Stimulation of the Gerbil's Gustatory Receptors by Some Potently Sweet Terpenoids†

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The gerbil was investigated as a model for sweet taste among several highly sweet plant terpenoids and the sweet dihydroisocoumarin phyllodulcin. Although the gerbil's chorda tympani nerve did not respond in electrophysiological experiments to rebaudiosides B and C, steviolbioside, and phyllodulcin, concentration–response curves were obtained for the stimulatory sweeteners hernandulcin, mogroside V, periandrin III, rebaudioside A, and stevioside. These compounds were more effective stimuli in the gerbil than sucrose, in the following order of potency: rebaudioside A = stevioside = periandrin III > hernandulcin > mogroside V > sucrose. In conditioned-taste aversion studies, gerbils trained to avoid these five stimulatory compounds generalized an avoidance to sucrose but not to hydrochloric acid, and except for the perception of a concomitant salty taste, our data show that these substances taste like sucrose to gerbils, as in humans. Support is thus provided for the potential involvement of this methodology to guide the purification of natural sweeteners from plant extracts.

There is an increasing interest in highly sweet nonnutritive and noncariogenic natural sweeteners, and over 50 such substances in more than 15 structural classes are biosynthesized by members of the plant kingdom (Kinghorn and Soejarto, 1986, 1989). Several sweet plant constituents, in either pure or partially purified form, are used commercially as sucrose substitutes in Japan, including the terpenoid glycosides glycyrrhizin, mogroside V, stevioside, and rebaudioside A, the dihydroisocoumarin phyllodulcin, and the protein thaumatin (Ishikawa et al., 1991; Kinghorn and Compadre, 1991). In addition, semisynthetic compounds based on plant constituents such as neohesperidin dihydrochalcone and perillartine are approved sweeteners in a number of countries (Horowitz and Gentili, 1991; Kinghorn and Compadre, 1991).

In electrophysiological gustatory experiments using the Mongolian gerbil, many different classes of naturally occurring and synthetic sweet compounds have been shown to stimulate the animal's taste nerve, including sweet monosaccharides, disaccharides, and polyols, as well as more potently sweet substances such as chlorosucrose, L-cyanosuccinanilic acid, dulcin, sodium saccharin, stevioside, and 6-chloro-D-tryptophan (Jakinovich, 1976, 1981; Jakinovich and Goldstein, 1976; Jakinovich and Oakley, 1976). Furthermore, the behavioral conditioned aversion technique has proven to be successful in rodent studies to identify the taste qualities of many compounds, such as alcohols, amino acids, aspartame, sodium saccharin, and sugars (Garcia et al., 1974; Herness and Pfaffmann, 1986; Jakinovich, 1981, 1982; Kasahara et al., 1987; Kiefer and Lawrence, 1988; Myers et al., 1989; Nachman and Cole, 1971; Ninomiya et al., 1984; Nissenbaum and Scafani, 1987; Nowlis et al., 1980; Pritchard and Scott, 1982; Smith and Theodore, 1984; Spector and Grill, 1988; Steward and Krafczek, 1988; Thomesen et al., 1988). Accordingly, using sweet compounds found to stimulate the gerbil's chorda tympani nerve in electrophysiological experiments, the majority of such substances were found to resemble sucrose in behavioral experiments using the Mongolian gerbil. However, some of these sweet substances were not avoided by animals trained to avoid sucrose, so it cannot be assumed that all compounds that are "sweet" to man are "sweet" to the gerbil (Jakinovich, 1981, 1982a,b).

