Stevia rebaudiana: Its agricultural, biological, and chemical properties

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Brandle, J. E., Starratt, A. N. and Gijzen, M. 1998. ***Stevia rebaudiana*: Its agricultural, biological, and chemical properties.** Can. J. Plant Sci. **78**: 527–536. *Stevia rebaudiana* is a member of the Compositae, native to Paraguay. It produces a number of high-potency low-calorie sweeteners in its leaf tissue. The sweeteners are diterpene glycosides and range between 30 and 320 times sweeter than sugar. Increasing consumer interest in natural food ingredients means that products like stevia sweeteners will be subject to increasing demand. Such demand will need to be supported by a modern mechanised production system. The purpose of this review is to summarize the existing agricultural, chemical and biochemical literature to provide a baseline for new research.

Key words: Stevia, diterpene, steviol glycoside, sweeteners

Brandle, J. E., Starratt, A. N. et Gijzen, M. 1998. ***Stevia rebaudiana* : qualités agricoles, biologiques et chimiques**. Can. J. Plant Sci. **78**: 527–536. Stevia rebaudiana, plante originaire du Paraguay, appartient à la famille des Composées. Il produit dans les tissus foliaires un certain nombre d'agents édulcorants puissants mais hypocaloriques. Ces produits, des glycosides diterpéniques sont de 30 à 320 fois plus sucrés que le sucre. Compte tenu de l'intérêt croissant du consommateur pour les ingrédients alimentaires naturels, la demande de produits comme les édulcorants du stevia ne pourra qu'aller en croissant, mais il faudra que cette demande soit étayée par un système moderne de production mécanisée. Voulue comme une base pour les recherches futures, cette mise au point vise à compulser la bibliographie actuelle de cette espèce sur les plans tant agricoles que chimiques et biochimiques.

Mots clés: Stevia, diterpène, glycoside de steviol, édulcorants

The worldwide demand for high-potency sweeteners is expected to increase, especially with the new practice of blending different sweeteners. The sweet herb of Paraguay, *Stevia rebaudiana* Bert. produces, in its leaves, just such an alternative with the added advantage that stevia sweeteners are natural plant products. In addition, the sweet steviol glycosides have functional and sensory properties superior to those of many other high-potency sweeteners. Stevia is likely to become a major source of high-potency sweetener for the growing natural food market in the future. The task at hand is to convert stevia from a wild plant to a modern crop well suited to efficient mechanized production. For Canada, the necessary steps are the development of a seed, seedling and crop production system, including information on optimized crop inputs, weed and disease control, harvest and handling methods and a breeding program aimed at optimizing glycoside content and sensory characteristics. Understanding the biology of the stevia plant and the chemistry and biochemistry of the sweet glycosides are prerequisites for conversion of stevia to a modern crop. This review summarizes the existing literature and provides a baseline for new research.

BIOLOGY, ETHNOBOTANY AND HISTORY OF CULTIVATION

Stevia rebaudiana Bert. is one of 154 members of the genus *Stevia* and one of only two that produce sweet steviol glycosides (Robinson 1930; Soejarto et al. 1982, 1983). It is native to the valley of the Rio Monday in the highlands of Paraguay, between 25 and 26° S latitude, where it grows in sandy soils near streams (Katayama et al. 1976). Stevia was first brought to the attention of Europeans in 1887 when M. S. Bertoni learned of its unique properties from the Paraguayan Indians and Mestizos (Lewis 1992). Various reports cited by Lewis (1992) indicate that it was long known to the Guarani Indians of the Paraguayan highlands who called it *caá-êhê*, meaning "sweet herb". The leaves were used either to sweeten *maté* or as a general sweetening agent. Seeds were sent to England in 1942 in an unsuccessful attempt to establish production. The first reports of commercial cultivation in Paraguay were in 1964 (Katayama et al. 1976; Lewis 1992). A large effort aimed at establishing stevia as a crop in Japan was begun by Sumida (1968). Since then, stevia has been introduced as a crop in a number of countries including Brazil, Korea, Mexico, United States, Indonesia, Tanzania, and since 1990, Canada (Lee et al. 1979; Donalisio et al. 1982; Schock 1982; Goenadi 1983; Saxena and Ming 1988; Brandle and Rosa 1992; Fors 1995). Currently, stevia production is centered in China and the major market is in Japan (Kinghorn and Soejarto 1985). No large-scale mechanized production has been established and stevia sweeteners are not yet found in maintstream food

Abbreviations: **CPP**, copalyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate

products in most countries of the world. Progress towards large-scale commercialization has been slow, largely due to difficulties in producing the crop, the poor quality of stevia extracts and the absence of regulatory approvals essential for stevia sweeteners in the North American and European markets.

