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Highly Sweet Compounds from North and South American Medicinal Plants*

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Abstract—Nearly 50 highly sweet substances have been isolated and structurally characterized from green plants, and such compounds comprise mainly various types of terpenoids, flavonoids, and proteins. Among the sweet substances that have been studied as constituents of North and South American medicinal plants are the sesquiterpene, hernandulcin, the triterpene glycosides, abrusosides A-D, the steroidal saponins, polypodosides A and B, and the dihydroflavonol, dihydroquercetin-3-acetate. In addition, safety studies have been performed on the potently sweet substance, stevioside, from the "sweet herb of Paraguay" (*Stevia rebaudiana*), a compound now produced on a commercial scale.

Keywords—Green plants • sweet constituents • hernandulcin • abrusosides A-D. polypodosides A and B • sweet dihydroflavonols • *Stevia rebaudiana* • stevioside • steviol • safety assessment

Introduction

There is a great societal demand for new noncaloric and noncariogenic "intense" sweetening agents, for use in diabetic, dietetic and oral hygeine products. This is because the consumption of sucrose is increasing on a worldwide basis, both as a nutritional agent and as a sweetener, and it is now recognized that this substance is the primary cause of dental caries in many countries.[1,2] Strict criteria must be adhered to before the introduction of a synthetic or naturally occurring sucrose substitute onto the market. Thus, in addition to being pleasantly sweet like sucrose, such compounds should have no toxic or cariogenic effects, when either in the unmetabolized or metabolized form, and should be odorless and colorless, and exhibit liberal water solubility and hydrolytic and thermal stability. Furthermore, each new sweetener should be economical to either synthesize or extract from a readily cultivable plant of origin, so that is economically competitive with already-used sweeteners in terms of costs of production. Finally, a new sweet compound should be adaptable to existing technology for the application of sweeteners.[3] No single artificial or natural sucrose substitute adheres to all of these diverse criteria. Shortcomings are

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therefore evident, with respect to either perceived safety, quality of sweetness, chemical stability, and/or cost of production, by all of the major currently approved sweeteners in western countries, namely, saccharin, aspartame, acesulfame K, and cyclamate.[3~5]

There are presently nearly 50 natural products, within some 15 distinct structural classes, that are known to be intensely sweet (i.e., at least 50 times sweeter than sucrose). Not included in the category of intense sweeteners are the sugar and sugar alcohol plant-derived bulk sweeteners. Naturally occurring highly sweet compounds have been only found as constituents of green plants (ferns, monocotyledons and dicotyledons) to date, and occur in an apparent randomly distributed manner in about 20 plant families. The currently known intensely sweet plant constituents are mainly terpenoids, flavonoids and proteins.[6~8] A number of potently sweet plant-derived substances have are commercial use in one or more countries as sweetening, flavoring, or taste-modifying agents, such as the triterpene glycoside, glycyrrhizin (from *Glycyrrhiza glabra* L., Fabaceae); stevioside, a diterpene glycoside from *Stevia rebaudiana* (Bertoni) Bertoni (Asteraceae); thaumatin, a protein from *Thaumatococcus daniellii* (Bennett) Benth. (Marantaceae); and phyllodulcin, a dihydroisocoumarin from *Hydrangea macrophylla* Seringe var. *thunbergii* (Siebold) Makino (Saxifragaceae). Modified highly sweet plant constituents also have use as additives for foodstuffs, medicines, or tobacco, such as ammonium glycyrrhizin, the fully ammoniated salt of glycyrrhizin; perillartine, the *α-syn*-oxime of perillaldehyde, a compound obtained from the essential oil of *Perilla frutescens* (L.) Britton (Labiatae); and neohesperidin dihydrochalcone, which is obtained from the flavonone glycoside, neohesperidin, isolated from the Seville orange, *Citrus auranthicum* L. (Rutaceae).[6~8]

While Tanaka and co-workers at Hiroshima University in Japan have found various Chinese medicinal plants to be excellent sources of novel highly sweet substances like baiyunoside, carnosifloside V and rubusoside,[7] we have concentrated our efforts to discover new natural sweeteners on North and South American medicinal plants. In the following paragraphs, several novel terpenoidal (hernandulcin, abrusosides A-D), steroidal saponin (polypodosides A and B) and dihydroflavonol (e.g., quercetin 3-acetate 4′-methyl ether) intense sweeteners investigated in our laboratory will be mentioned. Also reviewed are the approaches we have taken toward the discovery of new natural sweeteners, and several of the problems we have encountered. Finally safety studies carried out to date will be discussed on the commercially important natural sweetener, stevioside (obtained from the Paraguayan plant, *Stevia rebaudiana*).

