported to have a sweet taste, but the degree of sweetness was not stated. Scandenoside R11 (**40**) has an unusual structure within this class, with a β-epoxide group between C-5 and C-6 and glycosylation at both the C-3 and C-26 positions. Several highly sweet cucurbitane-type triterpene glycosides have been isolated from the Chinese medicinal plant "lo han kuo" [*Siraitia grosvenorii* (Swingle) Lu & Zhang]. Mogrosides IV (**37**) and V (**2**), and siamenoside I (**41**) were isolated from this plant species and their sweetness intensities were rated as 233-392, 250-425, and 563 times sweeter than sucrose, respectively (Kasai *et al.*, 1989; Matsumoto *et al.*, 1990). These are some of the sweetest plant glycosides known (Kinghorn *et al.*, 1998). Siamenoside I (**41**) was also isolated as a minor constituent from another species in the genus *Siraitia*, *S. siamensis*, together with 11-oxomogroside V (**38**), although the sweetness intensity of the latter compound was not reported (Kasai *et al.*, 1989).

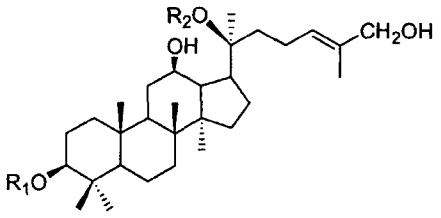
Abrusosides A-D (**42-45**) are the prototype members of a group of cycloartane-type triterpenoid sweeteners, and were isolated initially at the University of Illinois at Chicago from the leaves of *Abrus precatorius* L. and *A. fruticulosus* Wall et W. & A. (Leguminosae) (Choi *et al.*, 1989a; Choi *et al.*, 1989b; Fullas *et al.*, 1990). A fifth sweet-tasting compound of this series was isolated more recently, namely, abrusoside E (**46**) (Kennelly *et al.*, 1996a). The structure of the aglycone of these compounds, abrusogenin, was found to possess a novel carbon skeleton, by single crystal X-ray crystallography of abrusogenin methyl ester. The abrusoside glycosides differ in their type of saccharide substitution at the C-3 position. The sweetness intensities of the ammonium salts of abrusosides A-D were rated as 30, 100, 50, and 75 times sweeter than 2% w/w sucrose solution, respectively. Although the sweetness intensity of abrusoside E was not determined, the semi-synthetic monomethyl ester [the 6″-methyl-β-D-glucuronopyranosyl-(1→2)-β-D-glucopyranosyl derivative] of abruso-



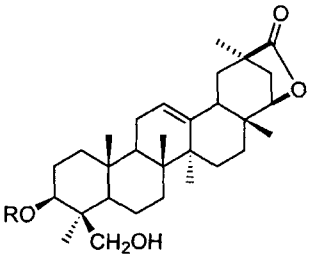
42 R = β-glc
43 R = β-glcA-6-CH_3^2-β-glc
44 R = β-glc^2-β-glc
45 R = β-glcA^2-β-glc
46 R = β-glc^2-β-glcA



	R_1	R_2
47	α-ara-5-Ac	α-rha
48	α-ara	β-qui



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



50 R = β-glcA^2-β-glcA^2-α-rha
52 R = β-glcA^2-β-glc^2-α-rha
|^3
β-glc
53 R = β-glcA^2-β-glc^2-α-rha
|^3
β-xyl
54 R = β-glcA^2-β-glcA^2-α-rha
|^3
β-xyl

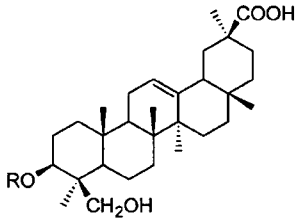
side E exhibited about 150 times the sweetness potency of 2% sucrose, making it the sweetest compound in this series. When the aglycone carboxylic acid group was methylated, as in abrusoside E dimethyl ester, no sweetness was apparent (Kennelly *et al.*, 1996b; Kinghorn *et al.*, 1999). In order to modify the saccharide moiety of the naturally occurring abrusogenin glycosides, reaction conditions have been determined for the glucosylation of the sterically hindered C-3 hydroxyl group in abrusogenin methyl ester (Kim *et al.*, 1999). Thus far, abrusosides A-E (**42-46**) appear to be the only cycloartane-type triterpenoids to have been isolated from the genus *Abrus* (Kinjo and Nohara, 1998; Kim, N.-C. *et al.*, 2002).