Stevia is a member of the Compositae family. It is a small shrubby perennial growing up to 65 cm tall, with sessile, oppositely arranged lanceolate to oblancoelate leaves, serrated above the middle. Trichome structures on the leaf surface are of two distinct sizes, one large (4–5 μm), one small (2.5 μm) (Shaffert and Chetobar 1994b). The flowers are small (7–15 mm), white and arranged in an irregular cyme. The seed is an achene with a feathery pappus (Robinson 1930).

Stevia is an obligate short-day plant with a critical day length of about 13 h. Extensive variability within populations for day length sensitivity has been reported (Valio and Rocha 1966; Zaidan et al. 1980). Plants can initiate flowering after a minimum of four true leaves have been produced (Carneiro 1990). Sumida reported the results from a complete diallel cross with eight parents and found that the amount of selfing ranged between 0 and 0.5%, while outcrossing ranged from 0.7 to 68.7%, indicating that some form of self-incompatibility system is operating (cited in Katayama et al. [1976]). The reproductive anatomy of the male and female gametophytes is typical for angiosperms (Shaffert and Chetobar 1992, 1994a). Stevia is diploid and has 11 chromosome pairs, which is characteristic for most of the South American members of the genus (Frederico et al. 1996).

CROP PRODUCTION

Stevia plants can be propagated from cuttings or seed. Since germination rates are poor and seedlings very slow to establish it is best grown as an annual or perrenial transplanted crop. Clonal propagation is practical for small scale production, but is probably not economically viable for large scale stevia production where labor costs are high. The cost of producing vegetatively propagated transplants in Canada is high, so low cost transplants produced from seed is the only viable method on which to base stevia production in Canada. Only production as an annual is possible in most regions of Canada. The discussion of crop production in this review will therefore be limited to seed-based propagation of an annual transplanted crop.

In the temperate latitudes of the Northern hemisphere and South Western Ontario in Canada more specifically, the production cycle for annual stevia begins with 6- to 7-wk-old plants grown from seed, in cells, in heated greenhouses. Seedlings are transplanted to the field in mid- to late May. Fertilizer is banded along with the transplants. The crop is irrigated as required. Stevia is slow to establish under Canadian conditions and growth is sluggish until mid-July. Most of the leaf yield is accumulated from July until mid- to late September. The whole plant is harvested just above ground level, elevated into wagons and then dried. Following drying, the leaves are separated from the stems using a thesher. The leaves are then stored ready for processing.

Seed Production and Quality

Given stevia's daylength requirements, seed production in the Northern hemisphere would be best situated between 20 and 30°N latitude. The crop could be transplanted in February or March and seed collected in late summer. Flowering under these conditions should occur between 54 and 104 d following transplanting, depending on the daylength sensitivity of the cultivars used for seed production (Katayama et al. 1979). One-thousand-seed weights for stevia seed usually range between 0.15 and 0.30 g and, depending on plant density, seed yields of up to 8.1 kg ha^{-1} are possible (Carniero 1990). Seed germination is often poor and rates of less than 50% are common (Miyazaki and Wantenabe 1974). Given the aforementioned conditions, seed produced on 1 ha could be enough to supply transplants for up to 200 ha of leaf production. Seed viability and yield are affected by growing conditions during pollination and seed filling. Excessive rainfall during pollination can affect both seed yield and germination (Carneiro 1990; Shuping and Shizhen 1995). Seed is best stored at 0°C, but even under low temperature conditions germination will still decline 50% over 3 yr (Shuping and Shizhen 1995). Sealing of storage containers or using lower temperatures did not prevent the decrease in germination over time.

Cultural Practices

Planting densities ranging from 40 000 to 400 000 plants ha^{-1} have been tried in experiments conducted in Japan (Katayama et al. 1976). Leaf yield increased with increasing density up to 83 000 and 111 000 plants ha^{-1} for the first year of production. The concentration of stevioside in the leaves of stevia increases when the plants are grown under long days (Metvier and Viana 1979). Since glycoside synthesis is reduced at or just before flowering, delaying flowering with long days allows more time for glycoside accumulation. It follows that stevia production would be best situated in a long-day environment where vegetative period is longer and steviol glycoside yields will be higher.