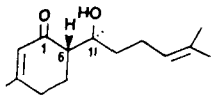
Novel Sweet Compounds from North and South American Plants Hernandulcin— Hernandulcin (**1**), the first known intensely sweet sesquiterpene, was discovered as a sweet constituent of *Lippia dulcis Trev.* (Verbenaceae), collected in Mexico, a plant known to the Aztecs as sweet-tasting herb.[9] Today, *L. dulcis* is sold in Mexican market-places for the treatment of coughs and for its reputed abortifacient activity.[10] Hernandulcin (**1**) was named in honor of the Spanish physician, Franscisco Hernández, who catalogued a number of New World medicinal plants in the 16th century, inclusive of *L. dulcis.* This non-polar sweet substance was isolated from the leaves and flowers of this plant after solvent partition into petroleum ether and chromatographic fractionation over silica gel. The structure of hernandulcin (**1**) was established as 6-(1, 5-dimethyl-1-hydroxyhex-4-enyl)-3-methylcyclohex-2-enone, as a result of the application of high-resolution mass spectrometry and two-dimensional NMR

spectroscopy. Racemic hernandulcin was synthesized in about 50% yield by directed aldol condensation with 3-methyl-2-cyclohexen-1-one and 6-methyl-5-hepten-2-one as starting materials. This reaction occurred in a predictable stereospecific manner, with larger amounts of hernandulcin (**1**) being produced than the epimeric compound, **2** (epihernandulcin). Synthetic (±)-hernandulcin (**1**) was not acutely toxic for mice at the doses tested, and was not active as a bacterial mutagen, both in the presence and absence of a metabolic activating system. Natural (+)-hernandulcin (**1**) was rated by a trained human panel as about 1,000 times sweeter than sucrose on a molar basis, although it was also found to exhibit certain undesirable hedonic effects such as possessing some bitterness and an unpleasant aftertaste.[9,11] Both natural (6S, 1'S)-[12,13] and (±)-hernandulcin[14] have served as synthetic target molecules for other research groups.

In response to the unpleasant taste of this new sweetener, several derivatives of hernandulcin (**1**) were synthesized in an effort to obtain sweet compounds with improved hedonic properties.[11,15] While none of the hernandulcin synthetic analogs turned out to be sweet, conclusions have been made on the role of the various functionalities of **1** in the exhibition of sweetness. Hernandulcin (**1**) is a very useful sweet molecule for this type of study, because of its limited number of functional groups. According to the hypothesis of Shallenberger and Acree, a characteristic of nearly all sweet substances is the presence of proton donor (AH) and proton acceptor (B) entities, with a separation of 2.86 A° between the atomic orbitals of A and B being optimal. The AH and B units act as an acid and a base, respectively, and the whole AH,B "glucophore" forms a double hydrogen-bonded complex with a reciprocal AH, B unit at the receptor site or sites.[16,17] Hernandulcin (**1**) seems to closely fit the Shallenberger model for sweet compounds, with the C-1' hydroxyl and C-1 carbonyl groups representing in turn the AH and B groups. Acetylation of the hydroxyl group or reduction of the keto group resulted in complete loss of sweetness.[11,15] When preferred conformations of compound **1** and some of its derivatives were determined by molecular mechanics calculations, support was obtained for additional theories on sweetness. Shallenberger and colleagues have also proposed the existence of a "spatial barrier" about 3-4 A° away from the AH, B unit, to explain the stereochemical requirements of certain sweet substances.[18] Mori and Kato have established by a stereospecific synthesis from (R)-limonene that naturally (+)-hernandulcin is the (6S, 1'S) diastereomer, and that the other three diastereomers of this compound are non-sweet.[13] The Shallenberger "spatial barrier" theory may account for why (6S, 1'S)-**1** is sweet but (6S, 1')-epihernandulcin (**2**) is not, since in the case of the latter compound, although it has the same AH,B unit as **1**, the bulky C-3' through C-7' fragment appears to prevent the molecule from fitting the appropriate receptor site(s). However, the "spatial barrier" can not be the only factor involved in determining the correct fit of hernandulcin (**1**) with the sweetness receptor, since compound **3**, which has the same conformation as **1**, but does not have a bulky group to hinder attachment to the receptor, was not found to be sweet. Since the hydrogenated compound **4**, again determined by molecular mechanics calculations to have the same conformation as **1**, was not sweet-tasting, it appears that an additional binding site at the C-4'-C-5' double bond is involved in mediating the sweetness of hernandulcin (**1**). Support has therefore been obtained for theories[19,20] that postulate a third binding site **X**, in sweet molecules, where interaction occurs with rece-

ptor(s) through dispersion or hydrophobic forces.[15]