Cyclocarioside A (**47**), 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 Peoples Republic of China as a treatment for diabetes (Yang *et al.*, 1992). Recently, another sweet-tasting principle, cyclocarioside I (**48**), was isolated from the same plant along with two other compounds with the same dammarane-type triterpenoid aglycone structure (Shu *et al.*, 1995). Cyclocarioside I was rated as about 250 times sweeter than sucrose (Shu *et al.*, 1995).

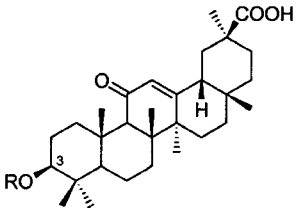
From the crude extract of the vine of *Gynostemma pentaphylum* Makino (Cucurbitaceae), which is used to make a sweet tea ("Amachazuru") in Japan, gypenoside XX (**49**) was isolated (Takemoto *et al.*, 1983b). Although the sweetness of this compound was not reported when it was first characterized, it was later stated to be sweet (Kinghorn *et al.*, 1999). The relative sweetness intensity of gypenoside XX (**49**) to sucrose has not been published, but this compound seems to be the first sweet dammarane-type triterpenoid to have been isolated from a plant source.

Recently, five oleanane-type triterpene saponins, namely, albiziasaponins A-E (**50-54**) have been reported as sweet principles of stems of *Albizia myriophylla* Benth. (Leguminosae), 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 C-20, 22 positions in ring E of the aglycone portion of albiziasaponin A and C-E (**50**,**52-54**). Albiziasaponin B (**51**), which has C-29 carboxyl group instead, was rated as about 600 times sweeter than sucrose (Yoshikawa *et al.*, 2002).

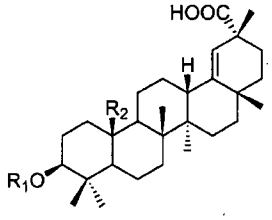
As mentioned ealier, glycyrrhizin (**1**) and its ammonium



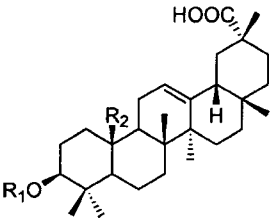
51 R = β-glcA^2-β-glcA^2-α-rha



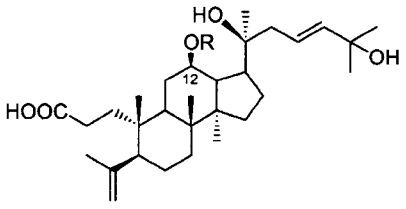
55 R = β-glcA^2-β-api
56 R = β-glcA^2-α-ara