Fertility requirements for stevia grown as an annual crop are moderate. Results from Japan demonstrate that, at the point of maximum dry matter accumulation, stevia plants consist of 1.4% N, 0.3% P, and 2.4% K (Katayama et al. 1976). In Ontario, total biomass production of 7500 kg ha^{-1} are possible and of that total, 26% would be roots, 35% stems, and 39% leaves (R. Beyaert personal communication). Based on the composition observed by Katayama (1976) such biomass would require approximately 105 kg N, 23 kg P and 180 kg K from both soil and fertilizer. The actual rates of application will vary according to soil type and production environment, and need to be optimized for each specific situation.

Two fungal diseases, *Septoria steviae* and *Sclerotinia sclerotiorum*, have been reported in stevia grown in Canada (Lovering and Reeleder 1996; Chang et al. 1997). *Septoria* disease was characterized by depressed, angular, shiny olive gray lesions, sometimes surrounded by a chlorotic halo, that rapidly coalesce. *Sclerotinia* disease was characterized by brown lesions on the stem, near the soil line, followed by wilting and eventually by the complete collapse of affected

individuals. No means of controlling these diseases have yet been published. Since stevia is very slow to establish and does not compete well with weeds, herbicides or other means will be essential to control weed growth to produce ample yield and a clean crop. The herbicide trifluralin appears to be well tollerated by stevia (Katayama 1976).

Stevia is harvested just prior to flowering when steviol glycoside content in the leaves is at its maximum (Sumida 1980; Xiang 1983). Following harvest, the whole plant is dried and the leaves separated from the stems for further processing (Murai 1988). The stems have very low concentrations of sweet glycosides and are removed to minimize processing costs (Brandle and Rosa 1992). Drying stevia under artificial conditions is affected by a number of factors including loading rate, temperature, and ambient air conditions (Van Hooren and Lester 1992). The effect of drying conditions on glycoside levels or processing quality of the leaves has not been investigated.

Cultivar Development

A variety of plant-breeding procedures have been used to improve leaf yield and rebaudioside A concentration in the leaves. Based on cultivar descriptions from Japan, China and Korea and our own work, it appears that sufficient genetic variability exists to make significant genetic gains in leaf yield, rebaudioside A content and the ratio of rebaudioside A to stevioside (Brandle and Rosa 1992; Lee et al. 1979; Morita 1987; Shyu et al. 1994; Shizhen 1995). Brandle and Rosa (1992) found that the heritability of stevioside content to be high (83%), based on calculations from a group of half-sib families. Heritabilities for leaf yield (75%) and leaf-to-stem ratio (83%) were also substantial, indicating that selection would be effective. Total sweet glycoside concentration in some lines from China was reported to be as high as 20.5%, and a rebaudioside A to stevioside ratio of 9:1 was disclosed in the Japanese patent literature (Morita 1987; Shizhen 1995). Two breeding methods reported by the latter authors were phenotypic mass selection and recurrent selection for phenotype, where selected plants are intercrossed before another round of selection. Some cultivars, such as the high rebaudioside A selection from Japan and Suweon 2 and 11 from Korea, are based on the selection of a single plant and because of self-incompatibility can only be reproduced vegetatively, which limits their utility.

Nakamura and Tamura (1985) studied a population of 300 random individuals and found that total glycoside concentrations at the seedling and harvest stages were not correlated, suggesting that early selection for total glycosides would not be effective. However, the proportion of individual glycosides relative to the total was correlated between seedlings and mature plants making early selection for glycoside composition possible. The authors also observed a wide range of variation in the four main glycosides and found that dulcoside A and stevioside, and rebaudioside A and C, were positively correlated with each other. Stevioside and rebaudioside A, and dulcoside and rebaudioside C, were negatively correlated with each other. These correlations can be partially explained by the biosynthetic relationships between the individual glycosides. For example, stevioside is the substrate for the synthesis of rebaudioside A, plants high in rebaudioside A will probably be low in stevioside (Shibata et al. 1991).

