



**CZECH UNIVERSITY OF
AGRICULTURE PRAGUE**



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Tropical and Subtropical Plants with Sweetening Properties: The Potential Source of Low-Calorie Sweeteners

review

1. Introduction

- Plant species with unusual taste properties such as bitterness, sourness or sweetness, and others with a taste-modifying components, have long been known to man, although their exploitation has been limited.
- In recent years, the number of diabetic and overweight people have greatly increased worldwide. For the prevention of obesity, dental caries or the therapy of diabetes, it is important to limit the ingestion of sugars.
- Together with this trend, there is also an increase in the demand for healthy and natural food products. Therefore, and in order to address this need, there is an intense and ongoing search for alternative sweeteners.

1. Introduction

- Efforts to find additional examples of highly sweet plant constituents have been stimulated both by a public demand for natural flavours, as well as perceived problems with the toxicity, taste quality, stability or price of existing synthetic high-potency sweeteners.
- At this time in Japan, China, USA, Australia and Europe, some of these natural nonsaccharide sweeteners are being consumed.
- Thus, with the increase in their demand there is a necessity for identification of these nonsacchariferous plant species, as well as the identification of their active sweet principles.

2. Approaches to the discovery of Highly Sweet Molecules from Higher Plants

- In the search for new sweeteners, one of the most demanding aspects is to find good candidate sweet-tasting plants, which might contain novel sweet compounds.
- There is very difficult to predict the occurrence of sweet compounds on a taxonomic basis. For example *Stevia rebaudiana* is well-known for producing the potently sweet glycosides stevioside and rebaudioside A. When the scientists examined organoleptically and phytochemically over 100 herbarium leaf specimens in the genus *Stevia* only one species other than *S. rebaudiana* was found to contain stevioside, namely, *S. phlebophylla* A. Gray.

2. Approaches to the discovery of Highly Sweet Molecules from Higher Plants

- According to these results I think that prior to phytochemical investigation there must be done detailed ethnobotanical screening in two major ways:
 - Through field investigation
 - Through literature sources

Followed by screening of biological activity of crude extracts

Field Investigations

- One of the most successful ways to locate new sweet plants is to survey local populations, especially in marketplaces.
- Generally speaking, humans view sweet-tasting plants as safe to consume, while bitter-tasting plants are frequently considered toxic.
- Other plants may be known as slightly sweet to the indigenous peoples, but are not used as sweeteners due to the low levels of the compounds, or the presence of bitter or bioactive compounds along with the sweet compound. So, some of the plants may not necessarily be identified as distinctly sweet by local people.

Literature Sources

- Another potential route to identifying candidate sweet plants are ethnobotanical literature sources. There is most likely a general understanding of sweet perception among all human cultures on earth. Therefore, a notation that a plant tastes sweet by a particular indigenous group of people, is frequently validated sometimes by the discovery of new intensely sweet compounds, but more often by the identification of high levels of free sugars.
- One shortcoming of this type of ethnobotanical screening is that ‚sweet‘ may also refer to the odour of the plant, which is not related to sweet-taste perception.

Literature Sources

- Another approach involves a search of *Index Kewensis*, a source of all of the published scientific names of seed plants, using specific epithets which might be indicative of sweetness and yields a number of new possible lead plants, as well as some well-known sweet plants.
- For example, a search of the epithet *dulcis* or *dulcificum* (Latin name for sweet)

3. Assays for sweetness

- Currently, there is no reliable *in vitro* method.
- *In vivo* models:
 - In order for humans to taste chromatographic fractions or pure isolates, preliminary safety testing comprised of mouse acute toxicity and bacterial mutagenicity studies must be performed. These tests represent a significant use of time and resources.
 - **Electrophysiological and behavioural experiments using the Mongolian gerbil:** in the electrophysiological assay, a potentially sweet plant extract, fraction, or pure compound is applied to the tongue of an anaesthetized gerbil, and electrophysiological recordings are taken from the chorda typhani nerve, evaluating up to 25 samples with a single gerbil. This is backed by a **conditioned-taste aversion assay** using gerbils that are trained to avoid sucrose.

3. Assays for sweetness

- The combination of these methods appears to be helpful in selecting extracts of different polarities from plants for the presence or absence of sweet constituents and about 2/3 of the pure compounds tested that were known to be sweet to humans were evaluated as ‘sweet’ to the gerbil.
- While the gerbil is not a perfect model for human sweet taste it does respond well to many „bulk“ (i.e., sugars and polyols) and „intense“ sweeteners, and the assay is more economic to perform than other *in vivo* options currently available.