	R_1	R_2
57	β-glcA^2-β-glcA	CHO
59	β-glcA^2-β-glcA	CH_2OH
61	β-glcA^2-β-xyl	CHO



	R_1	R_2
58	β-glcA^2-β-glc	CHO
60	β-glcA^2-β-glcA	CH_2OH



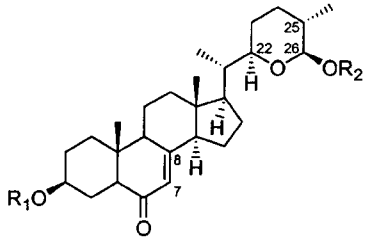
62 R = β-qui
63 R = α-ara

salts are available commercially for sweetening and flavoring purposes, and glycyrrhizin 3-*O*-D-glucuronide (MGGR, **7**) is a promising new intense sweetener (Tanaka, 1997; Mizutani *et al.*, 1998; Kinghorn and Compadre, 2001; Dalton, 2002). Apioglycyrrhizin (**55**) and araboglycyrrhizin (**56**) have been isolated from the roots of *Glycyrrhiza inflata* Batal. (Leguminosae) (Kitagawa *et al.*, 1989). While glycyrrhizin has a C-3-affixed diglucuronate unit, apioglycyrrhizin (**55**) has a β-D-apiofuranosyl-(1→2)-β-D-glucuronopyranosyl group and araboglycyrrhizin (**56**) an α-L-arabinopyranosyl-(1→2)-β-D-glucuronopyranosyl group at the C-3 position of the aglycone, glycyrrhetinic acid. The sweetness intensities of apioglycyrrhizin (**55**) and araboglycyrrhizin (**56**) were rated as 300 and 150 times sweeter than sucrose, respectively. In a recent review of 13 *Glycyrrhiza* glucuronide saponins, it was pointed out that 11-deoxoglycyrrhizin is bitter, thereby showing a requirement for the presence of the C-11 carbonyl group for the mediation of sweetness in glycyrrhizin (**1**) and its sweet derivatives (Kitagawa, 2002).

Periandrins I-IV (**57-60**) were characterized in the 1980s as oleanane-type triterpenoid glycoside sweeteners from *Periandra dulcis* Mart. (Leguminosae) (Brazilian licorice) by the Hashimoto group at Kobe Pharmaceutical University in Japan, and the sweetness potency was determined as about 900 times sweeter than sucrose for each compound. Periandrins I-IV (**57-60**) were also found in another species, *P. mediterranea* (Vell.) Taub. (reviewed by Kinghorn and Soejarto, 1986). A fifth compound in this series, periandrin V (**61**), was isolated from the roots of *P. dulcis* and found to be based on the same aglycone as periandrin I (**57**) (Suttisri *et al.*, 1993). The terminal D-glucuronic acid residue of periandrin I (**57**) was substituted by a D-xylose moiety in periandrin V (**61**). Periandrin V (**61**) exhibited 220 times the sweetness of 2% sucrose and was accordingly ranked as the sweetest substance obtained so far in the periandrin series (Suttisri *et al.*, 1993).

Two new sweet secodammarane glycosides, pterocaryosides A (**62**) and B (**63**), were isolated and structurally determined from the leaves and stems of *Pterocarya paliurus* Batal. (Juglandaceae) (Kennelly *et al.*, 1995). *Pterocarya paliurus* Batal. is a preferred taxonomic name for *Cyclocarya paliurus* (Batal.) Iljinsk (see above). The leaves of *P. paliurus* are used by local populations in Hubei Province of the Peoples Republic of China to sweeten cooked foods. While pterocaryoside A (**62**), which has a β-quinovose unit attached to the C-12 position, is 50 times sweeter than sucrose, pterocaryoside B (**63**), with an α-arabinose unit at C-12, was rated as 100 times sweeter than sucrose (Kennelly *et al.*, 1995). These are the first highly sweet secodammarane glycosides to have been isolated and structurally characterized, and represent interesting lead compounds for potential synthetic optimization.

The steroidal saponin osladin (**64**) was isolated as a sweet principle from the fern *Polypodium vulgare* L. (Poly-



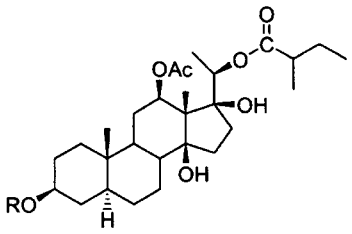
	R_1	R_2	Other
64	β-glc^2-α-rha	α-rha	7,8-dihydro
65	β-glc^2-α-rha	α-rha	-
66	β-glc	α-rha	-

podiaceae) nearly 40 years ago (reviewed by Kinghorn and Soejarto, 1986, 1989). However, the original structure proposed was later revised because a synthetic version produced was not sweet at all. The correct structure of osladin (**64**) 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 as 500 times, rather than 3,000 times sweeter than sucrose (reviewed by Nishizawa and Yamada, 1996). Polypodosides A (**65**) and B (**66**) were isolated from the rhizomes of North American fern *Polypodium glycyrrhiza* DC. Eaton (Polypodiaceae) as additional highly sweet steroidal glycosides (Kim *et al.*, 1988; Kim and Kinghorn, 1989). Their aglycone, polypodogenin, is the $\Delta^{7,8}$-derivative of the aglycone of osladin. The structure of polypodoside A (**65**) was also revised as 22*R*, 25*S*, 26*R*, by a chemical interconversion procedure (Nishizawa *et al.*, 1994; Kinghorn *et al.*, 1998). Polypodoside A (**65**) shows a high sweetness potency and was rated as 600 times sweeter than sucrose (Kim *et al.*, 1988).