Abrusosides A–D—The roots and leaves of the widely distributed subtropical plant, *Abrus precatorius* L. (Fabaceae), have a long history of human internal consumption, both as a substitute for licorice and for other medicinal purposes. In contrast, the seeds of this species are well-known as the source of the lethal glycoprotein, abrin.[21] There have been a number of reports suggesting that the sweetness of *A. precatorius* leaves is attributable to the presence of high levels of the oleanane-type triterpene, glycyrrhizin.[21,22] However, when a sample of *A. precatorius* leaves collected in Florida was examined in our laboratory, glycyrrhizin was found to be absent, and four novel sweet cycloartane-type triterpene glycosides, abrusosides A∼D (**5**∼**8**), were obtained from a butanol-soluble extract after extensive chromatographic fractionation. Acid hydrolysis of these glycosides afforded a common aglycone, abrusogenin, whose structure was determined as (20S, 22S)-3β, 22-dihydroxy-9, 19-cyclolanost-24-en-26, 29-dioic acid lactone (**9**), in which use was made of ^{1}H–^{1}H COSY, ^{1}H–^{13}C HETCOR and selective INEPT NMR experiments. The structure and stereochemistry of this aglycone was confirmed by single-crystal X-ray crystallography of the methyl ester of abrusogenin.[23] The final structures of the four glycosides 5∼8 were fixed by comparison of chemical shifts in their ^{13}C-NMR spectra with published data on other natural product glycosides having common saccharide moieties. The positions of sugar attachment and the linkages of the saccharide moieties were confirmed by application of the selective INEPT technique on the intact heterosides.[21,24] Therefore, the structures of abrusosides A∼D were determined as the β-D-glucopyranosyl (**5**), the β-D-glucopyranosyl-(1→2)-β-D-6-methylglucuronopyranosyl (**6**), the β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl (**7**), and the β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl (**8**) derivative of abrusogenin, respectively.[21,24]



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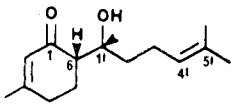

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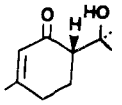

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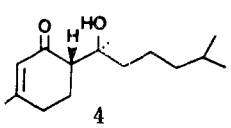

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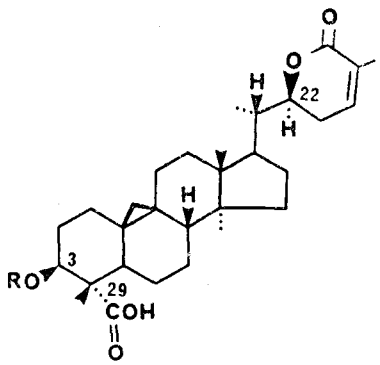
Abrusosides A∼D (**5**∼**8**) were found to be nonmutagenic and not acutely toxic for mice in preliminary safety tests, and the water-soluble ammonium salts of these substances exhibited sweetness potencies of between 30 and 100 times sweeter than sucrose. Abrusoside D, the most abundant of these new sweeteners in *A. precatorius* leaves, was rated by a small human taste panel as being about 75× sweeter than a 2% w/v sucrose solution.[21] Therefore, these compounds are equivalent in sweetness potency to the commercially available glycyrrhizin,[6,8] and offer potential advantages over the latter compound in being easier to produce by cultivation (in being produced by the leaves of the plant



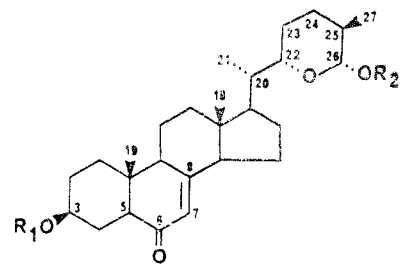
R

5 β-D-glc
6 β-D-glcA-6-CH_3^2-β-D-glc
7 β-D-glc^2-β-D-glc
8 β-D-glcA^2-β-D-glc
9 H

rather than the roots) and in not possessing an $\alpha\beta$-unstaturated carbonyl group in their glycone (**9**). The 11-oxo-12, 13-dehydro-group of glycyrrhizin is considered to be responsible for its adrenocorticomimetic effects that are manifested by edema and hypertension.[25] Recently, we have shown that abrusosides A～D (**5**～**8**) also occur in the leaves of *Abrus fruticulosus* Wall ex W. & A., which is used in Thai traditional medical practice for the sweetening of medicines.[26] Since it is unlikely that the abrusoside sweeteners will produce the same toxic effects as glycrrhizin, efforts are being made to evaluate plant extracts containing abrusosides A～D (**5**～**8**) to potentially substitute for glycyrrhizin in the sweetening of foods, beverages and medicines.

Polypodosides A and B—The rhizomes of the North American fern, *Polypodium glycyrrhiza* D.C. Eaton (Polypodiaceae) exhibit a bittersweet taste, and have a history of use by humans as a foodstuff and as a medicinal agent.[27,28] When *P. glycyrhiza* was initially studied as a potential substitute for licorice, a fluidextract of the rhizomes were found to be nontoxic for rats, and glycyrrhizin was claimed as the highly sweet constituent of this species.[27] In a subsequent investigation, glycyrrhizin was found to be absent from *P. glycyrrhiza* rhizomes, and the sweetness was attributed to sugars such as sucrose as well as an uncharacterized substance.[29] Our group became interested in *P. glycyrrhiza* rhizomes as a potential source of the steroidal saponin, osladin (**10**), a compound isolated from *Polypodium vulgare* by Czechoslovakian workers in 1971.[30] Osladin (**10**) has been rated as up to 3,000 times sweeter than sucrose, and thus represents one of the sweetest known natural products.[6,8] Phytochemical investigation of a butanol-soluble extract of the rhizomes of *P. glycyrrhiza* did not yield osladin (**10**), but rather the closely related compound, polypodoside A (**11**), which was established as 26-O-α-L-rhamnopyranopyranosyl-polypodogenin-3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside after the performance of spectroscopic and hydrolytic studies.[28] It can be postulated on biogenetic grounds that polypodoside A (**12**) is the $\Delta^{7,8}$ derivative of osladin (**10**), although the configuration of the C-26 rhamnose substituent has not yet been established.[28,30] Polypodoside A (**11**) was nonmutagenic and not acutely toxic for mice at the doses tested, and was rated by a taste panel as possessing 600 times the sweetness intensity of a 6% aqueous sucrose solution. Polypodoside A (**12**) occurred in a rather low yield in its plant of origin (0.29% w/w) and proved to be very insoluble in water, and also to exhibit a lingering aftertaste and a licorice-like off-taste, thus will probably not be commercially useful as a sucrose substitute.[28]