4. Examples of Recently Discovered Natural-Occurring Sweet Compounds

- Terpenoids and Steroids
- Dihydroisocoumarin
- Flavonoids
- Sweet-Tasting Proteins
- Sweetness-Modifying Substances

Terpenoids and Steroids

- Many of these compounds are glycosides containing one or more saccharide units, which results in enhanced water solubility.
- Among the terpenoids, certain naturally occurring, mono-, sesqui-, di- and triterpenoids, or their derivatives, are known to be intensely sweet.
- Several of these compounds have commercial application as sucrose substitutes.

Perilla frutescens (L.) Britton

- **Family:** *Lamiaceae*
- **Synonyms:** *Ocimum frutescens* L., *P. ocymoides* L., *P. nankinensis* (Lour.) J. Decaisne
- **Vernacular names:** Perilla, Perilla mint, Chinese basil (En.), Bhanjira (Hindi)
- **Origin:** Mountainous areas of India
- **Description:** Erect, aromatic, annual herb, 0,3-2 m tall, green or purplish.
- **Uses:** *volatile oil* – spice, perfumery; *fatty oil* – cooking; *anthocyanins* – for colouring pickled fruits; *medicine* – diaphoretic, anodyne, sedative, diuretic, anti-inflammatory and remedy for cough.

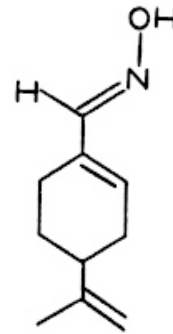


Perilla frutescens (L.) Britton

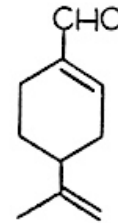
Sweet principle:

Perillartin

- Is a monoterpenoid constituent of the slightly sweet volatile oil (α -syn-oxime of Perillaldehyd).
- About 350 times sweeter than sucrose
- It is used in Japan for the sweetening of tobacco.
- The bitter after-taste and low water solubility have restricted its more general use as a sweetener.



PERILLARTINE (2)



PERILLALDEHYDE (1)

Lippia dulcis Trevir.

- **Family:** *Verbenaceae*
- **Synonyms:** *Phylla scaberrima* (Juss. ex Pers.) Moldenke.
- **Vernacular names:** Mexican lippia, Sweet lippia.
- **Origin:** Central America
- **Description:** Fast growing, low perennial creeper (up to 30 cm) with small white flowers.
- **Uses:** *medicine* - for coughs, colds, bronchitis, asthma, and colic.

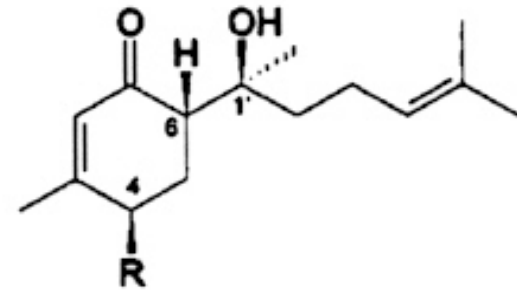


Lippia dulcis Trevir.

Sweet principle:

Hernandulcin

- Bisabolane sesquiterpenoid from the aerial parts of the plant. Present in the amount of 0.04 – 0.15% w/w dry weight.
- Despite being about 1,000 times sweeter than sucrose, naturally occurring Hernandulcin is somewhat thermolabile and has an unpleasant aftertaste and some inherent bitterness (due to presence of camphor), which could restrict its potential sweetening applications



- 1 Hernandulcin (R = H)
- 2 4β-Hydroxyhernandulcin (R = OH)

Stevia rebaudiana Bertoni

- **Family:** *Asteraceae*
- **Synonyms:** *Eupatorium rebaudianum* Bertoni
- **Vernacular names:** Stevia, Sweet herb of Paraguay, Honey-yerba (En.)
- **Origin:** Sierra Amambay in North Eastern Paraguay
- **Description:** A slender, erect, perennial herb 50-100 cm tall in natural stands and up to 120 cm under cultivation.
- **Uses:** natural low-calorie sweetener; *medicine* – treatment of diabetes, obesity and could lower blood pressure; *cosmetic products*