In an ongoing program to discover novel highly sweet natural products (Kaneda et al., 1992), it has been our practice to subject extracts of sweet-tasting plants to acute toxicity tests in mice and bacterial mutagenicity testing, prior to evaluation for sweetness by human participants. To investigate the possibility of circumventing such a costly and rather inconvenient safety procedure, we have found that a combination of gerbil electrophysiological and conditioned taste aversion experiments could be used in a generally reliable fashion to detect the presence or absence of sweet-tasting terpenoid glycosides in extracts of different polarities of three well-known sweet-tasting plants, namely, *Abrus precatorius*, *Stevia rebaudiana*, and *Thladiantha grosvenorii* (Jakinovich et al., 1990). The present study extends our previous effort by investigating the effect on the gerbil's receptors of several of the purified sweet-tasting diterpene constituents of *S. rebaudiana* (rebaudiosides A–C, stevioside, steviolbioside) (Kinghorn and Soejarto, 1986; Tanaka, 1982) and the major triterpene glycoside sweet principle of *T. grosvenorii* (mogroside V) (Takemoto et al., 1983). In addition, several other pure plant-derived sweeteners have been evaluated in the gerbil model: hernandulcin, a sesquiterpene constituent from *Lippia dulcis* (Compadre et al., 1985); periandrin III, a triterpene glycoside from *Periandra dulcis* (Hashimoto et al., 1982); and phyllodulcin, a dihydroisocoumarin obtained from the crushed or fermented leaves of *Hy-*

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drangea macrophylla var. *Thunbergii* (Arakawa and Nakazaki, 1959; Kinghorn and Soejarto, 1986). Since many of these potent natural sweeteners were observed in the present study to stimulate the gerbil's chorda tympani nerve and to taste like sucrose in behavioral experiments, further support has been obtained for the inclusion of experiments on the gerbil in the fractionation of sweet-tasting plants, thereby partially offsetting the need for human volunteer subjects.

MATERIALS AND METHODS

General Procedures. Melting points were determined on a Kofler hot-stage instrument and are uncorrected. Optical rotations, UV, IR, ^{1}H NMR, ^{13}C NMR, and low-resolution mass spectrometry were performed as described previously (Kaneda et al., 1992). Analytical TLC was carried out on Merck silica gel G plates, with 250 μm thick layers, which were visualized in short-wave UV light and/or after spraying with 60% w/v H_2SO_4 and heating at 110 °C for 10 min. Where suitable, HPLC analysis was performed to further verify purity (Makapugay et al., 1984, 1985; Compadre et al., 1987).

Test Compounds. Hernandulcin (1) was synthesized in the racemic form by directed-aldol condensation from 3-methyl-2-cyclohexen-1-one and 6 methyl-5-hepten-2-one and purified as previously described. This compound was indistinguishable spectroscopically and chromatographically from its naturally occurring (+) form, (6*S*,1′*S*)-hernandulcin (Compadre et al., 1985, 1987). Mogroside V (2) was purified from Lo Han Kuo fruits [*T. grosvenorii* (Swingle) C. Jeffrey; recently renamed *Siraitia grosvenorii* (Swingle) C. Jeffrey] (Kinghorn and Compadre, 1991) and characterized as described previously (Makapugay et al., 1985). The trisodium salt of periandrin III (3) was generously donated by Yamasa Shoyu Co., Ltd. (Chosi, Chiba, Japan) and, after conversion to the parent compound and workup, exhibited physical and spectroscopic data identical to published values for periandrin III (3) (Hashimoto et al., 1982).

The dihydroisocoumarin, phyllodulcin (4), was isolated from the crushed leaves of *H. macrophylla* Seringe var. *Thunbergii* (Siebold) Makino, kindly supplied by the late Prof. T. Takemoto. An initial methanol–water (4:1) extract was prepared from the dried milled plant material (7 kg) from which the solvent was removed. On suspension in water and partitioning with ethyl acetate, the ethyl acetate residue (350 g) was purified by gravity column chromatography over silica gel (Merck, Darmstadt, Germany), and phyllodulcin was eluted with mixtures of petroleum ether–chloroform in proportions of 13:7 and 1:1. Isolated phyllodulcin (4, 90 g, 1.28% w/w) was recrystallized as white needles from chloroform–petroleum ether [mp 118–119 °C, $[\alpha]^{25}_D$ +80.5° (*c* 2.9, $CHCl_3$) [lit. mp 119–121 °C; $[\alpha]^{25}_D$ +70.7–80.8° (*c* 1.02, Me_2CO)] (Arakawa and Nakazaki, 1959)] and exhibited spectroscopic data closely comparable to published values for this compound (Suzuki et al., 1978). The identity of 4 as phyllodulcin was confirmed by direct comparison (mmp, EI-MS, ^{1}H NMR, co-TLC) to a reference sample kindly supplied by Prof. M. Yamamoto.