A second novel sweet steroidal saponin was obtained from *P. glycyrrhiza* rhizomes, namely, polypodoside B (**12**), which was assigned as 26-O-α-L-rhamnopyranosyl-polypodogenin-3-O-β-D-glucopyranoside. Polypodoside B (**12**) was obtained as a less abundant and apparently less sweet *P. glycyrrhiza* constituent than polypodoside A (**11**). The quantity of compound **12** obtained did not permit the determination of its sweetness intensity relative to sucrose by a human taste panel.[31] A third steroidal saponin

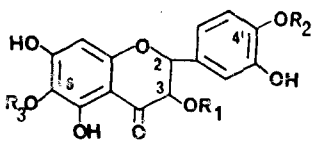


	R_1	R_2	
10	β-D-glc^2-α-L-rha	rha	(7,8-dihydro)
11	β-D-glc^2-α-L-rha	α-L-rha	
12	β-D-glc	α-L-rha	
13	β-D-glc	α-L-rha-3-Me	
14	β-D-glc^2-α-L-rha	H	

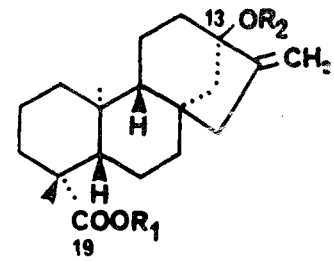
was isolated from the rhizomes of *P. glycyrrhiza*, namely, polypodoside C (**13**) (26-O-L-acofriopyranosyl-polypodogenin-3-O-β-D-glucopyranoside). Despite being the 3″-O-methyl derivative of polypodoside B (**12**), polypodoside C (**13**) was dovoid of an sweet taste. Since the monodesmosidic polypodogenin glycoside, polypodosaponin (**14**) was not reported to have a sweet taste,[32] we have concluded that it appears to be necessary for polypodogenin glycosides to be bisdesmosidic in order to exhibit a sweet taste, with saccharide substitution occurring at both the C-3 and C-26 positions. Even among such compounds, minor structural differences in the sugar units seem to profoundly affect the sweet taste.[27,31]

Dihydroflavonol Sweeteners—During field work in Paraguay in 1981, the plant *Tessaria dodoneifolia* (Hook. & Arn.) (Asteraceae) was obtained from a medicinal plants maket in Asuncíon, where it was on sale as an emmenogogue under the Guarani name, "kaá-hê-é" ("sweet herb"). The plant was subsequently cultivated from seed at the University of Illinois Pharmacognosy Field Station, and its sweet taste was found to be associated with only the young shoots. The sweetness of an ethyl acetate extract of *T. dodoneifolia* shoots was traced to the known dihydroflavonol, dihydroquercetin-3-acetate (**15**).[33] Compound **15** was initially isolated from this plant source by Kavka and co-workers,[34] although it was not recognized as being sweet at the time of its original isolation. After a preliminary safety evaluation, dihydroquercetin-3-acetate (**15**) was rated as 80 times the sweetness of sucrose, and is the first dihydroflavonol to have been reported as intensely sweet. The stereochemistry of **15** at C-2 and C-3 was confirmed as 2R, 3R by conversion to the pentacetate derivative of a commercial sample of (+)-dihydroquercetin (taxifolin, **16**), a compound of known absolute stereochemistry (2R, 3R).[33] In constrast, neoastilbin (**17**) a dihydroflavonol rhamnoside with 2S, 3S stereochemistry, has been obtained by the Tanaka group as a sweet constituent of the Chinese medicinal plant, *Engelhardtia chrysolepis* Hance (Juglandaceae).[35] The three other diastereoisomers of compound **17**, including the (2R, 3R)-derivative, astilbin (**18**), were not sweet.[35]

Compound **15** underwent slow spontaneous oxidation in neutral and basic media, and it was suspected that the introduction of a 4′-methoxy group in ring B would create a more stable compound, since free-radical formation at this position would be inhibited.[33] Furthermore, by analogy to the dihydroisocoumarin and dihydrochalcone sweetening agents, it was also reasoned that the sweetness potency of **15** could be



	R_1	R_2	R_3
15	Ac	H	H(2R, 3R)
16	H	H	H(2R, 3R)
17	α-L-rha	H	H(2S, 3S)
18	α-L-rha	H	H(2R, 3R)
19	Ac	Me	H
20	H	Me	H
21	Ac	Me	OMe(2R, 3R)