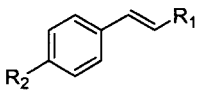
Telosmosides A_8-A_{18} (**67-77**), pregnane-type steroidal saponins, were isolated as sweet principles of the stems of *Telosma procumbens* (Hance) Merr. (Asclepiadaceae) (Huan *et al.*, 2001). This plant has been used as a medicinal plant in certain Asian countries traditionally and employed as a 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 aglycone of these compounds. Telosmoside A_{15} (**74**) was reported to exhibit a sweetness intensity of 1,000 times greater than that of sucrose (Huan *et al.*, 2001).

Phenylpropanoids

The phenylpropanoids *trans*-anethole (**78**) and *trans*-cinnamaldehyde (**79**) are used as flavoring agents in foods in the United States and some other countries (Kinghorn and Soejarto, 1989). *trans*-Cinnamaldehyde (**79**) was isolated from *Cinnamomum osmophloeum* Kanehira (Lauraceae) as a sweet principle, while *trans*-anethole (**78**) was isolated as the volatile oil constituent responsible for the sweet taste of several plant species, as listed in Table I (Hussain *et al.*, 1990b). 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 a dereplication procedure using gas chromatography-mass spectrometry (GC/MS) (Hussain *et al.*, 1990b).



67 R = β-dig^4-β-cym^4-β-ole^4-β-glc
68 R = β-dig^4-β-ole^4-β-the^4-β-glc
69 R = β-dig^4-β-cym^4-β-ole^4-β-ole^4-β-ole
70 R = β-dig^4-β-cym^4-β-ole^4-β-ole^4-β-the
71 R = β-dig^4-β-cym^4-β-ole^4-β-ole^4-β-glc
72 R = β-dig^4-β-dig^4-β-ole^4-β-ole^4-β-the
73 R = β-dig^4-β-cym^4-β-ole^4-β-ole^4-β-ole^4-β-glc
74 R = β-dig^4-β-cym^4-β-ole^4-β-ole^4-β-the^4-β-glc
75 R = β-dig^4-β-cym^4-β-ole^4-β-ole^4-β-glc^4-β-glc
76 R = β-dig^4-β-cym^4-β-ole^4-β-ole^4-β-alm^4-β-glc
77 R = β-dig^4-β-cym^4-β-ole^4-β-the^4-β-glc^4-β-glc



	R_1	R_2
78	CH_3	OCH_3
79	CHO	H

Dihydroisocoumarins

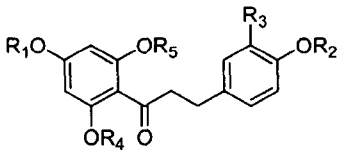
The dihydroisocoumarin, 3*R*-phyllodulcin (**3**), obtained from the leaves of *Hydrangea macrophylla* var. *thunbergii* via enzymatic hydrolysis, was mentioned earlier in the chapter as having commercial use. Recently, it has been demonstrated that this sweet substance occurs naturally in unprocessed leaves of its plant of origin as a 5:1 enantiomer with the previously undescribed compound, 3*S*-phyllodulcin (Yoshikawa *et al.*, 1999). Also reported in this study were the novel 3*R*- and 3*S*-phyllodulcin 3′-*O*-glycosides, although the presence or absence of a sweet taste in these three new phyllodulcin analogs was not disclosed (Yoshikawa *et al.*, 1999). Merlini and associates have recently summarized their research data on the effects on sweetness of the structural modification of phyllodulcin (**3**), wherein 120 compounds containing an isovanillyl unit were produced (Bassoli *et al.*, 2002).