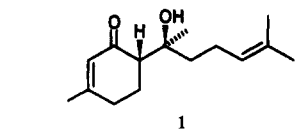
Rebaudioside A (5), rebaudioside C (7), and stevioside (9) were isolated and characterized from *S. rebaudiana* (Bertoni) Bertoni leaves, as described previously. Rebaudioside B (6) and steviolbioside (8) were obtained from compounds 5 and 9, respectively, by alkaline hydrolysis. All five of these sweet diterpene glycosides exhibited physical and spectroscopic data consistent with literature values (Makapugay et al., 1984).

Prior to being used in the present study, all compounds were tested for purity by analytical TLC and/or HPLC. The structures of test compounds 1–9 are shown in Figure 1.

Experimental Animals. Mongolian gerbils (*Meriones unguiculatus*) were obtained from Tumblebrook Farms, West Brookfield, MA. For electrophysiological experiments, male and female animals, and were less than 1 year old and weighing 50–70 g, were used. For behavioral experiments, male gerbils aged 7–12 weeks were utilized and were 50–60 g in weight.

Methods. *Electrophysiological Methods.* (*a*) *Anesthetic.* Gerbils were injected with ketamine as the primary anesthetic because it produces complete anesthesia in 5–10 min. The ketamine (100 mg/mL) was injected at a dose of 330 mg/kg into the gerbil's thigh muscle. If an animal required further anesthetic during the experiment, sodium pentobarbital (5 mg/mL; 0.15 mL) injected intraperitoneally was employed (Somenerain and Jakinovich, 1990).

(*b*) *Electrophysiology.* Each animal was secured to a head-holder (Oakley and Schaffer, 1978) which immobilized the skull.



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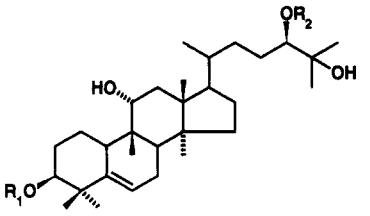
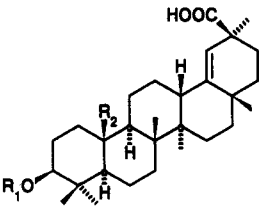
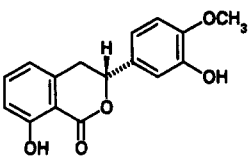


	R_1	R_2
2	β-glc^{6}–β-glc	β-glc^{6}–β-glc |^{2} β-glc



	R_1	R_2
3	β-glcA^{2}–β-glcA	CH_2OH



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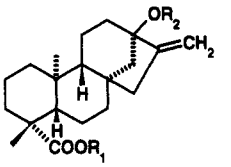

	R_1	R_2
5	β-glc	β-glc^{2}–β-glc |^{3} β-glc
6	H	β-glc^{2}–β-glc |^{3} β-glc
7	β-glc	β-glc^{2}–α-rha |^{3} β-glc
8	H	β-glc^{2}–β-glc
9	β-glc	β-glc^{2}–β-glc

Figure 1. Chemical structures of the highly sweet terpenoids (1–3, 5–9) and the dihydroisocoumarin (4) investigated in this study. Sugar units: β–glc = β-D-glucopyranosyl; β-glcA = β-D-glucuronopyranosyl; α-rha = α-L-rhamnopyranosyl.

The method for exposing and recording from the intact chorda tympani nerve has been reported in detail (Somenerain and Jakinovich, 1990).

(c) *Stimulation.* Chemical stimulation of the tongue was effected by a gravity-flow funnel-tubing system through which deionized water flowed continuously (0.13–0.17 mL/s). Test solutions (2–4 mL) were alternated with water without interruption of the flow. The temperatures of the water and the taste solutions were identical, 25 ± 1 °C. Each compound was tested twice, before and after a standard. Whenever the standard solution elicited responses differing by more than 15%, all interjacent responses were rejected.