THE CHEMISTRY OF THE DITERPENE GLYCOSIDE SWEETENERS

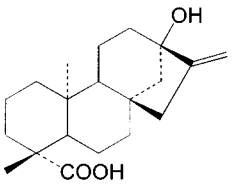
The sweet diterpene glycosides of stevia have been the subject of a number of reviews (Kinghorn and Soejarto 1985; Crammer and Ikan 1986; Hanson and De Oliveira 1993). Although interest in the chemistry of the sweet principles dates from early in the century, significant progress towards chemical characterization was not made until 1931, with the isolation of stevioside (Bridel and Lavieille 1931a). Treatment of this substance with the digestive juice of a snail yielded 3 mol of glucose and 1 mol of steviol, while acid hydrolysis gave isosteviol (Bridel and Lavieille 1931b). Isosteviol was also obtained when steviol was heated in dilute sulfuric acid. Subsequent studies have led to the isolation of seven other sweet glycosides of steviol. Typical proportions, on a dry weight basis, for the four major glycosides found in the leaves of wild stevia plants is 0.3% dulcoside, 0.6% rebaudioside C, 3.8% rebaudioside A and 9.1% stevioside.

Structure of Steviol, Isosteviol and Stevioside

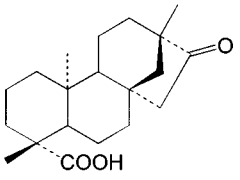
The structure, steriochemistry and absolute configuration of steviol and isosteviol were established, through a series of chemical reactions and correlations over 20 yr after the pioneering work of Bridel and Lavieille (Mosettig and Nes 1955; Dolder et al. 1960; Djerassi et al. 1961; Mosettig et al. 1963). Structures of these and other diterpenes and diterpene glucosides are presented in Fig. 1. Concurrent studies on the parent glycoside indicated that one D-glucopyranose residue, hydrolyzed under alkaline conditions yielding steviolbioside, was attached to a carboxyl group (Wood et al. 1955) while the other two were components of a sophorosyl group (Vis and Fletcher 1956) bound to the aglycone through a β-glycosidic linkage (Yamasaki et al. 1976). Support for the proposed stereochemistry was achieved by the synthetic transformation of steviol into stevioside (Ogawa et al. 1980). Earlier, several approaches to the in vitro synthesis of steviol had been reported (Cook and Knox 1970; Nakahara et al. 1971; Mori et al. 1972; Ziegler and Kloek 1977). Recently, spectroscopic data concerning stevioside and steviolbioside were published (Van Calsteren et al. 1993).

Other Diterpenoid Glycosides

Further investigation of extracts of *S. rebaudiana* leaves resulted in the isolation and identification of seven other sweet diterpenoid glycosides. Kohda et al. (1976) obtained the first two of these, rebaudiosides A and B, from methanol extracts together with the major sweet substance stevioside and steviolbioside, a minor constituent, which was first prepared from stevioside by alkaline hydrolysis (Wood et al. 1955). Subsequently, it was suggested that rebaudioside B was an artifact formed from rebaudioside A during the isolation (Kaneda et al. 1977; Sakamoto et al. 1977b).

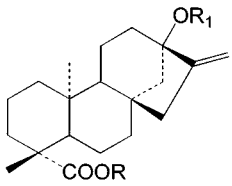


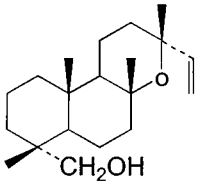
Steviol



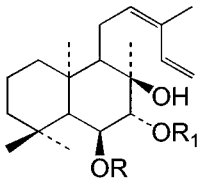
Isosteviol

	R	R_1
Stevioside	β-Glc	β-Glc(2)–β-Glc (3 β-Glc)
Steviolbioside	H	β-Glc(2)–β-Glc (3 β-Glc)
Rebaudioside A	β-Glc	β-Glc(2)–β-Glc (3 β-Glc)
Rebaudioside B	H	β-Glc(2)–β-Glc (3 β-Glc)
Rebaudioside C	β-Glc	β-Glc(2)–α-Rha (3 β-Glc)
Rebaudioside D	β-Glc(2)–β-Glc	β-Glc(2)–β-Glc (3 β-Glc)
Rebaudioside E	β-Glc(2)–β-Glc	β-Glc(2)–β-Glc
Dulcoside A	β-Glc	β-Glc(2)–α-Rha

