Discovery of terpenoid and phenolic sweeteners from plants*

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Abstract: Several plant-derived compounds of the terpenoid and phenolic types have commercial use as sweeteners. In our research program directed toward the discovery of additional sweet compounds of these chemical classes, candidate sweet plants for laboratory investigation may be selected after scrutiny of the available literature, as a result of making inquiries in the field, and/or from a limited amount of organoleptic testing. Sweet-tasting plants are extracted according to a standard protocol, and preliminary safety testing is conducted before crude extracts or pure compounds are tasted. The practicality of using Mongolian gerbil electrophysiological and behavioral assays to monitor plant extracts and pure isolates has been examined. A number of sweet-tasting, plant-derived terpenoids and phenolics have been isolated and characterized, including the bisabolane sesquiterpenoids, hernandulcin and 4β-hydroxyhernandulcin, the labdane diterpene glycoside, gaudichaudioside A, the oleanane triterpenoid glycoside, periandrin V, the cycloartane triterpene glycosides, abrusosides A–E, the 3,4-*seco*-dammarane triterpene glycosides, pterocaryosides A and B, the semisynthetic dihydroflavonol, dihydroquercetin 3-acetate (4′-methyl ether), and the proanthocyanidin, selligueain A. Most of these new compounds are prototype "high-intensity" sweeteners that may be worthy targets for chemical synthesis or for semi-synthetic modification to produce substances with enhanced sweetness properties.

INTRODUCTION

Approximately 150 million persons in the United States use sugar-free low-calorie products, with their use having tripled over the last 20 years [1]. It has been estimated that the consumption of both nutritive and non-nutritive sweeteners will increase about 3 % per year over the next few years [2], with the market value of food additives inclusive of artificial sweeteners accounting for about $1.5 billion in the United States [3].

All of the currently approved "high-intensity" sweeteners in the United States are synthetic substances (aspartame, acesulfame K, saccharin, and sucralose) [4]. Thus far, there are about 80 sweet compounds exclusive of monosaccharides, disaccharides, and polyols obtained from natural sources, with all of these from vascular plants. These plant-derived compounds mainly belong to three major structural classes, namely, the terpenoids, flavonoids, and proteins [5]. At present, none of these highly sweet compounds is approved for use as a "high-intensity" sweetener in the United States, although plant-derived compounds such as glycyrrhizin, neohesperidin dihydrochalcone, stevioside, and thaumatin are

used commercially in some other countries for sweetening purposes [6–8]. However, in the United States there is an increasing use of plant extractives known to contain highly sweet terpenoids. An ammoniated derivative of the oleanane-type triterpene glycoside, glycyrrhizin, has been available for several years on the generally recognized as safe (GRAS) list of approved natural flavors [8]. More recently, purified extracts of *Stevia rebaudiana* (Bertoni) Bertoni (Compositae) containing the sweet *ent*-kaurane-type diterpene glycosides stevioside and rebaudioside A have become popular as "dietary supplements" [9]. Soft drinks incorporating extracts of *Siraitia grosvenorii* (Swingle) Lu & Zhang (Cucurbitaceae) fruits (also known as "Lo Han Kuo"), containing sweet cucurbitane-type triterpene glycosides such as mogroside V [8], are now on the market.

In the present review, we describe the approaches taken in our ongoing project to discover new prototype highly sweet terpenoids and phenols from plants, including botanical and ethnobotanical considerations, phytochemical protocols, preliminary safety procedures, and the evaluation of sweetness by using small human taste panels and the Mongolian gerbil. Examples of small-molecule plant secondary metabolites representative of the terpenoid and flavonoid (phenolic) structural types will be presented in general order of biogenetic complexity.