(d) *Taste Solutions.* All compounds were dissolved in deionized tap water (>1 megohm). When not used immediately, the sweetener solutions were stored in frozen form or at 2 °C for later use, when they were brought to room temperature.

(e) *Mixtures.* To determine if nonstimulating sweeteners were interacting with the sweetener taste receptor sites, responses to mixtures of sucrose and a nonstimulating sweetener and of rebaudioside A and a nonstimulating sweetener were compared to responses to sucrose and rebaudioside A (Jakinovich, 1981, 1983; Vlahopoulos and Jakinovich, 1986).

Behavioral Methods. (a) *General Scope of Study.* This behavior study comprised two sets of experiments, with the first to determine how the gerbils perceived the taste of mogroside V (2, 0.001 M) and stevioside (9, 0.002 M). The second set of experiments dealt with the perception of the taste by the gerbil of hernandulcin (1, 0.01 M), periandrin III (3, sodium salt) (0.003 M), and rebaudioside A (5, 0.002 M). The concentrations used were the CR_{50}'s (concentrations that produced half-maximal responses), as determined from the appropriate electrophoretic concentration–response curve, or else the maximum solubility obtained in water.

(b) *Conditioned Taste Aversion.* The following training procedures were used in all experiments:

(1) *Water Intake Training.* One day after arrival, the animals were housed in individual plastic cages with wood chip bedding, rather than in individual wire-bottom cages, to avoid health problems (Jakinovich, 1981, 1982; Jakinovich et al., 1990; Myers et al., 1989). Two days later, all test animals were placed on a drinking schedule whereby they received deionized water twice daily (from 09:00 to 10:00 a.m. and from 3:00 to 4:00 p.m.). Animals were fed Purina Rat Chow (Ralston Purina Co.) *ad libitum.*

(2) *Conditioned Avoidance Training.* After 6 days of the water training, the animals were randomly divided into three groups of 12 each, with the first group trained to avoid mogroside V (2, 0.001 M), the second group trained to avoid stevioside (9, 0.002 M), and a control group trained to avoid water. The mogroside V (0.001 M) and stevioside (0.002 M) solutions were used as conditioning solutions in the following manner: On the Friday of each of the *consecutive* weeks, during the usual morning drinking period one group of gerbils was offered a drinking bottle containing mogroside V, one group was offered stevioside, and the third group, acting as control, received only water. These solutions were offered for 15 min during the morning watering period. When each animal finished drinking, its drinking tube was again placed in its mouth, leaving a few drops behind, and, immediately after that, the gerbil was injected with LiCl (0.3 M) at 1% of its body weight. Shortly after the injections, the animals showed lethargic appearances which lasted for several hours. This entire avoidance training procedure, as described above, was repeated on the following Friday, and on the Friday of each consecutive week.

(3) *Conditioned Avoidance Testing.* On the Monday of the third week, having allowed 2 days for the animals to recuperate, half of the animals in each group were offered water bottles containing sucrose (0.03 M), while the other half received bottles containing NaCl solution (0.01 M). Then, the following morning the animals received these two solutions in reverse order. On Wednesday and Thursday, the above procedure was repeated. Measurements of the amounts of particular fluids consumed were made by weighing the drinking bottles immediately after each animal stopped drinking. During the afternoon drinking period, all animals received only deionized water.

On the Monday of the fourth week, 50% of the animals in each group were offered water bottles containing HCl (0.01 M), while the other half received bottles of quinine hydrochloride solution (0.001 M). Next, on the following morning, the animals received those solutions in reverse order. On Wednesday and Thursday, the above procedure was repeated.

To strengthen and reinforce the aversion, the entire conditioning and testing was repeated. The entire above-indicated behavioral procedure was repeated with solutions of hernandulcin (1, 0.01 M), periandrin III (3, sodium salt, 0.003 M), and rebaudioside A (5, 0.002 M).

(c) *Statistics.* One-way analyses of variance (ANOVA) were applied to the behavioral results. When significant differences were observed, pairwise analyses (*t*-test) were performed between the control and experimental groups (see Results).