Flavonoids

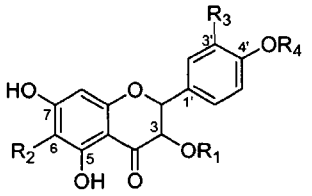
Glycyphyllin (**80**), phlorizin (**83**), and trilobatin (**84**) are sweet dihydrochalcone glycosides and were isolated from *Smilax glycyphylla* Sm. (Liliaceae), *Symplocos lancifolia* Sieb. et Zucc., and *Symplocos microcalyx* Hayata (Symplocaceae), respectively (Kinghorn and Soejarto, 1986). Naringin dihydrochalcone (**81**) and neohesperidin dihydrochalcone (**82**) are semisynthetic dihydrochalcone glycosides and can be obtained as by-products of the citrus industry Neohesperidin dihydrochalcone (**82**) is sweeter than compound **81** (600-1,500 times sweeter than sucrose), and has acceptable hedonic properties, and is used in a wide variety of foodstuffs as a sweetener and flavor ingredient (Borrego and Montijano, 2001). There have been a large number of attempts to synthesize improved dihydrochalcones, with such compounds requiring 3-hydroxy-4-alkoxy substitution in ring B (reviewed in Kinghorn *et al.*, 1995).

The seeds of *Aframomum hanburyi* K. Schum. (Zingiberaceae) are used as an antidote and ingredient in certain medicinal preparations in Cameroon (Tsopmo *et al.*, 1996). From an acetone extract of the seeds of this plant, two sweet dihydroflavonols, 3-acetoxy-5,7-dihydroxy-4′-methoxyflavanone (**85**) and 2*R*,3*R*-(+)-3-acetoxy-5,7,4′-trihydroxyflavanone (**86**), were isolated (Tsopmo *et al.*, 1996). 3-Acetoxy-5,7-dihydroxy-4′-methoxyflavanone (**85**) was previously isolated from a different species, *Aframomum pruinosum* Gagnepain (Ayafor and Connolly, 1981). However, the sweetness intensities of these compounds were not indicated (Ayafor and Connolly, 1981; Tsopmo *et al.*, 1996). The previously known (2*R*,3*R*)-dihydroquercetin 3-*O*-acetate (**88**) which was rated as 80 times sweeter than sucrose, was isolated from *Tessaria dodoneifolia* (Hook. & Arn.) Cabrera and *Hymenoxys turneri* K. Parker (Compositae) (Kinghorn and Soejarto, 1989). The sweetness of this compound was increased to 400 times that of sucrose by methylation at the C-4′ hydroxyl to form an isovanillyl derivative (**87**) (Kinghorn and Soejarto, 1989). Two dihydroflavonols, huangqioside E (**92**) and neoastilbin (**93**), were isolated from *Engelhardtia chrysolepis* Hance (Juglandaceae), although their sweetness was not evaluated (Kasai *et al.*, 1991). A series of three sweet additional dihydroflavonols (**89-91**) was isolated from *H. turneri* (Gao *et al.*, 1990).

No additional sweet-tasting dihydrochalcones appear to have been isolated and characterized from plant sources in recent years.



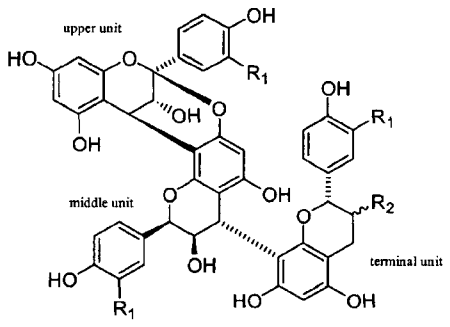
	R_1	R_2	R_3	R_4	R_5
80	H	H	H	α-rha	H
81	β-glc[2]-α-rha	CH_3	H	H	H
82	β-glc[2]-α-rha	CH_3	OH	H	H
83	H	H	H	H	β-glc
84	β-glc	H	H	H	H