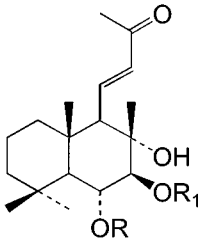




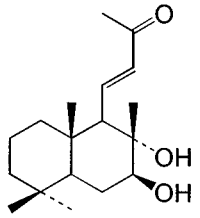
Jhanol



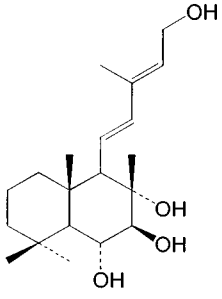
$R = R_1 = H$ Austroinulin
$R = Ac, R_1 = H$ 6-*0*-Acetylaustroinulin
$R = H, R_1 = Ac$ 7-*0*-Acetylaustroinulin



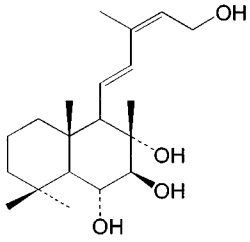
$R = R_1 = H$ Sterebin A
$R = Ac, R_1 = H$ Sterebin B
$R = H, R_1 = Ac$ Sterebin C



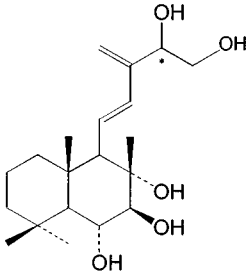
Sterebin D



Sterebin E



Sterebin F



Sterebin G
Sterebin H

Fig. 1. Structures precursors of the eight sweet glycosides, the glycosides themselves and those of other significant diterpenes found in the leaves of *Stevia rebaudiana*.

Stevioside has been converted by enzymatic and chemical procedures to rebaudioside A (Kaneda et al. 1977). Further fractionation of leaf extracts led to the isolation and identification, which was aided by ^{13}C NMR spectroscopy, of three other new sweet glycosides named rebaudioside C, D and E (Sakamoto et al. 1977a, b). Both rebaudioside A and rebaudioside D could be converted to rebaudioside B by alkaline hydrolysis showing that only the ester functionality differed (Kohda et al. 1976; Sakamoto et al. 1977b). Dulcosides A and B, the latter having the same structure as rebaudioside C, were reported by another laboratory (Kobayashi et al. 1977).

Methods of Diterpenoid Glycoside Analysis

A wide range of analytical techniques has been employed to assess the distribution and level of sweet diterpenoid glycosides in *S. rebaudiana*. These include thin layer chromatography (Metivier and Viana 1979; Tanaka 1982; Kinghorn et al. 1984; Nikolova-Damyanova et al. 1994), over-pressured layer chromatography (Fullas et al. 1989), droplet countercurrent chromatography (Kinghorn et al. 1982), and capillary eletrophoresis (Liu and Li 1995; Mauri et al. 1996).

Stevioside levels have also been determined enzymatically (Mizukami et al. 1982) and by near infrared reflectance spectroscopy (Nishiyama et al. 1992) in plants strains producing mainly stevioside. The most common analytical method, however, has been high performance liquid chromatography. Although separations have been also achieved using silica gel (Nikolova-Damyanova et al. 1994), hydroxyapatite (Kasai et al. 1987), hydrophilic (Hashimoto et al. 1978), and size exclusion (Ahmed and Dobberstein 1982a, b) columns, amino-bonded columns have been used most frequently for the analysis of the sweet glycosides (Kinghorn et al. 1984; Makapugay et al. 1984; Striedner et al. 1991; Liu and and Li 1995). Amino columns have also been used to measure stevioside and related glycosides in foods and beverages (Chang and Cook 1982; Fujinuma et al. 1986; Kitada et al. 1989). In our laboratories, a carbohydrate cartridge column with a propylamine bonded phase, has been used to analyze the diterpenoid glycosides in more than 4000 stevia leaf samples (W.A. Court, unpublished data). Stevioside and rebaudioside A have also been analyzed by HPLC after conversion to the *p*-bromophenacyl esters of steviolbioside and rebaudioside B (Ahmed et al. 1980).

