

POTENTIAL SWEETENING AGENTS OF PLANT ORIGIN. III. ORGANOLEPTIC EVALUATION OF *STEVIA* LEAF HERBARIUM SAMPLES FOR SWEETNESS¹

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ABSTRACT.—A total of 184 *Stevia* leaf samples taken from herbarium specimens, representing 110 species and 121 taxa, were screened organoleptically for their taste sensation. Fragments of a 62-year-old leaf of *S. rebaudiana* exhibited a potent and prolonged sensation of sweetness, thereby indicating the stability of its sweet *ent*-kaurene glycoside constituents to drying, preservation, mounting and storage. No other leaf samples exhibited an intensity of sweetness equivalent to that of *S. rebaudiana*, though 18 species and varieties were considered to exhibit a sweet taste. These taxa appear to be promising candidates for future phytochemical investigation for new and known *ent*-kaurene glycosides.

As an extension of our field search for the sweet-tasting species of *Stevia* (1), we have performed organoleptic tests on dried leaves of members of this genus. The leaf samples or fragments of leaves used for the present study were removed from herbarium specimens on deposit at the John G. Searle Herbarium of the Field Museum of Natural History in Chicago.

Stevia, a New World genus of the Compositae, belongs to the tribe Eupatorieae. The distribution range of this taxon extends from southwestern U.S.A. to northern Argentina, through Mexico, Central America, the South American Andes and the Brazilian highlands. Estimates on the number of species within the genus range from 150 to 300 (2-5), of which over 80 species are known to occur in North America, with at least 70 of these indigenous to Mexico (3,4). While the South American *Stevia* have not received recent taxonomic attention, perhaps 90 species occur in a triangular area bounded by Bolivia, southern Brazil and northern Argentina.³

Eight sweet *ent*-kaurene glycosides, stevioside, rebaudiosides A-E, dulcoside A and steviolbioside have been identified from *Stevia rebaudiana* (Bertoni) Bertoni (6-8). High concentrations of these sweet principles accumulate in the leaves of this species, with yields of over 10% w/w of stevioside, the most abundant representative of this series, having been reported (9,10). Extracts of *S. rebaudiana* are currently used commercially in Japan for sweetening a variety of products including pickled vegetables, sea foods, soft drinks, soy sauce and confectionary products (11-14). In addition, *S. rebaudiana* sweetening preparations were recently approved for sale in Brazil (15).

Within the genus *Stevia*, sweet *ent*-kaurene glycosides have to date been found only in *S. rebaudiana*, but paniculoses I-V, a series of non-sweet *ent*-kaurene derivatives, have been isolated from *S. paniculata* and *S. ovata* (6,7,16,17). Thus far no systematic attempt appears to have been made to investigate the distribution of these sweet glycosides within *Stevia*. Such a study may not only indicate the presence of novel compounds of this type, but may uncover *Stevia* species which biosynthesize higher proportions than *S. rebaudiana* of rebaudiosides A, D and E, which are claimed to be sweeter and to have a more pleasant taste than stevioside (6). In this communication, organoleptic data are presented recording the sensation of sweetness exhibited by fragments of herbarium leaf samples of species in the genus *Stevia*, including *S. rebaudiana*. Species found to exhibit a sweet

¹For the previous article in this series see reference 1.

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³D. D. Soejarto, unpublished data.

taste in this preliminary study will be screened for the presence of *ent*-kaurene glycosides, the results of which will be reported in a separate paper.

RESULTS AND DISCUSSION

The results from an organoleptic evaluation for sweetness of 184 *Stevia* leaf herbarium samples, inclusive of 110 species and 121 taxa, are shown in table 1.

TABLE 1. Organoleptic evaluation of *Stevia* leaf samples for sweetening effect.