STRATEGIES FOR THE SELECTION OF CANDIDATE SWEET-TASTING PLANTS

Botanical and ethnobotanical approaches

A guiding principle our group has followed in the past in searching for new sweet-tasting natural products is that the sweet taste of these substances tends to afford a pleasant oral gratification that is not easily forgotten. This type of pleasant sensation is often transmitted through word of mouth, and may eventually come to the attention of travelers, explorers, plant collectors, or other scientists, who might document such observations. We have identified three major approaches to access information on potentially sweet-tasting plants. The first of these is through perusal of the botanical and ethnobotanical literature, wherein accounts are searched on uses of plants by indigenous communities. On a worldwide scale, the record of plants that elicit a sweet taste is preserved in a compedium entitled *Index Kewensis*, which records all published Latin binomial names of angiosperms and gynosperms. Botanists aware that a plant part tastes sweet may assign an epithet to a new plant signifying "sweet" or a related meaning, such as "dulcificum", "dulcis", "glycyrrhiza", "mellifera", "mellosa", and "saccharum". In previous work, we have implemented this type of name search for candidate sweet-tasting plants from *Index Kewensis* [10]. On a regional or local scale, one can access books and articles on local flora. A second method to discover sweet-tasting plants is through field work and interviews with members of indigenous communities, and local healers or herbalists, followed up by checking on the sweet taste [11]. This can often be accomplished most easily in medicinal plant marketplaces. However, as a consequence of the signing in 1992 of the United National Convention on Biological Diversity in Rio de Janeiro (the so-called Rio Summit), it is now necessary to obtain prior informed consent, and scientific investigators must make provisions for providing compensation in lieu of the information obtained, such as the equitable sharing of benefits that may arise in the event of commercial development of indigenous traditional knowledge. Finally, previously undocumented sweet-tasting plants may be accessed in a third manner through a limited amount of organoleptic testing. This can be performed either in the field as part of a plant collection expedition, or in the laboratory [11]. In the latter context, on obtaining institutional permission, we have accessed selected species in the John G. Searle Herbarium of the Field Museum, Chicago, Illinois, in our sweetener research program. Thus, following appropriate safety precautions, dried leaves of approximately 110 species of the genus *Stevia* were tasted, and, as a result of this exercise, stevioside was found not only in a 70-year old leaf specimen of *S. rebaudiana*, but also in a second species, *Stevia phlebophylla* A. Gray [12,13]. This third approach, however, would be very difficult if not impossible to undertake in a comprehensive fashion, since there are between 215 000 and 240 000 species of angiosperms [14,15], the plant group that has yielded the

most highly sweet natural products [16]. The particular approach that led us to investigate each sweet plant mentioned below in this review will be indicated in turn.

Phytochemical methods and preliminary safety protocols

Based on information derived from any of the three above-mentioned collection strategies, candidate sweet-tasting plants are extracted initially with the general solvent methanol–water (4:1). It is necessary at this stage to test each dried extract for the presence or absence of sweetness, but this is not performed until a preliminary safety evaluation is carried out. The latter consists of acute toxicity testing in mice, with the extract administered intraperitoneally at a dose of up to 2 g/kg in suspension in sodium carboxymethylcellulose, with animals examined for up to 14 days after treatment for both mortality and loss of weight [11,17]. This is coupled to a forward mutation assay using *Salmonella typhimurium* strain TM677, both in the absence and the presence of a metabolic activator [11,17]. If neither acute toxicity nor mutagenicity is observed for the initial crude extract, it is then tasted. If found to be sweet, the extract is then taken up in methanol–water (1:1), and partitioned sequentially with petroleum ether, ethyl acetate, and 1-butanol. When sweetness is detected in one or other of these extracts, a useful guide to the polarity (and, hence, general chemical class) of the pure sweet plant constituent(s) may be afforded [11].

In practice, only relatively few plants found to be sweet-tasting in the manner described above tend to contain highly sweet secondary metabolites. The presence of a sweet taste is most likely to be due to high concentration levels of sugars and polyols, which will selectively partition into the residual methanol–water (1:1) extract in our standard extraction scheme, and, on concentration over a charcoal column, may be rapidly identified and quantified by gas chromatography-mass spectrometry (GC-MS) [18,19]. We have found that in order to taste overtly sweet, the saccharide and/or polyol content of a given plant part should be over 5 % w/w dry wt. In situations where levels of a sweet-tasting plant part contain smaller concentrations of sugars and polyols, there is the possibility of highly sweet compounds co-occurring [18,19]. Another group of common plant constituents that may taste sweet are phenylpropanoids such as *trans*-anethole and *trans*-cinnamaldehyde [11]. If these compounds are in high enough concentration, they may confer a sweet taste to the plant part under consideration. Such compounds partition selectively into the petroleum ether extract in our extraction scheme, and can be identified by GC-MS [20].