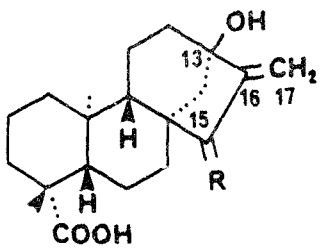
	R_1	R_2
22	β-D-glc	β-D-glc^2-β-D-glc
23	β-D-glc	β-D-glc^2-β-D-glc ^{3} β-D-glc
24	H	H

enhanced by the presence of such a methyl ether group. Racemic dihydroquercetin-3-acetate-4′-methyl ether (**19**), a previously unknown compound, was prepared from 2,4-bis-(benzyloxy)-6-(methoxymethoxy)acetophenone and 3-(benzyloxy)-4-methoxybenzaldehyde, according to a known method for the synthesis of dihydroflavonols.[33,36] After the conduct of preliminary safety tests, compound **19** was found to be 400× sweeter than 3% w/v sucrose, and to exhibit a pleasant sweet taste with no bitterness, although it was found to have a somewhat slow onset of action. The lack of any appreciable water solubility will probably limit the useful applications of this synthetic dihydroflavonol sweetener.[33]

Preliminary structure-sweetness studies among the analogues of **15** have shown that the 3-acetate group is not essential for the exhibition of sweetness, since the synthetic compound, racemic dihydroquercetin-4′-methyl ether (**20**) exhibited about one-tenth of the sweetness intensity of compound **19**. The unsubstituted compound, (+)-dihydroquercetin (**16**), was devoid of any sweet taste. In an unrelated phytochemical investigation on *Hymenoxys turneri* K. Parker (Asteraceae), a plant collected in Texas, compound **15** and several less sweet 6-methyoxylated analogs were isolated. One such derivative, compound **21**, was considered by a small teste panel to possess only half the sweetness intensity of **15** relative to a sucrose standard solution.[37]

Approaches Toward the Discovery of Novel Highly Sweet-Tasting Compounds from Plants

In the years of operation of our program to discover and evaluate novel sweet-tasting natural products from plants, we have attempted to streamline our investigations in the field and in the laboratory, so as to optimize our chances of obtaining new compounds. Particular effort



R	
25	α-OH, β-H
26	O

has gone into the selection of candidate sweet plants for testing, which is the most crucial part of any natural sweetener discovery research program. We have also developed phytochemical procedures to rapidly detect sweet sugars, sugar alcohols and phenylpropanoids, since compounds in these classes can represent false leads by occurring in sufficiently high concentration as to suggest that a particular plant part might contain one or more highly sweet substances. Finally, we have began to look at the feasibility of using gerbil bioassays to monitor sweetness, to try to avoid our current practice of subjecting plant extracts to time-consuming and expensive acute toxicity tests in mice and bacterial mutagenicity tests. These aspects will be briefly considered in turn.

Selection of Sweet-Tasting Plants—The two most useful methods of identifying suitable sweet plants on which to work involve either ethnobotanical observations in the field or careful analysis of published botanical literature. Field observations represent the most direct manner of obtaining interesting leads, as previously reviewed for *Stevia rebaudiana* and other plants that have afforded new intense sweeteners.[6] Vendors of medicinal plants are often aware that certain species are slightly sweet-tasting, as was the case for *Tessaria dodoneifolia*, which ultimately led to the isolation of a dihydroflavonol intense sweetener, as described earlier in this review.[33] Literature information

on sweet-tasting plants is found in old herbals and numerous other types of botanical articles.[6] Another literature approach which might pay dividends in the selection of candidate sweet plants is information contained in *Index Kewensis*, a repository of all of the published names of the seed plants. Often a specific epithet such as "*dulcis*" may indicate the production of highly sweet compounds by the plant concerned.[38]

Phytochemical Procedures—It has been our experience that if monosaccharides, disaccharides and/or sugar alcohols are present in a plant part at levels of above 5% w/w, then these compounds will most likely impart a distinct sweet taste. Such compounds will preferentially partition into methanol-water (1:1), or occasionally butanol, and, after concentration by passage over charcoal, may be detected and quantitated by gas chromatography-mass spectrometry (GC/MS).[39] Similarly, high concentrations of sweet-tasting volatile oil components like *trans*-cinnamaldehyde[40] and *trans*-anethole [41] may also lead to ouvert sweetness in a plant part. Such nonpolar compounds are taken up in petroleum ether, and again can be screened out by GC/MS.[41] It has been found that, on most occasions, the perception of sweetness by a plant collected in the field is due to the presence of high concentrations of either sugars or phenylpropanoids, rather than lower amounts of intensely sweet constituents.