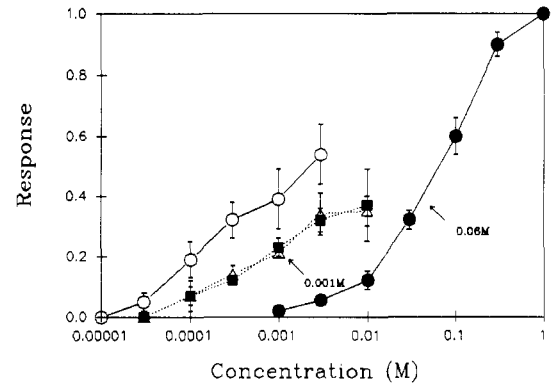

Figure 2. Integrated neural discharge from the gerbil's chorda tympani nerve in response to various concentrations (log scale) of purified rebaudioside A (△), Nutrilite stevioside (impure commercial source) (○), purified stevioside (■), and sucrose (●). The CR_{50}'s are shown. Bars indicate ±2SE; N for sucrose = 14, N for purified stevioside = 5, and N for rebaudioside A = 8.

RESULTS

No Electrophysiological Responses. The following compounds did not produce responses in the gerbil's chorda tympani nerve at the concentrations used: phyllodulcin (4), rebaudioside B (6), rebaudioside C (7), and steviolbioside (8). Their maximum solubility in water ranged from 1×10^{-3} to 5×10^{-4} M.

Electrophysiological Responses—Concentration–Response Curves Determined. Hernandulcin (1), mogroside V (2), periandrin III (3, sodium salt), rebaudioside A (5), and stevioside (9) produced responses in the gerbil's chorda tympani nerve. The maximum solubility of the compounds in water was around 0.01 M, which was lower than that of sucrose in all cases. Responses to two compounds, rebaudioside A and stevioside, that produced concentration–response curves with a shape similar to that of sucrose, are shown in Figure 2. These concentration curves exhibited a sigmoidal shape with a maximum response (R_{max}) evident, which is a characteristic of a normal neural sweetener taste response (Jakinovich and Sugarman, 1989). The R_{max} values of rebaudioside A and stevioside were 0.4 (sucrose R_{max}, 1.0). The previously published concentration–response curve of stevioside obtained from a commercial source (Nutrilite Products Inc., Buena Park, CA) is included in Figure 2 for comparison purposes (Jakinovich, 1981). Since R_{max} values were determined, CR_{50} data could be used as one measure of potency (Jakinovich, 1976), and the results obtained were rebaudioside A CR_{50} = 0.001 M, stevioside CR_{50} = 0.001 M, and sucrose CR_{50} = 0.06 M. Another measure of potency, the K_d value (dissociation constant, representing the efficacy of the sweet compounds) (Biedler, 1954), was determined for three sweeteners using the reciprocal plot, as expressed in Figure 3 (sucrose, 0.07 M; stevioside, 0.0013 M; rebaudioside A, 0.0014 M). A third measure of potency, threshold value, was determined directly from Figure 2