Sweetness evaluation of plant extracts and pure isolates

Once pure compounds are obtained in our program on sweeteners, they are subjected to a preliminary evaluation of safety via the mouse acute toxicity and bacterial mutagenicity tests mentioned above. For several years we have used a threshold sensory test method using a small panel of human volunteers in good health to evaluate the sweetness potency of our pure compounds relative to 2 % w/v sucrose [e.g., 21]. However, such preliminary safety testing is time-consuming and may use up a large proportion of the available amount of a particular sweet-tasting pure natural product on hand. Accordingly, it was thought desirable to seek out a method of sweetness evaluation using a laboratory animal model, for crude extracts, chromatographic fractions, and pure compounds from plants. Using a combination of electrophysiological and behavioral (conditioned taste-aversion) assays on the Mongolian gerbil (*Meriones unguiculatus*) some progress has been made toward meeting this aim. In an initial study, we found that, when these two methods were used in tandem, it was possible to detect in a reliable manner the presence or absence of sweet-tasting glycosides in extracts of different polarities of three plants known to contain highly sweet terpenoids {*Stevia rebaudiana*, *Siraitia grosvenorii* [formerly *Momordica grosvenorii* Swingle and *Thladiantha grosvenorii* (Swingle) C. Jeffrey], and *Abrus precatorius*} [22]. This was followed up by work on a group of pure highly sweet natural products, using the same methodology, and it was found that hernandulcin (see next section), mogroside V, periandrin III, rebaudioside A, and stevioside all are more effective electrophysiological stimuli to the gerbil than

sucrose. However, the ranking order of sweetness potency in the gerbil method was not found to be the same as perceived by humans [23].