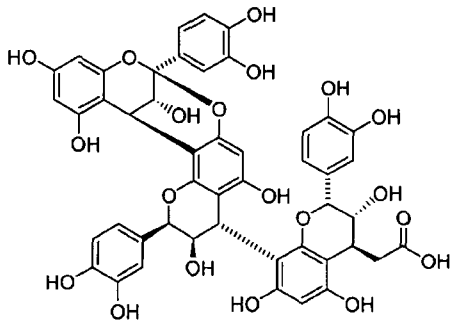
	R_1	R_2	R_3	R_4	Other
85	Ac	H	H	CH_3	2*R*,3*R*
86	Ac	H	H	H	2*R*,3*R*
87	Ac	H	OH	CH_3	-
88	Ac	H	OH	H	2*R*,3*R*
89	Ac	CH_3O	OH	H	2*R*,3*R*
90	H	CH_3O	OH	H	2*R*,3*R*
91	Ac	CH_3O	H	H	2*R*,3*R*
92	α-rha[3]-β-glc	H	OH	H	2*R*,3*R*
93	α-rha	H	OH	H	2*S*,3*S*

Proanthocyanidins

Several doubly linked ring-A proanthocyanidins are known to be sweet-tasting (Morimoto *et al.*, 1985; Tanaka *et al.*, 1991). For example, two proanthocyanidins, cinnamtannin B-1 (**94**) and cinnamtannin D-1 (**95**), isolated from the roots of *Cinnamomum sieboldii* Meisner (Lauraceae) showed sweet properties (Morimoto *et al.*, 1985). Other sweet-tasting proanthocyanidins with carboxylic acid (**97**) and lactone (**98**) functionalities, were isolated from the ferns *Arachniodes sporadosora* Nakaike and *A. exilis* Ching (Aspidiaceae) (Tanaka *et al.*, 1991). However, none of these proanthocyanidins was ever quantitatively rated for its sweetness intensity relative to sucrose. A sweet-tasting proanthocyanidin, selligueain A (**96**) was isolated



	R_1	R_2
94	OH	β-OH
95	OH	α-OH
96	H	β-OH



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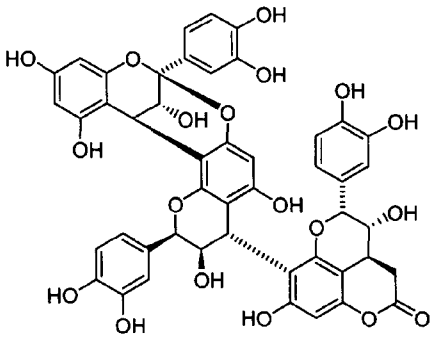

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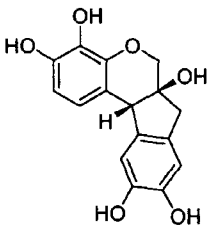

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from the rhizomes of the fern *Selliguea feei* Bory (Polypodiaceae), collected in Indonesia (Baek *et al.*, 1993). Selligueain A may be distinguished from the previously known sweet-tasting proanthocyanidins since it has an afzelechin residue rather than an epicatechin moiety as the lower terminal unit of the molecule. When evaluated by a small human taste panel, selligueain A (**96**) showed 35 times the sweetness of a 2% sucrose solution and was not perceived as astringent when in solution (Baek *et al.*, 1993). 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 (Baek *et al.*, 1994). As a result of the investigation of selligueain A (**96**) 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 (Baek *et al.*, 1993). Bohlin and co-workers have demonstrated that selligueain A (**96**) is present in low yields in an additional five *Polypodium* species collected in Honduras, and that this sweet-tasting compound is also a elastase inhibitor in human neutrophils (Vasaenge *et al.*, 1997). Moreover, Subarnas and Wagner have reported the analgesic and anti-inflammatory activities of selligueain A (**96**) in two *in vivo* models (Subarnas and Wagner, 2000).