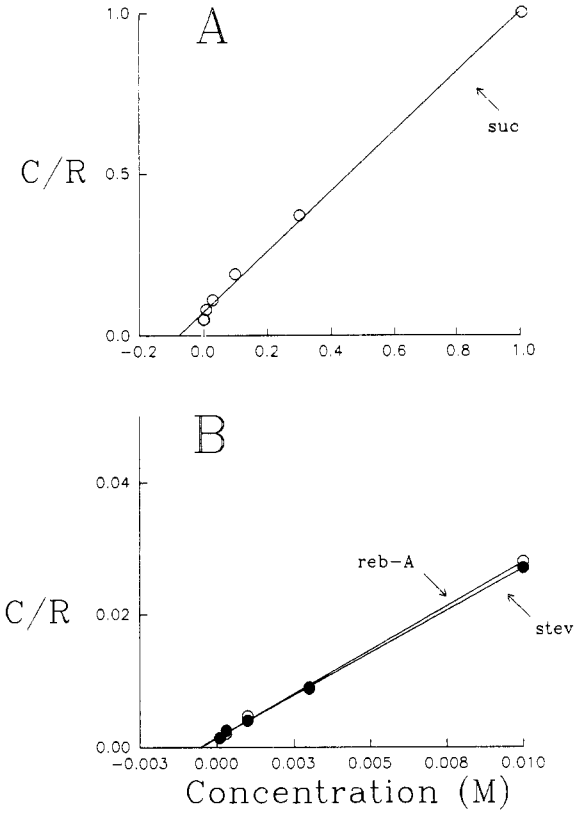

Figure 3. (A) Reciprocal plot of gerbil integrated chorda tympani nerve responses to sucrose (suc, O). (B) Reciprocal plot of gerbil integrated chorda tympani nerve responses to rebaudioside A (reb-A, O) and stevioside (stev, ●). C = concentration, R = response, slope = $1/R_{max}$, K_d/R_{max} = y intercept, and $-K_d$ = x intercept.

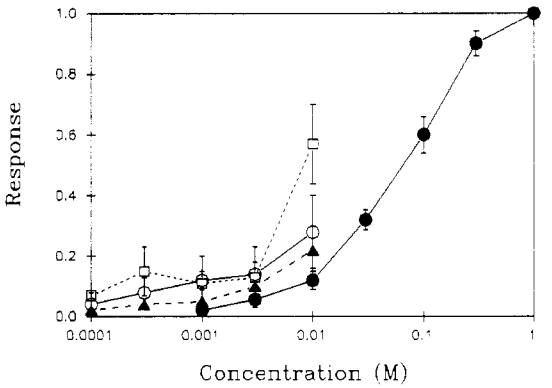

Figure 4. Integrated neural discharge from the gerbil's chorda tympani nerve in response to various concentrations (log scale) of hernandulcin (▲), mogroside V (□), periandrin III (sodium salt) (O), and sucrose (●). Bars indicate ±2SE; N for sucrose = 14, N for hernandulcin = 5, N for mogroside V = 6, and N for periandrin III (sodium salt) = 5.

(sucrose, 0.003 M; stevioside, 0.0001 M; rebaudioside A, 0.0001 M).

We were unable to obtain complete sigmoidally shaped (with R_{max}) concentration–response curves for three compounds, hernandulcin (1), mogroside V (2), and periandrin III (3; sodium salt) (Figure 4). However, since the R_{max}'s were not present, the potency of each compound was determined from its K_d in the reciprocal plot (Figure 5) (Beidler, 1954), with the following values obtained: periandrin III, sodium salt, K_d = 0.0006 M; mogroside V, K_d = 0.003 M; hernandulcin, K_d = 0.002 M. Thresholds were determined from Figure 4: periandrin III (sodium salt) threshold, 0.0001 M; mogroside V threshold, 0.0001 M; and hernandulcin threshold, 0.0003 M.

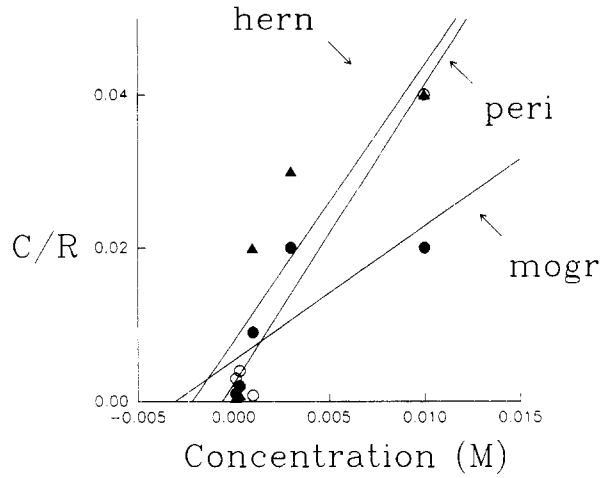

Figure 5. Reciprocal plot of gerbil integrated chorda tympani nerve responses to hernandulcin (hern, ▲), mogroside V (mogro, ●), and periandrin III (sodium salt) (peri, O).

Mixtures. The taste responses to sucrose or rebaudioside A were not influenced by the presence of a nonstimulating sweetener.