Gerbil Electrophysiological and Behavioral Assays—In order to protect human subjects monitoring sweetness against the possibility of sweet-tasting plants from also containing toxic substances, we have routinely subjected all initial plant extracts investigated to preliminary safety tests invoving a mouse acute toxicity assessment and a bacterial mutagenicity evaluation. In this manner, only innocuous extracts are assessed for sweetness by volunteer subjects. However, encouraging results have been obtained in a pilot investigation in which sweet and non-sweet extracts of three plants were evaluated by a combination of electrophysiological and behavioral tests using the Mongolian gerbil. In the former model, electrophysiological stimulation of the intact chorda tympani nerve is recorded when sweet substances are present, while in behavioral conditioned taste aversion tests, gerbils trained to avoid sweet and other taste qualities provide an estimation of the degree of similarity of taste to sucrose of an unknown solution.[42] Data obtained thus far have shown a good correlation between the prediction of sweetness by the gerbils and the actual presence of sweet-tasting diterpene or triterpene glycosides in the plant extracts evaluated.[42]

Safety Studies on Stevioside and Extracts of *Stevia rebaudiana*

Sweetening Applications—The powdered leaves of the Paraguayan plant, *Stevia rebaudiana*, have been used for a long period of time by the Guarani Indians and others to sweeten the bitter stimulant beverage, maté. Stevioside (**22**), the most abundant *ent*-kaurene glycosidic constituent of the plant, and extracts of *S. rebaudiana* leaves are used in Japan as sucrose substitutes to many food items, such as dried seafoods, pickles, fish meat products, chewing gum, and confectionery. For sweetening Japanese foods, stevioside (**22**) offers particular advantages over other sweeteners in being both non-fermentive and heat-stable. *S. rebaudiana* products are also used commerciallys in Korea, the People's Republic of China, Brazil, and Paraguay.[43~45] Stevioside (**22**) is very unusual as a plant secondary metabolite in occurring at concentration levels in *S. rebaudiana* leaves up to over 8% w/w dry weight, depending upon the geographic origin of the sample.[46] However, rebaudioside A (**23**), in being more polar, is

more water-soluble than stevioside (22), and in also being sweeter and more pleasant-tasting, offers the prospects of ultimately being a more useful sucrose substitute.[43,45] The structure of stevioside (22) was established by groups at the National Institutes of Health, Bethesda, Maryland,[47,48] while rebaudioside A (23) was first isolated and characterized by Tanaka and co-workers.[49]

Mutagenic Activity of Steviol—Steviol (24, *ent*-13-hydroxykaur-16-en-19-oic acid) is the aglycone of both stevioside (22) and rebaudioside A (23), and may be obtained from these glycosides by enzymatic hydrolysis.[43~45] While we and several other investigators have observed no evidence of mutagenicity by *Stevia rebaudiana* extracts or any of the sweet *ent*-kaurene diterpene constituents of this species,[43~45] our group has found that steviol is mutagenic toward *Salmonella typhimurium* strain TM677, when metabolically activated.[50] Having evaluated a series of analogs of steviol (24) in this manner, it was concluded that the functionalities necessary for the conferment of mutagenicity under the conditions used are the C-13 tertiary hydroxyl and the C-16, C-17 exomethylene groups.[50,51] The *in vitro* metabolism of steviol (24) has been examined in the presence of a rat liver enzyme preparation, under the same conditions found to produce a mutagenic response. The major pathway of such mammalian metabolism of steviol (24) appears to allylic oxidation, as evidenced by the production of 15α-hydroxysteviol (25) as a major metabolite. Compound 25 was not found to be mutagenic, either with or without metabolic activation, but its closely related analog, 15-oxosteviol (26), did prove to be a direct-acting mutagen and also highly toxic to *S. typhimurium* cells.[52] However, 15-oxosteviol was not detected as a metabolite of steviol (24) under the conditions used.[52] Our observations on the activity of steviol (24) in forward mutation assays utilizing *S. typhimurium* strain TM677 have been confirmed by workers at the National Institute of Hygienic Sciences, Tokyo, Japan.[53] Although there are only limited *in vitro* and *in vivo* data available on the metabolism of the sweet *S. rebaudiana* constituents, a study published in 1980 showed that stevioside (22) and rebaudioside A (23) were degraded to steviol (24) by rat intestinal flora *in vitro*.[54] More recently, steviol (24) has been shown to be a major metabolite of stevioside (22) when a tritiated form of this glycoside was fed to rats.[55]

Chronic Toxicity Studies—The above mutagenicity and metabolism studies do suggest that follow up safety tests on stevioside (22), rebaudioside A (23) and steviol (24) would be helpful, because of well-known value of mutation assays in predicting carcinogenicity. However, a chronic toxicity test performed on extracts of *Stevia rebaudiana* did not demonstrate any higher incidence of cancer in dosed test animals compared with controls. In this study, male and female rats were given a hot-water extract of *S. rebaudiana* leaves, containing about 75% of stevioside (22) and 16% rebaudioside A (23) for up to two years.[56]

Human Exposure—*Stevia rebaudiana* extracts have been used on an increasing basis in Japan for the last 15 years, and the Japanese human consumption of stevioside (22) was estimated as 200 metric tons in 1987.[57] There do not seem to have been any adverse reports as a result of the ingestion of these materials. Thus, on this basis, stevioside (22) and *S. rebaudiana* products appear to be safe.