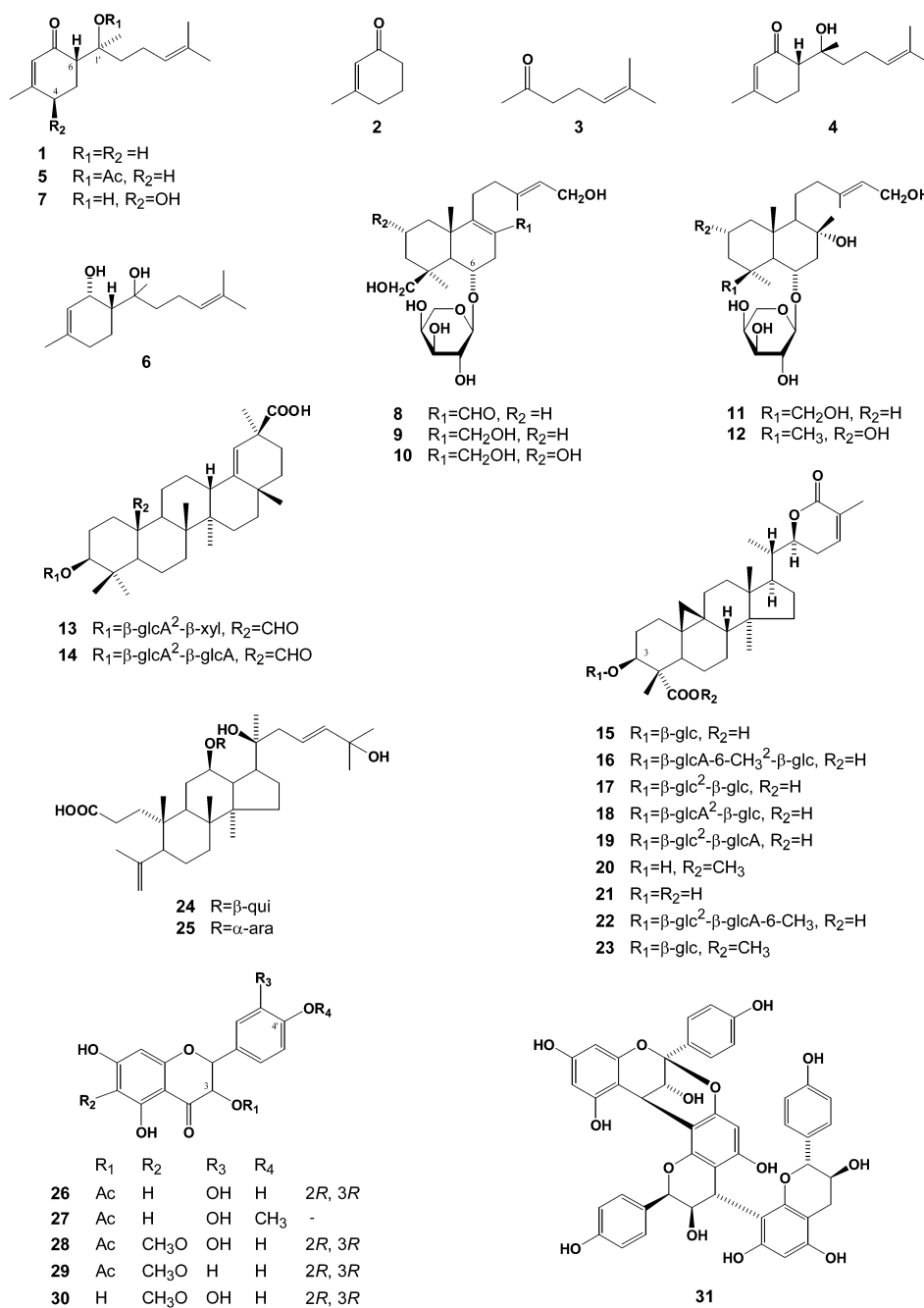## NEW HIGHLY SWEET PLANT-DERIVED TERPENOIDS AND PHENOLS

Sesquiterpenoids

(+)-Hernandulcin [6-(1,5-dimethyl-1-hydroxy-hex-4-enyl)-3-methylcyclohex-2-enone, **1**] was isolated from the leaves and flowers of *Lippia dulcis* Trev. (Verbenaceae) collected in Mexico. This plant was identified as being sweet as a result of a combination of scrutiny of the literature followed by organoleptic tests in the field and in a herbarium. Following preliminary safety evaluation in a forward mutation assay using *Salmonella typhimurium* and by acute toxicity testing in mice, this new bisabolane sesquiterpene was determined to be about 1000 times sweeter than sucrose on a molar basis by a trained taste panel [24]. Compound **1** was named after the Spanish physician Francisco Hernández, since in the 16th century he drew attention to this same remarkably sweet plant, which was known to the Aztecs under the Nahuatl name "Tzonpelic xihuitl" ("sweet herb") [24]. Racemic hernandulin was synthesized in our laboratory by a directed aldol condensation, using 3-methyl-2-cyclohexen-1-one (**2**) and 6-methyl-5-hepten-2-one (**3**) as starting materials, and afforded (±)-**1** and its diastereomer (±)-epihernandulcin (**4**) in a 95:5 ratio [24,25]. It was shown by Mori and Kato by synthesis from (*R*)-(+)-limonene that (+)-hernandulcin is the (6*S*,1′*S*) form, and it is the only one of the four possible diastereomers to exhibit a sweet taste [26,27]. It was found that the 1′-*O*-acetyl derivative (**5**) and the product (**6**) of a hydride reduction of hernandulcin were also not sweet [25]. Additional derivatives of hernandulcin (**1**) were made synthetically by our group, so that the functional groups responsible for the potent sweetness of this sesquiterpene could be probed further, and, as a result, it was postulated that this compound binds to its putative receptor through a potential three-point interaction, involving the C-1′ hydroxyl group (AH group), the C-1 carbonyl (B group), and the double bond between C-4′ and C-5′ (X region) [28]. There have been several other synthetic procedures published for hernandulcin, including a method from a cyclohexadiene derivative using boron and silicon enolates [29], preparation from (2*Z*,6*E*)-farnesal oxime by an intramolecular nitrile oxide cycloaddition route [30,31], and generation from an *E*-dienyl carbamate by titanium chloride catalysis [32]. The crystal structure of an intermediate in the synthesis of (+)-**1** has been published, namely, (5*R*)-3,5-dimethyl-5-[(1*R*,2*R*,4*R*)-2-acetoxy-4-methylcyclohexyl]-Δ^2-isoxazole [33]. Natural (+)-hernandulcin (**1**) was obtained from hairy roots and shoot cultures of *Lippia dulcis*, in yields of 0.025 % w/w [34] and 2.9 % w/w [35], respectively.