OTHER CONSTITUENTS

In addition to the sweet diterpenoid glycosides, several other diterpenes have been isolated from stevia. Since these compounds may be part of the waste stream produced during stevia processing, their availability in large quantitites could make them into valuable co-products. The first to be characterized were jhanol and austroinulin, previously obtained from other plants, and 6-*O*-acetylaustroinulin (Sholichin et al.1980). Also reported were the triterpenes β-amyrin acetate and three esters of lupeol and the sterols stigmasterol and β-sitosterol, previously isolated from leaves by Nabeta et al. (1976). Jhanol, austroinulin, 6-*O*-acetylaustroinulin and 7-*O*-acetylaustroinulin as well as steviosde and rebaudioside A have been obtained from stevia flowers (Darise et al. 1983). Eight additional diterpenes, called sterebins A–H, have been isolated from leaves and identified (Oshima et al. 1986, 1988).

Other chemical constituents of stevia have been reported. Rajbhandari and Roberts (1983) identified six flavonoid glycosides in an aqueous methanol extract of leaves: apigenin-4′-*O*-glucoside, luteolin-7-*O*-glucoside, kaempferol-3-*O*-rhamnoside, quercitrin, quercetin-3-*O*-glucoside and quercetin-3-*O*-arabinoside and 5, 7, 3′-trihydroxy-3, 6, 4′-trimethoxyflavone (centaureidin). The major identified components in the essential oil were the sesquiterpenes β-caryophyllene, trans-β-farnesene, α-humulene, δ-cadinene, caryophyllene oxide and nerolidol and the monoterpenes linalool, terpinen-4-ol and α-terpineol (Fujita et al. 1977). Later, Martelli et al. (1985) identified 54 components of a steam distillate of dried leaves from Brazil. Of these, caryophyllene oxide and spathulenol were the main components, totaling 43%. Interestingly, these substances were not the major components in an essential oil preparation from a fresh sample of cultivated stevia plants from Italy.

BIOSYNTHESIS OF THE SWEET GLYCOSIDES

Steviol glycosides are derived from the mevalonic acid pathway. This is a fundamental metabolic route that provides the two C_5 building block molecules, isopentenyl pyrophosphate and dimethylallyl pyrophosphate, that are required for synthesis of all isoprenoid compounds (Chappell 1995; McGarvey and Croteau 1995).

Steviol Biosynthesis from Geranylgeranyl Pyrophosphate

Steviol biosynthesis was first investigated more than 30 yr ago (Fig. 2) (Ruddat et al. 1965; Bennett et al. 1967; Hanson and White 1968). This early work established that the initial steps leading to the steviol glycosides from GGPP are identical to those in gibberellin biosynthesis. Thus, GGPP is converted to *ent*-copalyl pyrophosphate (CPP) by CPP synthase (also called *ent*-kaurene synthase A) and *ent*-kaurene is produced from CPP by *ent*-kaurene synthase (also called ent-kaurene synthase B). Subsequent oxidation of this product at the C-19 position to *ent*-kaurenoic acid is assumed to occur via the action of one or more P450 monooxygenases that have yet to be identified (Hedden and Kamiya 1997). At this point the pathways to the steviol glycosides and the gibberellins diverge. Steviol is produced by further hydroxylation of *ent*-kaurenoic acid at the C-13 position. This *ent*-kaurenoic acid 13-hydroxylase has been purified from stevia leaf extracts and partially characterized (Kim et al. 1996a). The native enzyme is a 160 kD homotetramer that requires NADPH and O_2 for catalysis.

The Glycoside Side Chains

The two oxygenated functional groups of steviol, the C-19 carboxylate and the C-13 alcohol, provide attachment points for the sugar side chains that determine the identity of the eight different glycosides identified to date. These glycan side chains are comprised predominately of glucose residues but may also contain rhamnose (Fig. 1). The biosynthetic