Conclusions

In all probaility, there will be a continued future need for new noncaloric and noncariogenic sucrose substitutes. Plant-derived highly

sweet substances already play an important role in satisfying such needs in Japan[57] and it can be expected that additional natural product highly sweet substances will be introduced onto the Japanese market. Also, natural product sweeteners, even with less than ideal properties, could find use in speciality markets.[58] Plant-derived sweeteners that prove to be altogether inappropriate for direct commercial exploitation, should serve as good lead molecules for synthetic modification and computer-aided sweetener design. It may be contended that, with the careful selection of candidate sweet plants for subsequent phytochemical investigation, green plants may be used for the discovery of novel highly sweet molecules on a systematic basis.

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Literature Cited

1. Nicol, W.M.: in Birch, G.G. and Parker, K.J. (Eds.) *Nutritive Sweeteners*, Applied Science, London, U.K., pp.17-35 (1982).
2. Sheiham, A.: in Guggenheim, B. (Ed.) *Cariology Today, International Congress, Zurich, 1983*, Karger, Basel, Switzerland, pp.33-39 (1984).
3. Crosby, G.A., DuBois, G.E. and Wingard, Jr., R.E.: in Ariens, E.J. (Ed.) *Drug Design*, vol.8, Academic, New York, pp.215-310 (1979).
4. Stegink, L.D. and Filer, Jr, L.J. (Eds.): *Aspartame: Physiology and Biochemistry*, Marcel Dekker, New York, 670pp. (1986).
5. O'Brien Nabors, L. and Gelardi, R.C., (Eds.): *Alternative Sweeteners*, Marcel Dekker, New York, p.355 (1986).
6. Kinghorn, A.D. and Soejarto, D.D.: *CRC Crit. Rev. Plant Sci.* **4**, 79(1986).
7. Tanaka, O.: *Kagaku to Kogyo* (*Osaka*) **61**, 404 (1987).
8. Kinghorn, A.D. and Soejarto, D.D: *Medicinal Res. Rev.* **9**, 91(1989).
9. Compadre, C.M., Pezzuto, J.M., Kinghorn, A.D. and Kamath, S.K.: *Science* (*Washington D.C.*) **227**, 417(1985).
10. Compadre, C.M., Robbins, E.F. and Kinghorn, A.D.: *J. Ethnopharmacol.* **15**, 89(1986).
11. Compadre, C.M., Hussain, R.A., Lopez de Compadre, R.L., Pezzuto, J.M. and Kinghorn, A.D.: *J. Agric. Food Chem.* **35**, 273(1987).
12. Mori, K. and Kato, M.: *Tetrahedron Lett.* **27**, 981(1986).
13. Mori, K. and Kato, M.: *Tetrahedron* **42**, 5895 (1986).
14. De Cusati, P.F. and Olofson, R.A.: *Tetrahedron Lett.* **31**, 1409(1990).
15. Compadre, C.M., Hussain, R.A., Lopez de Compadre, R.L., Pezzuto, J.M. and Kinghorn, A.D.: *Experientia* **44**, 447(1988).
16. Shallenberger, R.S. and Acree, T.A.: *Nature* (*London*) **216**, 490(1967).
17. Birch, G.G.: in Dobbing, J. (Ed.) *Sweetness*, Springer Verlag, London, U.K., pp.3-13, (1987).
18. Shallenberger, R.S., Acree, T.E. and Lee, C.Y.: *Nature* (*London*) **221**, 555(1969).
19. Deutsch, E.W. and Hansch, C.: *Nature*(*London*) **211**, 75(1966).
20. Kier, J.B.: *J. Pharm. Sci.* **61**, 1394(1972).
21. Choi, Y.H., Hussain, R.A., Pezzuto, J.M., Kinghorn, A.D. and Morton, J.E.: *J. Nat. Prod.* **52**, 1118(1989).
22. Akinloye, B.A. and Adalumo, L.A.: *Nigerian*