A second sweet-tasting sesquiterpene, (+)-4β-hydroxyhernandulcin (**7**), was isolated from another collection of *L. dulcis* leaves and flowers, made in Panama. In addition, (–)-epihernandulcin (**4**) was obtained as a natural product. Moreover, in contrast to the initial collection of *L. dulcis* from Mexico, where the yield of (+)-hernandulcin (**1**) was quite low (0.004 % w/w), this sweet compound was obtained in much higher yield (0.154 % w/w) in the Panamanian *L. dulcis* sample [36]. The quantity of compound **7** isolated (9.5 mg; 0.0008 % w/w) was too small to perform preliminary safety testing and evaluation for sweetness potency relative to sucrose using a human taste panel. However, it could be observed that the C-4 β-OH group offers a potential point of attachment for sugars or other polar moieties, so that more water-soluble hernandulcin derivatives can be prepared. Witczak has generated new analogs of hernandulcin with a sugar unit as the six-membered ring, although it was not indicated if such compounds are sweet or not [37]. Based on our earlier observation of the high levels of camphor in the *L. dulcis* sample collected in Mexico [38], a Puerto Rican group has proposed that there are two chemotypes for this species, a hernandulcin type and a camphor type [39,40].

Although hernandulcin (**1**) has been patented as a potential noncaloric sweetener [41], and its high sweetness potency relative to sucrose confirmed by another group [27], the compound does have disadvantages as a sweetener. Thus, it is not very water soluble, and decomposes to ketones **2** and **3** on

heating [25]. Moreover, the compound exhibits definite off- and aftertastes as well as some bitterness when tasted [24]. However, this sweet compound may be suitable for use in oral dentifrices when formulated with *l*-menthol and the ketones menthone, isomenthone, and piperitone [42]. Owing to its structural simplicity and high sweetness potency, hernandulcin remains an excellent model for further studies on the relationship between sweetness and chemical structure [37].

1 $R_1=R_2=H$
5 $R_1=Ac$, $R_2=H$
7 $R_1=H$, $R_2=OH$

8 $R_1=CHO$, $R_2=H$
9 $R_1=CH_2OH$, $R_2=H$
10 $R_1=CH_2OH$, $R_2=OH$

11 $R_1=CH_2OH$, $R_2=H$
12 $R_1=CH_3$, $R_2=OH$

13 $R_1=\beta\text{-glcA}^2\text{-}\beta\text{-xyl}$, $R_2=CHO$
14 $R_1=\beta\text{-glcA}^2\text{-}\beta\text{-glcA}$, $R_2=CHO$

15 $R_1=\beta\text{-glc}$, $R_2=H$
16 $R_1=\beta\text{-glcA-6-CH}_3^2\text{-}\beta\text{-glc}$, $R_2=H$
17 $R_1=\beta\text{-glc}^2\text{-}\beta\text{-glc}$, $R_2=H$
18 $R_1=\beta\text{-glcA}^2\text{-}\beta\text{-glc}$, $R_2=H$
19 $R_1=\beta\text{-glc}^2\text{-}\beta\text{-glcA}$, $R_2=H$
20 $R_1=H$, $R_2=CH_3$
21 $R_1=R_2=H$
22 $R_1=\beta\text{-glc}^2\text{-}\beta\text{-glcA-6-CH}_3$, $R_2=H$
23 $R_1=\beta\text{-glc}$, $R_2=CH_3$

24 R=β-qui
25 R=α-ara

	R_1	R_2	R_3	R_4	
26	Ac	H	OH	H	2*R*, 3*R*
27	Ac	H	OH	CH₃	-
28	Ac	CH₃O	OH	H	2*R*, 3*R*
29	Ac	CH₃O	H	H	2*R*, 3*R*
30	H	CH₃O	OH	H	2*R*, 3*R*

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Fig. 1 Structures of sweet terpenoids, phenols, and some derivatives from plants.