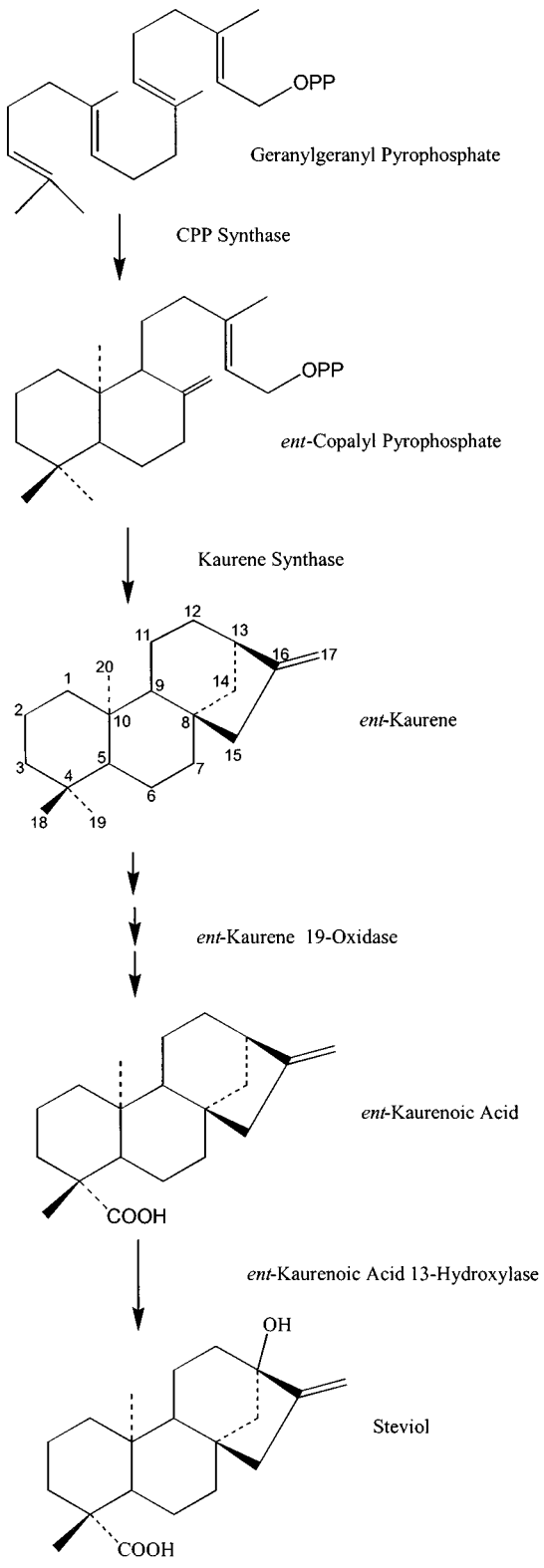


Fig. 2. The enzymes and chemical changes involved in the biosynthesis of steviol, the precursor for all of the sweet glycosides of stevia, from geranylgeranyl pyrophosphate.

sequence of glycosylations that gives rise to the different glycan side chains is still in the early stages of elucidation. At least three distinct glycosyltransferase activities have been identified (Shibata et al. 1991, 1995). Two of these activities have been purified and characterized. Activity I transfers glucose from UDP-glucose to the 13-hydroxy position of steviol to afford steviolmonoside. Activity IIb has a much broader substrate specificity, using steviol, steviolmonoside, steviolbioside, or stevioside as substrate for further glycosylation by UDP-glucose (Shibata et al. 1995).

Compartmentation of Biosynthesis and Storage

Diterpene biosynthesis has been found to occur generally in plastids of plant cells (McGarvey and Croteau 1995; Hedden and Kamiya 1997). There is good evidence that steviol biosynthesis conforms to this pattern and is localized in leaf chloroplasts. High levels of HMG-CoA reductase activity can be extracted from isolated stevia chloroplasts and the *ent*-kaurenoic acid 13-hydroxylase that converts *ent*-kaurenoic acid to steviol was purified from the chloroplast stroma (Kim et al. 1996a, b). In contrast, the UDP-glucosyl transferases performing the glycosylations on the steviol skeleton are operationally soluble enzymes, indicating that these reactions happen outside of the chloroplast. Steviol glycosides are transported to the cell vacuole where they are stored. The glycosides accumulate in stevia leaves where they may comprise from 10 to 20% of the leaf dry weight. Thus, a large fraction of total plant metabolism is committed to the synthesis of these structurally complex molecules. The conditions that favoured selection of such high diterpene glycoside producers are not known. Like other plant secondary metabolites, the steviol glycosides may function in a defensive capacity as feeding deterrents or anti-microbial agents against specific herbivores, pests, or pathogens.

FUNCTIONAL AND SENSORY PROPERTIES OF STEVIOL GLYCOSIDE SWEETENERS

Of the four major sweet diterpene glycoside sweeteners present in stevia leaves only two, stevioside and rebaudioside A, have had their physical and sensory properties well characterized. Stevioside and rebaudioside A were tested for stability in carbonated beverages and found to be both heat and pH stable (Chang and Cook 1983). However, rebaudioside A was subject to degradation upon long-term exposure to sunlight. Kinghorn and Soejarto (1985) also cite numerous Japanese studies that demonstrate that stevioside is very stable.