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

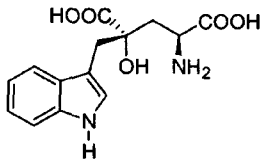
From the extract of the heartwood of *Haematoxylon campechianum* L. (Leguminosae), a sweet principle was isolated, namely, (+)-hematoxylin (**99**) (Masuda *et al.*, 1991). This compound has been used for a long time as a microscopic staining reagent, but the sweetness of this compound was not recognized previously. Also, in the same study, brazilin, the 4-deoxy derivative of (+)-hematoxylin, and a constituent of *Caesalpinia echinata* Lam. (Leguminosae), was found not to be sweet (Masuda *et al.*, 1991). In a follow-up study, (+)-hematoxylin (**99**) was rated as 120 times sweeter than 3% sucrose, while its synthetic (-)-enantiomer was only 50 times sweeter (Arnoldi *et al.*, 1995; Bassoli *et al.*, 2002).

Amino acids

A highly sweet amino acid, monatin (**100**), was isolated from an African plant, *Schlerochiton ilicifolius* A. Meeuse (Acanthaceae) (Vleggaar *et al.*, 1992). Monatin (**100**) was rated as being comparable to the synthetic amino acid, 6-chloro-D-tryptophan, which showed a sweetness intensity of 1,300 times that of sucrose. Monatin (**100**) 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 (Nakamura *et al.*, 2000). A structure-sweet-tasting activity relationship on synthetic analogs of monatin is currently underway in the laboratory of Merlini at the University of Milan (Bassoli *et al.*, 2001).

Proteins

Several plant-derived proteins have been reported previously as sweeteners, inclusive of curculin (Yamashita *et al.*, 1990), mabinlin (Hu and He, 1991; Kohmura and Ariyoshi, 1998), monellin (Van der Wel, 1972; Kurihara, 1992), pentadin (Van der Wel *et al.*, 1989), and thaumatin, with the latter compound mentioned earlier in this review as having commercial use as a sweetener and flavor



100

enhancer (Kurihara, 1992; Kinghorn and Compadre, 2001). Curculin, mabinlin, monellin, and thaumatin have been expressed in microorganisms, and solid-phase synthesis has been used to produce mabinlin and monellin (Kohmura *et al.*, 2002). Recently, a sixth sweet protein of plant origin, brazzein was isolated from the fruits of an African climbing vine, *Pentadiplandra brazzeana* Baillon (Pentadiplandraceae), which grows in Gabon, Congo, and Cameroon (Ming and Hellekant, 1994). Pentadin was also isolated from this same plant (Van der Wel *et al.*, 1989). Brazzein has 54 amino acid residues and a molecular weight of 6,473 daltons making it a relatively small protein compared to other sweet proteins such as curculin (12,491 daltons), mabinlin (12,441 daltons), monellin (11,086 daltons), and thaumatin (22,206 daltons) (Ming and Hellekant, 1994). Brazzein has four disulfide bridges and promising thermostability, since its sweetness was not destroyed at 80 °C for 4 hours exposure (Kohmura *et al.*, 1996). Most of the other protein sweeteners are unstable to heat and inappropriate for use at high temperature. The sweetness of brazzein was rated as 2,000 times sweeter than 2% sucrose (Ming and Hellekant, 1994). According to Markley and associates, there is a minor variant of brazzein (des-pGlu-1-brazzein) that is also naturally occurring, and possesses twice the sweetness intensity of the parent compound (DeRider *et al.*, 2001). Brazzein has considerable potential as a new naturally occurring sweetening agent, because of its favorable taste profile and thermostability.

CONCLUSIONS

Despite the relatively small number of highly (potently) sweet substances of natural origin, it is impressive that there are so many plant-derived substances that have some commercial value as sucrose substitutes and/or flavoring agents, with the primary examples being glycyrrhizin (**1**) and ammoniated glycyrrhizin (oleanane triterpene glycosides), rebaudioside A (**4**) and stevioside (**5**) (*ent*-kaurene diterpene glycosides), the semi-synthetic flavonoid glycoside, neohesperidin dihydrochalcone (**82**), and the protein thaumatin. Also used to some extent are the cucurbitaine glycoside, mogroside V (**2**), the dihydroisocoumarin, phyllodulcin (**3**), monoglucuronated glycyrrhetic acid (**7**), and semi-synthetic perillartine (**10**). However, like several of the synthetic low-calorie sweeteners previously mentioned in this review, there is some controversy about the perceived safety of some of these natural sweeteners, particularly the sweeteners from *Stevia rebaudiana*. On the one hand, refined extracts from this plant or as purified stevioside (**5**) are approved as food additives in Japan, Korea, and South American countries such Brazil, Argentina, and Paraguay. Moreover, since 1995, preparations from *S. rebaudiana* have been used extensively in the United States as a "dietary supplement" (Kinghorn, 2002). Several companies now package *Stevia rebaudiana* products for use as "table-top" sweeteners in the United States. In contrast, stevioside has been reviewed by both the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) and the Scientific Committee for Food (SCF) of the European Union (EU), and deemed unacceptable as a sweetener on the basis of the presently available safety data, which are considered insufficient (Kinghorn *et al.*, 2001). Moreover, the leaves of *Stevia rebaudiana* were considered by the SCF of the EU, with concern being expressed that insufficient data were provided to permit safety to be established (Kinghorn, 2002).