Diterpene glycosides

Baccharis gaudichaudiana DC. (Compositae) was recognized as being a sweet-tasting plant as a result of inquiries in a medicinal plant marketplace in Asuncíon, Paraguay, followed up by organoleptic tests in the field in its native habitat in eastern Paraguay [43]. From this species, a new prototype sweetener of the labdane diterpene glycoside type was isolated, namely, gaudichaudioside A [**8**; 15,19-dihydrox-ylabda-8(9),13(14)*E*-dien-17-al-6α-L-arabinopyranoside]. The structure of **8** was established spectroscopically, using a combination of one- and two-dimensional NMR methods, including the selective INEPT and COLOC techniques. After preliminary safety testing in the usual manner, compound **8** was found by a small human taste panel to be about 55 times sweeter than 2 % w/v sucrose when dissolved in water, and exhibited a pleasant taste [44]. Also obtained in this same investigation were four additional structural analogs of gaudichaudioside A, namely, gaudichaudiosides B–E (**9–12**), which like **8** were all characterized structurally as labdane 6α-L-arabinopyranosides. However, their differential functional group substitution pattern led to a transient sweet leading to a bitter (sweet bitter) taste (**9**), a neutral taste (**10**), an entirely bitter taste (**11**), and a sweet-bitter (**12**) taste, respectively, when evaluated as 0.5 % w/v aqueous solutions [43]. *Baccharis gaudichaudiana* appears to be unusual when compared with other species in the same genus, which are generally bitter or unpleasant tasting.

Triterpene glycosides

Periandrin V {3β-*O*-[β-D-xylopyranosyl-(1 → 2)-β-D-glucuronopyranosyl]-25-al-olean-18(19)-en-30-oic acid} (**13**) was obtained at the University of Illinois at Chicago as a fifth sweet-tasting oleanane-type glycoside from a donated sweet-tasting extract of the rhizomes of *Periandra dulcis* L. (Leguminosae) (Brazilian licorice) [45]. The prototype compound in this series, periandrin I (**14**), was characterized by Hashimoto and co-workers at what is now Kobe Pharmaceutical University in Japan [45]. Periandrin I, like periandrins II–IV, three closely related structural analogs also isolated at Kobe, was rated as about 90–100 times sweeter than sucrose. However, substitution of the terminal D-glucuronic acid unit of periandrin I (**14**) by a D-xylopyranosyl group as in periandrin V (**13**) led to a more highly sweet compound (about 200 times sweeter than 2 % w/v sucrose) [45]. Periandrin V was deemed innocuous in preliminary safety testing in the usual manner. As a result of the sensory evaluation of compound **13**, it was concluded that future synthetic modification of the saccharide units in the periandrin class could prove rewarding in terms of generating more potently sweet substances.

Abrusosides A–E (**15–19**) were isolated as prototype sweet-tasting cycloartane-type triterpene glycosides from the leaves of *Abrus precatorius* L. (Leguminosae) [46–49]. The sweetness of a sample of *A. precatorius* leaves grown in Florida was brought to our attention by the late Dr. Julia F. Morton, a renowned ethnobotanist. The novel structure of abrusoside A [3β-*O*-(β-D-glucopyranosyl)-(20*S*,22*S*)-3β,22-dihydroxy-9,19-cyclolanost-24-en-26,29-oic acid] was proposed as a result of spectroscopic data interpretation, as well as the single-crystal X-ray crystallography of the methyl ester of the aglycone (abrusogenin methyl ester, **20**) [46]. Abrusosides A–E (**15–19**) are differentially glycosylated at the C-3 position of their common aglycone, abrusogenin (**21**). Abrusosides A–D were obtained in sufficient quantities to perform preliminary safety testing (acute toxicity in mice and bacterial mutagenicity), and were found to be innocuous. When the ammonium salts of abrusosides A–D were tasted by a small panel, the compounds were rated, in turn, as 30, 100, 50, and 75 times sweeter than sucrose, although it was found that these glycosides elicit a delayed sweetness response. Somewhat unusually for natural product sweeteners, the compounds have a pleasant sweet taste without significant bitterness [47]. Abrusosides A–D (**15–18**) were also isolated from a second species, namely, *Abrus fruticulosus* Wall. ex W. & A., of Thai origin [50]. Since abrusoside E (**19**) was found to be only marginally sweet [49], it was unexpected that its semi-synthetic 6-*O*-monomethyl ester (**22**) was approximately 150 times sweeter than sucrose, making it the most highly sweet compound in the abrusoside series to date [51]. To facilitate the preparation of additional abrusoside analogs with modified saccharide units with poten-

tial improved sweetness potency compared to the presently known derivatives, recently we have determined reaction conditions for the glucosylation of the sterically hindered C-3 hydroxyl group in abrusogenin methyl ester (**20**). In this work, abrusogenin (**21**) was isolated from *A. precatorius* leaves and methylated with CH_2N_2 to produce **20**, in order to protect the C-4 carboxylic acid group. Abrusoside A methyl ester (**23**) was prepared by a coupling reaction with **20** using 1-chloro-2,3,4,6-tetra-*O*-acetyl-glucopyranose in the presence of AgOTf and TMU in dichloromethane, followed by deacetylation using K_2CO_3 in MeOH-H_2O [52].