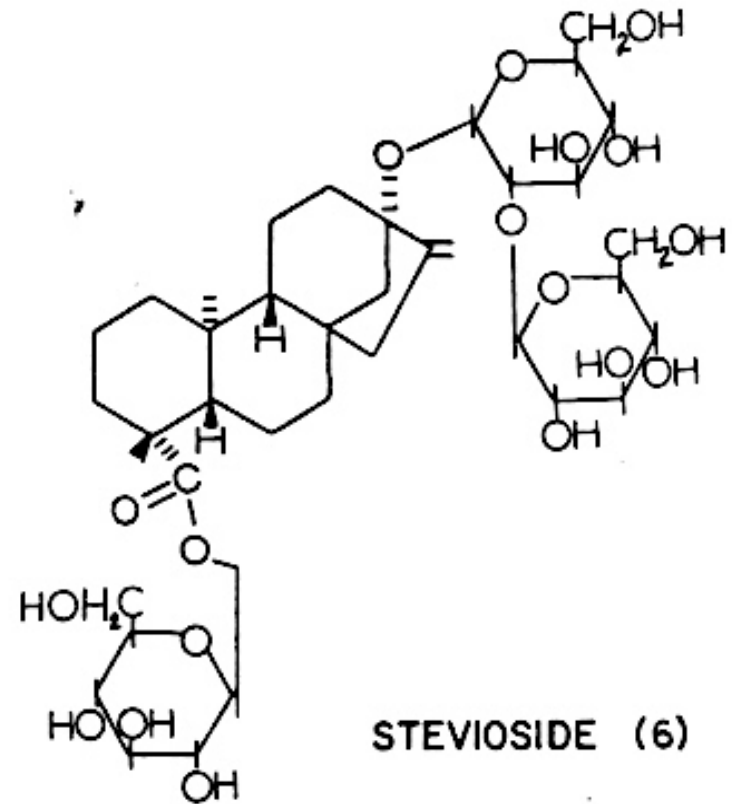


Stevia rebaudiana Bertoni

Sweet principle:

Stevioside

- Sweet *ent*-kaurene glycoside
- Stevioside – the most abundant sweet constituent of this plant (9%). It is stable over the pH range 3-9 for 1 hour at 100°C. Over 90% pure stevioside also exhibit a persistent aftertaste along with some bitterness. The sweetness intensity: 150-300 times sweeter than sucrose. Acute toxicity: LD₅₀ = 8.2 g/kg



Glycyrrhiza glabra L.

- **Family:** *Fabaceae*
- **Synonym:** *Glycyrrhiza glabra* L. var. *glabra*
- **Vernacular names:** Common licorice, licorice (En.)
- **Origin:** Mediterranean region
- **Description:** Perennial herb up to 1.8 m tall, has dark green leaflets, yellow, blue, or violet flowers, and sweet-flavoured rhizomes.
- **Uses:** natural sweetener in food and pharmaceutical industry; *medicine* - demulcent, diuretic, emollient, expectorant, laxative, pectoral, and stomachic agent

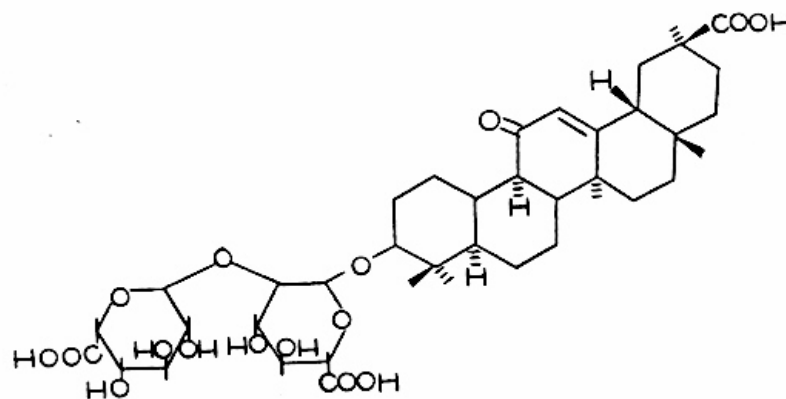


Glycyrrhiza glabra L.

Sweet principle:

Glycyrrhizin

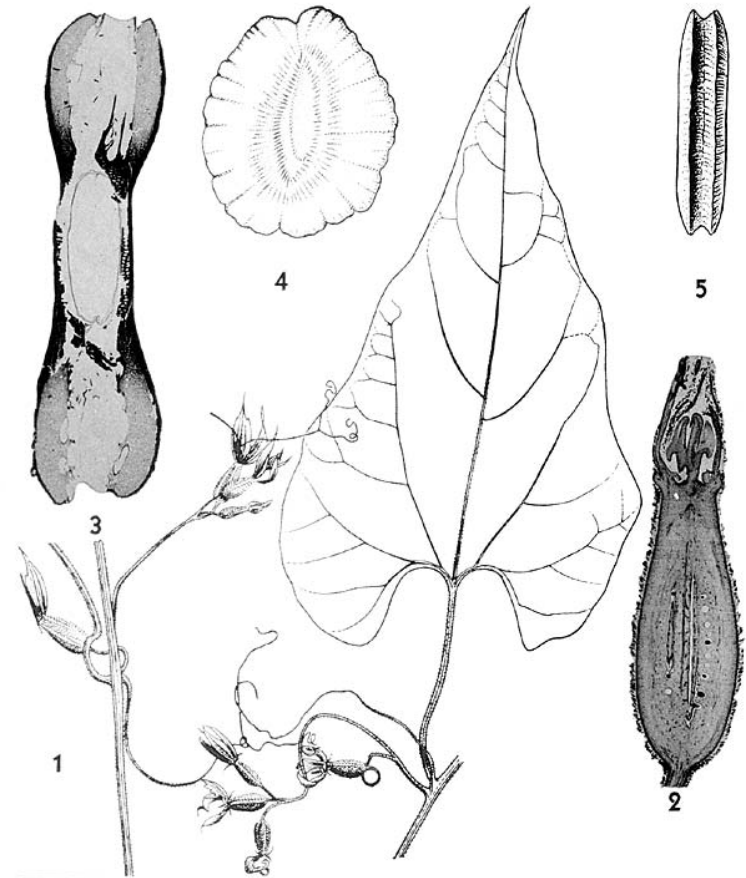
- Oleanane-type triterpenoid saponin
- Occurs in the roots in high yields (6-14%)
- 50-100 times sweeter than sucrose, it has a very slow onset of taste and a long aftertaste.
- Ammoniated glycyrrhizin is 50 times sweeter than sucrose and is considered GRAS (generally regarded as safe) by US Food and Drug Administration for use as a flavouring compound.



GLYCYRRHIZIN (7)

Momordica grosvenori Swingle

- **Family:** *Cucurbitaceae*
- **Synonym:** *Siraitia grosvenorii* (Swingle), *Thladiantha grosvenorii* (Swingle) C. Jeffrey
- **Vernacular names:** arhat fruit, longevity fruit (En.), luo han guo (Chinese)
- **Origin:** Southern China
- **Description:** Perennial, dioecious, herbaceous climbing vine, 2–5 m. in length. Roots tuberous, fusiform when young, finally subglobose, 10–15 cm in diameter.
- **Uses:** Low calorie sweetening agent in other juices or drinks, or it can be made into a desirable beverage itself. Dried fruit used often in Cantonese soups.



Momordica grosvenori Swingle

Sweet principle:

Mogroside IV and V

- Cucurbitane-type triterpenoid glycosides
- In aqueous extracts of the fruits
- Mogroside V – more abundant and more water soluble; it occurs in yields of greater than 1% of dried fruits
250 times sweeter than sucrose

Abrus precatorius L.

- **Family:** *Fabaceae*
- **Vernacular names:** Indian licorice, jequirity bean, crab's eye (En.), Akar saga (Malaysia)
- **Origin:** tropical Asia
- **Description:** a woody climber up to 6-9 m long, stem 1.5 cm in diameter, leaves with 16-34 oblong or ovate leaflets; flowers in dense robust clusters; fruit oblong 1-7 seeded pod; seeds ovoid scarlet with area around hilum black.
- **Uses:** *medicinal* – treatment of conjunctivitis, aphta and asthma, leaves – natural sweetener; seeds are used in ornamentals



Abrus precatorius L.

Sweet principle:

Abrusosides A-D

- Sweet-tasting cycloartane-type triterpene glycosides
- Abrusosides A-D were rated as being, respectively, 30, 100, 50 and 75 times sweeter than 2% sucrose and exhibit a pleasant sweet taste without any bitterness but they have delayed onset of sweet taste
- Yield < 1% in dry leaves

Dihydroisocoumarin

Hydrangea macrophylla Seringe var. *thunbergii* (Siebold) Makino

- **Family:** *Saxifragaceae*
- **Vernacular names:** Amacha
- **Origin:** Japan and China
- **Description:** Shrub about 4 m tall; leaves – elliptic to broadly ovate or obovate, 7-15 cm long, coarsely toothed and almost glabrous. Flowers – pink or blue; propagation by cuttings
- **Uses:** leaves are used in Japan to make a sweet tea which is imbibed in certain religious ceremonies