Although it does seem as stevioside (**5**) is safe at the low doses required for sweetening and so long as the daily intake is limited, there is a fairly large literature on the biological activities of stevioside other than its sweet properties (reviewed by Huxtable, 2002). In spite of this, there is an incomplete perspective on its absorption, distribution, metabolism, and excretion in non-rodent mammals and humans at present (Huxtable, 2002). A recent *in vitro* study using human intestinal microflora, however, has indicated that rebaudioside A (**4**) and stevioside (**5**) were both hydrolyzed to their aglycone steviol (Koyama *et al.*, 2001). Steviol itself has a number of biological effects (reviewed by Huxtable, 2002), and its mutagenicity to *Salmonella typhimurium* strain TM677 in the presence of a metabolic activating system, which was established over 15 years ago (Pezzuto *et al.*, 1985), still attracts additional attention (e.g., Matsui *et al.*, 1996; Terai *et al.*, 2002). Investigators at the National Institute of Health Science in Tokyo, however, have pointed out that the *in vitro* mutagenicity of steviol may not be significant. In a two-year feeding study of stevioside in F344 rats, in which steviol was positively identified and quantitated, there was no significant carcinogenic effect obtained (Toyoda *et al.*, 1997). Moreover, no cases of clinical toxicity due to the ingestion by humans of *Stevia rebaudiana* extracts or stevioside (**5**) have appeared in the literature, despite their ever-increasing use. This may be contrasted with the reports on pseudoaldosteronism caused by large intakes of glycyrrhizin (**1**) contained in licorice-flavored confectionery or when used as a drug (de Klerk *et al.*, 1997; Van Rossum *et al.*, 2001; Dalton, 2002). However, it can be expected that additional investigations on the safety of the *Stevia rebaudiana* sweeteners will continue to be conducted in the future, until a more complete understanding is eventually obtained.

The recent review of Konoshima and Takasaki (2002), in which it was indicated that glycyrrhizin (**1**), mogroside V (**2**), and stevioside (**5**) have potential cancer chemopreventive activities, is intriguing, and points to the potential use of these natural sweeteners in "nutraceutical" or "func-

tional food" compositions (Konoshima and Takasaki, 2002).

In terms of the prospects of discovery of future highly sweet natural products from plants using ethnobotanical approaches, it will probably be necessary to access more remote geographical areas than previously in order to obtain candidate sweet-tasting plants for the study of their chemical constituents. As a result of the passage of the United Nations Convention on Biological Diversity in Rio de Janeiro in 1992, it is now necessary to obtain "prior informed consent" and to develop benefit-sharing agreements before accessing indigenous traditional knowledge, such as imparting information on which plants in a given locality taste sweet. It is most advantageous in the sweetener discovery projects from natural sources to work in a multidisciplinary team composed of botanists, natural products chemists, and biologists (Kinghorn *et al.*, 1998; Kinghorn and Soejarto, 2002). There has been considerable recent progress leading to the identification of the T1R family of receptors that respond to sweet stimuli (Montmayeur and Matsunami, 2002), so it is possible that new receptor-binding assays can be developed to aid with the discovery of new natural sweeteners in the future, instead of relying on human panels to taste crude extracts, chromatographic fractions, and pure compounds.

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