The leaves of *Pterocarya paliurus* Batal. (Juglandaceae) are known in regions of Hubei Province in the People's Republic of China as "sweet leaf tree", and are used locally to sweeten foods in cooking. Work up of a sweet-tasting extract of the dried and milled leaves and stems of this plant provided by a Chinese colleague, led to the isolation of two new sweet-tasting 3,4-*seco*-dammarane glycosides, which were accorded the trivial names, pterocaryosides A (**24**) and B (**25**), respectively. The structure of the first of these compounds was assigned using spectroscopic methods as 12-*O*-β-D-quinovopyranosyl-(23*E*)-(12*R*,20*S*)-12,20,25-trihydroxy-3,4-*seco*-dammar-4(28),23-dien-3-oic acid, while in compound **25** an α-L-arabinose unit replaced the sugar unit of compound **24** [53]. Compounds **24** and **25** showed considerable structural resemblance to some C_{30} secodammaranes isolated earlier from the male flowers of *Alnus japonica* (Thunb.) Steudel (Betulaceae) [54]. After the performance of preliminary safety testing involving acute toxicity tests in mice and bacterial forward mutation tests, the ammonium salts of **24** and **25** were rated by a small human taste panel with 50 and 100 times, respectively, the sweetness intensity of 2 % w/v sucrose. While both compounds had a persistent, mildly bitter off-taste, their onset of sweet taste was almost instantaneous [53]. Also obtained from the same plant [under its other name, *Cyclocarya paliurus* (Batal.) Iljinskaya] are two sweet-tasting dammarane glycosides called cyclocarioside A [55] and cyclocarioside I [56], which were isolated and characterized by investigators at two different institutions in the People's Republic of China, and each rated as exhibiting about 200–250 times the sweetness intensity of sucrose. In addition, Shu et al. isolated two 3,4-*seco*-dammarane glycosides called cyclocariosides II and III from the same plant, although these compounds were not stated as being sweet-tasting [57]. Accordingly, pterocaryosides A (**24**) and (**25**) are the first examples of highly sweet *seco*-dammarane glycosides to have been obtained, and were isolated as a result of a previous ethnobotanical observation. Since compounds **24** and **25** differ only in their glycosidic units, but yet differ in their sweetness potencies, it is possible that derivatives with different sugar units might be synthesized with improved parameters as sweetening agents [53].

Dihydroflavonols

The herb *Tessaria dodoneifolia* (Hook. & Arn.) Cabrera (Compositae) was included in our sweetener research program, because it was obtained as a sweet herb at a medicinal plant marketplace in Asuncíon, Paraguay. The young shoots were collected from a cultivated plot of *T. dodoneifolia*, and sweetness was traced to a dihydroflavonol of previously known structure, (+)-dihydroquercetin 3-acetate (**26**) [57]. Owing to the general similarity between the dihydroflavonol and dihydroisocoumarin sweeteners, it was decided to synthetically modify this compound in ring B. Compound **27** was synthesized in racemic form in 7 % overall yield from 2,4-bis(benzyloxy)-6-(methoxymethoxy)acetophenone and 3-(benzyloxy)-4-methoxybenzaldehyde, based on a known method for dihydroflavonols. In contrast to compound **26**, which was rated after preliminary safety testing as about 80 times sweeter than 2 % w/v sucrose, the synthetic compound dihydroquercetin 3-acetate (4′-methyl ether) (**27**) was evaluated as 400 times sweeter [58]. However, compound **27** showed only very limited solubility in water, and in order to perform the sensory testing, it was necessary to use 3 % ethanol to dissolve the sample [58]. Compound **26** and three additional sweet-tasting dihydroflavonols (**28–30**) were isolated from the above-ground parts of *Hymenoxys turneri* K. Parker (Compositae) in the laboratory of Prof. Tom J. Mabry, at the University of Texas. When rated for sweetness intensity, the C-6 methoxylated com-

pounds **28–30** were rated as exhibiting about 25, 15, and, 20 times the sweetness intensity of 2 % w/v sucrose, respectively [59].