J. Pharm. **12**, 405(1981).

23. Choi, Y.H., Kinghorn, A.D., Shi, X., Zhang, H. and Teo, B.K.: *J. Chem. Soc., Chem. Commun.* **1989**, 887(1989).
24. Kim, J. and Kinghorn, A.D.: *Tetrahedron Lett.* **28**, 3655(1987).
25. Segal, R., Pisantry, S., Wormser, R., Azaz, E. and Sela, M.N.: *J. Pharm. Sci.* **74**, 79(1985).
26. Fullas, F., Choi, Y.H., Kinghorn, A.D. and Bunyapraphatsara, N.: *Planta Med.* **56**, 332 (1990).
27. Fischer, L. and Goodrich, F.J.: *J. Am. Pharm. Assoc.* **19**, 1063(1930).
28. Kim, J., Pezzuto, J.M., Soejarto, D.D., Lang, F.A. and Kinghorn, A.D.: *J. Nat. Prod.* **51**, 1166(1988).
29. Fischer, L. and Lynn, E.V.: *J. Am. Pharm. Assoc.* **32**, 1225(1933).
30. Jizba, J., Dolejs, J., Herout, V. and Sorm, F.: *Tetrahedron Lett.* 1329(1971).
31. Kim, J. and Kinghorn, A.D.: *Phytochemistry* **28**, 1225 (1989).
32. Jizba, J., Dolejs, L., Herout, V., Sorm, F., Fehlhabe, H.W., Snatzke, G., Tschesche, R. and Wulff, G.: *Chem. Ber.* **104**, 837(1971).
33. Nanayakkara, N.P.D., Hussain, R.A., Pezzuto, J.M., Soejarto, D.D. and Kinghorn, A.D.: *J. Med. Chem.* **31**, 1250(1988).
34. Kavka, J., Guerriero, E. and Giordano, O.S.: *An. Quím.* **73**, 305(1978).
35. Kasai, R., Hirono, S., Chou, W.H., Tanaka, O. and Chen F.H.: *Chem. Pharm. Bull.* **36**, 4167 (1988).
36. Takahashi, H., Kubota, Y., Miyazaki, H. and Onda, M.: *Heterocycles* **22**, 1147(1984).
37. Gao, F., Wang, H., Mabry, T.J. and Kinghorn, A.D.: *Phytochemistry* **29**, 2865(1990).
38. Hussain, R.A., Kinghorn, A.D. and Soejarto, D.D.: *Econ. Bot.* **42**, 267(1988).
39. Hussain, R.A., Poveda, L.J., Bordas, E., Chung, B.S., Pezzuto, J.M., Soejarto, D.D. and Kinghorn, A.D.: *J. Ethnopharmacol.* **28**, 103(1990).
40. Hussain, R.A., Kim, J., Hu, T.W., Pezzuto, J.M., Soejarto, D.D. and Kinghorn, A.D.: *Planta Med.* **52**, 403(1986).
41. Hussain, R.A., Poveda, L.J., Pezzuto, J.M., Soejarto, D.D. and Kinghorn, A.D.: *Econ. Bot.* **44**, 174(1990).
42. Jakinovich, Jr., W., Moon, C., Choi, Y.H. and Kinghorn, A.D.: *J. Nat. Prod.* **53**, 190(1990).
43. Kinghorn, A.D. and Soejarto, D.D.: in Wagner, H., Hikino, H. and Farnsworth, N.R. (Eds.) *Economic and Medicinal Plant Research*, vol. 1, Academic London, U.K., pp. 1-52, (1985).
44. Phillips, K.C.: in Grenby, T.H. (Ed.) *Developments in Sweeteners*, vol. 3, Elsevier Applied Science, London, U.K., pp. 1-43, (1987).
45. Crammer, B. and Ikan, R.: in Grenby, T.H. (Ed.) *Developments in Sweeteners*, vol. 3, Elsevier Applied Science, London, U.K., pp. 45-64, (1987).
46. Makapugay, H.C., Nanayakkara, N.P.D. and Kinghorn, A.D.: *J. Chromatogr.* **283**, 390(1984).
47. Wood, Jr., H.B., Allerton, R., Diehl, H.W. and Fletcher, Jr., H.G.: *J. Org. Chem.* **20**, 875 (1955).
48. Mosettig, E., Begliger, U., Dolder, F., Lichti, H., Quitt, P. and Waters, J.A.: *J. Am. Chem. Soc.* **85**, 2305(1963).
49. Kohda, H., Kasai, R., Yamasaki, K., Murakami, K. and Tanaka, O.: *Phytochemistry* **15**, 981 (1976).
50. Pezzuto, J.M., Compadre, C.M., Swanson, S.M., Nanayakkara, N.P.D. and Kinghorn, A.D.: *Proc. Natl. Acad. Sci., USA* **78**, 2482(1985).
51. Pezzuto, J.M., Nanayakkara, N.P.D., Compadre, C.M., Swanson, S.M., Kinghorn, A.D., Guenthner, T.M., Sparnins, V.L. and Lam, L.K.T.: *Mutation Res.* **169**, 93(1986).
52. Compadre, C.M., Hussain, R.A., Nanayakkara, N.P.D., Pezzuto, J.M. and Kinghorn, A.D.: *Biomed. Environ. Mass Spectrum.* **15**, 211(1988).
53. Matsui, M., Matsui, K., Nohmi, T., Mizusawa, H. and Ishidate, M.: *Bull. Natl. Inst. Hyg. Sci. Tokyo* **107**, 83(1989).
54. Wingard, Jr., R.E. Brown, J.P., Enderlin, F.E. Dale, J.A., Hale, R.L. and Seitz, C.T.: *Experientia*, **36**, 519(1980).
55. Nakayama, K., Kasahara, D. and Yamamoto, F. *J. Food Hyg. Soc., Japan* **27**, 1(1986).

56. Yamada, A., Ohgaki, S., Noda, T. and Shimizu, M.: *J. Food Hyg. Soc., Japan* **26**, 169(1985).
57. Anonymous: *Food Chemicals, Tokyo*, June issue, pp. 18-25(1988).
58. Crosby, G.E. and Wingard, Jr., R.E.: in Hough, C.A.M., Parker, K.J. and Vlitos, A.J. (Eds.) *Developments in Sweeteners*, vol. 1, Applied Science, London, U.K., pp. 135-163 (1979).