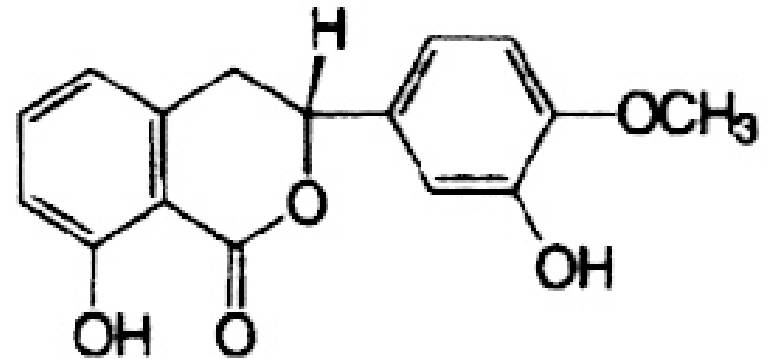


Hydrangea macrophylla Seringe var. *thunbergii* (Siebold) Makino

Sweet principle:

Phyllodulcin

- Obtained from fermented or crushed young leaves
- It is rated as 400 times sweeter than 3% dilution of sucrose
- But has several problems as an intense sweetener – delayed onset of sweetness, lingering aftertaste and a low solubility in water



5 Phyllodulcin

Flavonoids

- From the peel of *Citrus aurantium* L. and *C. paradisi* Macfad. (Rutaceae) naturally occurring flavanone glycosides: **Neohesperidin** and **Naringin** are bitter. Extraction with dilute alkali and hydrogen leads to intensely sweet dihydrochalcones. **Neohesperidin dihydrochalcone** is 250 up to 1,800 times sweeter than sucrose depending upon concentration. Is used as a saccharin replacement for utilization in chewing gums and various beverages.

Sweet-Tasting Proteins

- The existence, in nature, of sweet-tasting proteins has been known for many years. All of these proteins have been found in the fruits of tropical plants, and the indigenous peoples have frequently used them to sweeten their foodstuffs.
- With the commercialisation of Thaumatin, both as a sweetener and as a flavour enhancer, there has been an increase in the interest in these compounds.
- In recent years, an extraordinary number of sweet-tasting proteins have been discovered, studied, purified and characterized. Their genes have been cloned and sequenced, and in many cases have expressed in foreign hosts.

Thaumatococcus daniellii Benth

- **Family:** *Marantaceae*
- **Vernacular names:** sweet prayer plant, Katamfe
- **Origin:** tropical rainforests of West Africa
- **Description:** perennial herb with fleshy rhizome; the footstalk up to 1-2.5 m tall with alternate oblong leaves. Inflorescence – terminal spikes with violet flowers; fruit – 3-lobed capsule containing black seeds covered with yellow sweet aril.
- **Uses:** sweetening agent and substitution for agar



Thaumatococcus daniellii Benth

Sweet principle:

Thaumatocin I and II

- Protein 3 000 times sweeter than sucrose
- It has lingering aftertaste that might not be considered acceptable by some palates
- According to current price of thaumatin obtained by extraction from its natural source, microbial production of this protein would only be economically feasible if the recombinant microbes could produce 1 g of product per litre.

Dioscoreophyllum cumminsii Diels.

- **Family:** *Menispermaceae*
- **Vernacular names:** Serendipity Berry
- **Origin:** tropical West Africa
- **Description:** dioecious, herbaceous perennial plant, it has long, thin, twining stems and grows in the humid and heavily shaded understory vegetation of closed forests. The aerial vegetation dies back in the dry season and tubers resprout to produce climbing vines at the onset of the rains. Red berries are born in clusters of up to 100. The mucilage within each berry is intensely sweet.
- **Uses:** sweetening agent

Dioscoreophyllum cumminsii Diels.

Sweet principle:

Monellin

- Protein – 3,000 times sweeter than sucrose
- It has been shown to lose its sweetness when heated above 50°C at acidic pH.

Other sweet-tasting proteins

- *Capparis masaikai* Levl bears fruits that contains sweet-tasting protein **Mabinlin** (100 times sweeter than sucrose)
- *Pentadiplandra brazzeana* Baillon provide two sweet proteins from it's fruit – **Pentadin** and **Brazzein** (500 times sweeter than sucrose)
- *Curculigo latifolia* (Ridl.) D.J.L.Geerinck provide sweet and also sweet-modifying protein **Curculin** – it is able to turn sour taste into sweet one similarly like **Miraculin**
- *Synsepalum dulcificum* Baill. It's fruits contain a taste-modifier named **Miraculin**. Miraculin by itself does not elicit a sweet response. Like curculin however it can modify a sour taste into a sweet taste.

Conclusions

- **Future research needs:**
 - **New methods for detection of nonsacchariferous sweet compounds** (method based on evaluation of content of saccharides and polyols)
 - **Safety of natural sweeteners** (cheaper and faster methods)
 - **Evaluation of sweetness** (High Throughput Screening – immunoassays)
 - **Industrial production of sweet-tasting proteins through genetically modified microorganisms**

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