PROANTHOCYANIDINS

The sweet-tasting rhizomes of the fern *Selliguea feei* Bory [syn. *Polypodium feii* (Bory) Mett.] (Polypodiaceae) were chosen for study for chemotaxonomic reasons, in an attempt to see if novel sweet-tasting steroidal saponins related in structure to osladin [60] and polypodoside A [21] were present, as found in other *Polypodium* species. The sample obtained was collected in western Java, and it was noted that only the mature rhizomes were sweet, with the immature rhizomes being bitter tasting. Chromatographic fractionation of a 1-butanol extract of the mature *S. feei* rhizomes led to the isolation of a member of an unanticipated chemical class as the major sweet-tasting principle, namely, a new trimeric proanthocyanidin with a doubly linked ring A unit, with the structure epi-afzelchin-(4β → 8, 2β → *O* → 7)-epiafzelechin-(4β → 8)-afzelechin (**31**). This compound, which was accorded the trivial name, selligueain A, was purified in a high yield (0.69 % w/w) from *P. feei* rhizomes by several separations over silica gel columns and final purification from methanol. The structure of **31** was proved by peracetylation to an undecaacetate, and thiolysis with α-toluenethiol in the presence of glacial acetic acid to afford (+)-afzelechin and a known thioether. The latter was subjected to desulfuration using Raney nickel to afford a doubly linked ring A dimeric proanthocyanidin of known structure [61]. Selligueain A (**31**) was found not to be acutely toxic for mice (1 g/kg and 2 g/kg) and not mutagenic in a forward mutation test, and thus was rated for its sweetness properties by a small taste panel. When dissolved in water, the sweetness intensity of **31** was rated as equivalent to about 35 times sweeter than a 2 % w/v aqueous sucrose solution, and exhibited no appreciable off-taste or after-taste. At a higher concentration (0.5 % w/v), compound **31** showed a pleasantly sweet taste, with only a trace of bitterness and astringency [62]. Compound **31** differs from previously known sweet-tasting doubly-linked ring A proanthocyanidins in being the first such compound to possess a ring-C afzelechin unit [51]. Using a quantitative HPLC method, Bohlin and co-workers have shown the occurrence of selligueain A in the rhizomes of five additional *Polypodium* species from Honduras in the range 0.0028–0.016 % w/w, namely, *P. aureum* L., *P. decumanum* Willd., *P. loriceum* L., *P. lowei* C. Chr., and *P. triseriale* Sw. [62]. In addition, compound **31** was found in the leaves of these species in trace quantities [62]. Bohlin and co-workers reported selligueain A (**31**) as an elastase inhibitor in human neutrophils [63], and Subarnas and Wagner reported its analgesic and anti-inflammatory activities when evaluated with a writing method in mice and a rat paw edema method, respectively [64]. Further phytochemical investigation of *S. feei* rhizomes by our group led to the isolation and structure elucidation of a second new proanthocyanidin, selligueain B [epiafzelechin-(4β → 8,2β → *O* → 7)-epi-afzelechin-(4β → 8)-3′-deoxydryopteric acid methyl ester], which was not sweet-tasting and somewhat astringent [65]. Also isolated in this additional study was the known flavonoid glycoside kaempferol 3-*O*-β-D-glucopyranosyl-7-*O*-α-L-rhamnopyranoside, which was bitter tasting, and may account for the bitterness of the immature *S. feei* rhizomes [65]. The structural requirements for the exhibition of a sweet taste among the trimeric proanthocyanidins of plant origin seem to be quite specific, since the epimer of selligueain A (**31**), namely, epiafzelechin-(4β → 8,2β → *O* → 7)-epiapzelechin-(4β → 8)-epiafzelechin was astringent without any hint of sweetness [51,65].

CONCLUSIONS

Several plant-derived compounds of the terpenoid and phenolic (flavonoid) types have commercial value as substitutes for sucrose in foods, beverages, or medicines, in a number of countries. In this review, we have described a number of additional sweet-tasting compounds of plant origin representing these structural classes, which were discovered in a collaborative manner between scientists in several disciplines, including botanists, ethnobotanists, natural products chemists, and biologists. It is becom-

ing necessary to locate suitable candidate sweet-tasting plants for study in ever more remote geographical areas than previously has been the case. We feel that further new highly sweet compounds of natural origin will be able to be discovered most expeditiously through multidisciplinary collaborative research efforts in the future.

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