Behavioral Responses. In all cases, gerbils trained to avoid an intense natural sweetener consumed significantly less sucrose (0.03 M) than water (Table I). Concerning saltiness, gerbils trained to avoid hernandulcin, rebaudioside A, and periandrin III (sodium salt) consumed significantly less NaCl (0.01 M) than water. For sourness, intakes of HCl (0.01 M) by all trained gerbils were no different from water. Finally, for bitterness, gerbils trained to avoid mogroside V, hernandulcin, rebaudioside A, and periandrin III (sodium salt) consumed significantly less quinine hydrochloride (0.001 M) than water.

DISCUSSION

In the present gerbil electrophysiological investigation, the CR_{50} in the standard sucrose concentration–response curve was 0.06 M, which is in general agreement with our past gerbil studies in which the sucrose CR_{50} values ranged from 0.015 to 0.05 M (Somenerain and Jakinovich, 1990). Another consistency was that the sucrose concentration–response curve was sigmoidally shaped and reached an R_{max} (Figure 2). However, it was found in this study that the plant-derived sweeteners phyllodulcin (4), rebaudiosides B and C (6, 7), and steviolbioside (8) did not stimulate the gerbil's chorda tympani nerve in electrophysiological experiments (Table II). These observations are consistent with our previous gerbil study in which it was discerned that a lack of response in this regard was evident with the following natural and artificial potently sweet compounds: aspartame, beryllium acetate, furan acrylonitrile, 4-(methoxymethyl)-1,4-cyclohexadiene-1-carboxaldehyde *syn*-oxime, monellin, 5-nitro-2-propoxyaniline, and perillartine (Jakinovich, 1981).

The electrophysiological concentration–response curves of the purified stevioside (9) and rebaudioside A (5) used in this investigation were also sigmoidally shaped and reached an R_{max} of 0.4 (Figure 2). The CR_{50} of both these compounds was 0.001 M. The concentration–response curve of our previously published work using a commercially available stevioside sample is anomalous because it did not reach an R_{max} and was not sigmoidal (Jakinovich, 1981). This distortion is probably due to the presence of unspecified impurities, which are also thought to be responsible for distorted concentration–response curves in the gerbil's chorda tympani nerve responses reported for methyl β-D-galactopyranoside (Jakinovich, 1985) or in human psychophysics studies for D-ribitol (Jakinovich and

Table I. Amounts (Milliliters ± Standard Error) of Test and Control Solutions Consumed by Gerbils Trained To Avoid Highly Sweet Plant-Derived Constituents

		test solution			
conditioning solution	control solution	0.03 M sucrose	0.1 M NaCl	0.01 M HCl	0.001 M quinine hydrochloride
mogroside V (0.001 M)		0.66 ± 0.21[a]	3.19 ± 0.27	1.22 ± 0.22	0.54 ± 0.13[a]
stevioside (0.02 M)		0.39 ± 0.06[a]	3.32 ± 0.31	1.06 ± 0.17	1.01 ± 0.21
	water–sucrose	2.07 ± 0.29			
	water–NaCl		2.71 ± 0.18		
	water–HCl			1.63 ± 0.07	
	water–quinine hydrochloride				1.23 ± 0.12
hernandulcin (0.01 M)		0.95 ± 0.22[a]	1.52 ± 0.35[a]	1.6 ± 0.38	0.76 ± 0.17[a]
rebaudioside A (0.02 M)		0.21 ± 0.01[a]	1.05 ± 0.31[a]	1.1 ± 0.16	0.89 ± 0.23[a]
periandrin III (sodium salt) (0.003 M)		1.14 ± 0.27[a]	1.12 ± 0.34[a]	1.6 ± 0.31	0.90 ± 0.19[a]
	water–sucrose	1.81 ± 0.16			
	water–NaCl		2.38 ± 0.38		
	water–HCl			1.85 ± 0.25	
	water–quinine hydrochloride				1.58 ± 0.18

[a] Significant difference, $p > 0.01$; $N = 12$.