Phillips (1989) summarized the early sensory research. Stevioside was between 110 and 270 times sweeter than sucrose, rebaudioside A between 150 and 320 times sweeter, and rebaudioside C between 40 and 60 times sweeter, Dulcoside A was 30 times sweeter than sucrose. Rebaudioside A was the least astringent, the least bitter, had the least persistant aftertaste and was judged to have the most favourable sensory attributes of the four major steviol glycosides (Phillips 1989; Tanaka 1997). Dubois and Stephanson (1984) also confirmed that rebaudioside A is less bitter than stevioside and demonstrated that the bitter notes in stevioside and rebaudioside A are an inherent prop-

erty of the compounds and not necessarily the result of impurities in whole-plant extracts. Relative to other high potency sweeteners such as aspartame, bitterness tends to increase with concentration for both stevioside and rebaudioside A (Schiffman et al. 1994). Both stevioside and rebaudioside A are synergistic in mixtures with other high-potency sweeteners such as aspartame and are good candidates for inclusion in blends (Schiffman et al. 1995). Although specialty applications may exist for the other glycosides, increasing levels of rebaudioside A in stevia leaves is a clear objective for breeding work.

COMMERCIAL EXTRACTION OF STEVIOL GLYCOSIDES

Most of the commercial processing of stevia leaves occurs in Japan and there are dozens of patents describing methods for the extraction of steviol glycosides. Kinghorn and Soejarto (1985) have categorized the extraction patents into those based on solvent (Haga et al. 1976), solvent plus a decolorizing agent (Ogawa 1980), adsorption chromatography (Itagaki and Ito 1979), ion exchange (Uneshi et al. 1977), and selective precipitation of individual glycosides (Matsushita and Kitahara 1981). Phillips (1989) indicated that the most favored extraction processes involve four steps: aqueous or solvent extraction, ion exchange, precipitation or coagulation with filtration, then crystallization and drying. New methods based on ultra-filtration have been disclosed recently (Tan and Ueki 1994).

SAFETY OF STEVIA SWEETENERS

Stevia sweeteners have a long history of use in South America and now in Japan and there are no reports of adverse effects. Nonetheless, the safety of stevia sweeteners has been the subject of controversy for a number of years (e.g. Pendergast 1991; Bonvie et al. 1997). Planas and Kuc (1968) reported that a 5% solution of stevia leaf extract had a strong anti-fertility effect in both male and female rats. Subsequent studies conducted to confirm this result have all been negative (Sincholle and Marcorelles 1989; Yodyingyuad and Bunyawong 1991). In studies of acute toxicity, a LD_{50} of 8.2 g kg^{-1} for a refined stevioside extract was cited by Katayama et al. (1976). An acceptable daily stevioside intake of 7.9 mg kg^{-1} was suggested by Xili et al. (1992). Yodyingyuad and Bunyawong (1991) reported that neither growth nor reproduction was affected in hamsters fed pure stevioside at levels up to 2.5 g kg^{-1} d^{-1} for 4 mo. Stevioside and rebaudioside A are both non-cariogenic (Das et al. 1992).

Pezzuto and co-workers (1985) reported that metabolically activated steviol is mutagenic, a result that has been confirmed in another more recent study (Matsui et al. 1996). Kinghorn and Soejarto (1985) and Kinghorn (1992) conducted two reviews of the literature related to safety of stevia sweeteners and concluded that leaves and stevioside are safe for human consumption. However, the activated steviol metabolite that is mutagenic has not yet been identified and it is not known if the activation of steviol actually occurs in humans (Procinska et al. 1991; Matsui et al. 1996). Matsui et al. (1996) concluded that further work is required to determine what risk steviol glycosides pose to humans.

CONCLUSION

Stevia represents a new opportunity for researchers and farmers alike. A great deal of information relating to production practices and disease control is required to optimize annual transplant production for Canada. Such basic things as herbicide and fungicide registration, optimum planting and harvest times and fertilizer recommendations are all essential. Since markets now exist for stevia, production and optimization must occur in parallel. The production of remarkably high levels of one class of secondary metabolite is of significant interest to chemists, biochemists and geneticists and may prove to be a foundation for the production of new metabolites in the future. Because the safety of stevia for human consumption remains controversial, there is a clear need for further experimentation with respect to the metabolic fate of steviol glycosides.

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