Table II. Gerbil Electrophysiological Responses to Stimulatory Plant-Derived Sweeteners[a,b]

compound	threshold, M	K_d, M	CR_{50}, M	sweetness to humans[c] (× sucrose)	ref
sucrose	0.003	0.07	0.06	1	Beck (1974)
mogroside V	0.0001	0.002	ND[d]	340	Takemoto et al. (1983)
hernandulcin	0.0003	0.003	ND[d]	1,500	Compadre et al. (1985)
periandrin III (sodium salt)	0.0001	0.0006	ND[d]	80	Kinghorn and Soejarto (1986)
rebaudioside A	0.0001	0.0006	0.001	240	Kasai et al. (1981)
stevioside	0.0001	0.0006	0.001	140	Kasai et al. (1981)

[a] Test compounds are ranked according to K_d. [b] The following compounds in the study were nonstimulatory at the doses specified: phyllodulcin (5×10^{-4} M); rebaudioside B (1×10^{-3} M); rebaudioside C (1×10^{-3} M); steviolbioside (1×10^{-3} M). [c] Figures are expressed as approximate sweetness intensities on a weight comparison to sucrose. [d] ND, not determined.

Sugarman, 1989). The reduced R_{max}'s observed in the stevioside and rebaudioside A concentration–response curves when compared to that of sucrose suggested that stevioside and rebaudioside A either are partial agonists at the receptor site or else bind at a different receptor site from sucrose (Ariëns et al., 1964). Complete concentration–response curves were not obtained for mogroside V (**2**), periandrin III (**3**; used as the sodium salt), and hernandulcin (**1**). In spite of this, it was possible to rank the potency of these sweeteners as gustatory stimuli in the gerbil on the basis of their determined K_d values in the following order of decreasing magnitude: rebaudioside A = stevioside = periandrin III (sodium salt) > hernandulcin > mogroside V > sucrose (Table II). A similar ranking list of potency was obtained by considering threshold and CR_{50} values.

The results of the behavioral experiments showed that the gerbil's taste responses to hernandulcin, mogroside V, periandrin III (sodium salt), rebaudioside A, and stevioside resemble their effects in humans. To the human, representatives of these highly sweet natural sweeteners, for which hedonic data have been reported, have been found to taste either sweet (resembling the taste of sucrose) or sweet-bitter (resembling the taste of sucrose and quinine) (Compadre et al., 1985; Schiffman et al., 1979). In the human, all five of these natural sweeteners are sweeter than sucrose, in the following order of potency of sweetness intensity: hernandulcin > mogroside A > rebaudioside A > stevioside > periandrin III > sucrose (Table II) (Compadre et al., 1985; Kasai et al., 1981; Kinghorn and Soejarto, 1986; Takemoto et al., 1983). When the two *ent*-kaurene glycoside sweeteners in this group of stimulatory compounds are considered structurally, rebaudioside A possesses one more glucopyranosyl moiety in its C-13-affixed saccharide unit than stevioside, which accordingly confers greater sweetness and more pleasant hedonic attributes for humans. However, the sweetness potency of rebaudioside A is greatly diminished in human subjects either by removal of the glucose attached to C-19 or by substitution of a glucose moiety by rhamnose in the C-13 sugar unit, as in rebaudiosides B and C, respectively (Kinghorn and Soejarto, 1986; Tanaka, 1982).

In conclusion, a combination of two well-established gerbil electrophysiological and behavioral assays has been applied to a structurally diverse group of terpenoids that are highly sweet to humans, constituted by the bisabolane sesquiterpene hernandulcin, the cucurbitane triterpene glycoside mogroside V, the oleanane-type triterpene glycoside periandrin III, and the *ent*-kaurene-type diterpene glycosides rebaudioside A and stevioside. It is significant that the most abundant sweet constituents of *T. grosvenorii* fruits (mogroside V) and *S. rebaudiana* leaves (stevioside and rebaudioside A) have been found to stimulate the gerbil's taste receptors in this study, since we have earlier shown that extracts containing these compounds also gave positive data (Jakinovich et al., 1990). Although these gerbil assays do not respond to all classes of compounds perceived as sweet by humans, they do seem to have validity for evaluating sweet-tasting terpenoids of plant origin.

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