Species	Voucher Specimen, Country, Collection Date	Unit tested (mm ²)		
		1-2	4-6	8-10
<i>S. ambylolepis</i> Robins.....	Pringle 13655 Mexico, 1905	N*	B	B
<i>S. ammotropha</i> Robins.....	Jorgensen 4902 Paraguay, no date	BB	BB	—
<i>S. amplexicaulis</i> Hassler.....	Hassler 1011 Paraguay, 1908	B	B	—
<i>S. aristata</i> D. Don.....	Cabrera 11777 Argentina, 1954	T	T	—
<i>S. aristata</i> D. Don.....	Hassler 9033a Paraguay, 1905	BB	BB	—
<i>S. aschenborniana</i> Sch.-Bip.....	Pringle 9120 Mexico, 1900	B	BB	BB
<i>S. aschenborniana</i> Sch.-Bip.....	Cronquist & Sousa 10408 Mexico, 1965	B	B	—
<i>S. balansae</i> Hieron.....	Jorgensen 4702 Paraguay, 1931	N	B	B
<i>S. bangii</i> Rusby.....	Rusby 6885 Bolivia, no date	N	N	T
<i>S. benthamiana</i> Hieron.....	Apolinar-Maria 274 Colombia, 1938	B	B	B
<i>S. berlandieri</i> A. Gray.....	Pringle 11573 Mexico, 1903	B	BB	BB
<i>S. berlandieri</i> A. Gray var. <i>berlandieri</i>	Barkley, Webster & Rowell 7139 Mexico, 1947	B	BB	—
<i>S. bertholdii</i> Robins.....	Acosta-Solis 7849 Ecuador, 1944	B	B	BB
<i>S. boliviensis</i> Sch.-Bip. ex Rusby	Holway & Holway 542 Bolivia, 1920	N	N	B
<i>S. boliviensis</i> Sch.-Bip. ex Rusby	Buchtien s.n. Bolivia, 1912	B	BB	—
<i>S. breviaristata</i> Hook. & Arnott.	Cabrera 3068 Argentina, 1933	N	N	N
<i>S. calderillensis</i> Hieron.....	Fiebrig 3425 Bolivia, 1904	B	BB	—
<i>S. camporum</i> Baker.....	Irwin 2749 Brazil, 1959	B	B	B
<i>S. caracasana</i> DC.....	Molina 30283 Guatemala, 1974	N	N	S
<i>S. cathartica</i> Poepp. & Endl.....	Espinosa E-787 Ecuador, 1946	B	BB	BBB
<i>S. chamaedrys</i> Griseb.....	Lorentz & Hieronymus 171 Argentina, 1873	N	N	N
<i>S. cinerascens</i> Sch.-Bip.....	Matzenbacher 510 Brazil, 1976	N	N	B
<i>S. claussemi</i> Sch.-Bip.....	Barreto 8353 Brazil, 1936	BB	BBB	BBB
<i>S. claussemi</i> Sch.-Bip.....	Lenarte 2570 Brazil, 1950	B	BBB	—
<i>S. clinopodioides</i> Greenm.....	Sessé, Mociño & Maldonado 2613 Mexico, ca. 1804	BBB	BBB	—
<i>S. collina</i> Gardn.....	Warming 618 Brazil, 1865	BB	BB	—
<i>S. compacta</i> Benth.....	Buchtien 186 Bolivia, 1912	N	N	B
<i>S. conmixta</i> Robins.....	Hatschbach 18764 Brazil, 1968	N	N	B
<i>S. connata</i> Lag.....	Pringle 4580 Mexico, 1893	BB	BB	—

TABLE 1. *Continued.*

Species	Voucher Specimen, Country, Collection Date	Unit tested (mm ²)		
		1-2	4-6	8-10
<i>S. connata</i> Lag.....	Smith 255 Mexico, 1894	B	B	BB
<i>S. connata</i> Lag.....	Pringle 4580 Mexico, 1893	B	BB	—
<i>S. crenulata</i> Baker.....	Hatschbach 18770 Brazil, 1968	B	BB	—
<i>S. cuneata</i> Hassler.....	Hassler 10286 Paraguay, 1908	B	BB	—
<i>S. cuzcoensis</i> Hieron.....	Pennell 13553 Peru, 1925	B	B	B
<i>S. cuzcoensis</i> Hieron.....	Herrera 2353 Peru, 1929	B	B	—
<i>S. dianthoidea</i> Hieron.....	Penland & Summers 564 Ecuador, 1939	N	N	—
<i>S. elatior</i> HBK.....	Acosta-Solia 10184 Ecuador, 1945	N	N	—
<i>S. elatior</i> HBK.....	Apollinar-Maria 448 Colombia	N	B	B
<i>S. elatior</i> HBK.....	O. Kuntze s.n. Bolivia, no date	N	N	N
<i>S. elatior</i> HBK.....	Breedlove 7036 Mexico, 1964	B	B	B
<i>S. elongata</i> HBK.....	Haenke 329 Mexico, 1791	N	N	N
<i>S. elongata</i> HBK.....	Linden 475 Venezuela, 1842	B	BB	—
<i>S. entreriensis</i> Hieron.....	Cabrera 3922 Uruguay, 1936	N	B	B
<i>S. eupatoria</i> (Spreng.) Willd....	Taylor 13 Mexico, 1936	BB	BB	—
<i>S. filipes</i> Rusby.....	Brooke 6209 Bolivia, 1950	B	BB	—
<i>S. galeopsidifolia</i> Hieron.....	Vargas 9531 Peru, 1950	N	N	N
<i>S. galeopsidifolia</i> Hieron.....	Vargas 4310 Peru, 1944	B	B	BB
<i>S. glandulosa</i> Hook. & Arnott var. <i>gentryi</i> Grashoff.....	Gentry 1204 Mexico, 1934	BB	BBB	—
<i>S. glandulosa</i> Hook. & Arnott var. <i>glandulosa</i>	Palmer 1821 Mexico, 1892	BBB	—	—
<i>S. glutinosa</i> HBK.....	Lehmann 4727 Colombia	B	B	B
<i>S. heptachaeta</i> DC.....	No Collector Brazil, 1863	N	N	N
<i>S. herrerae</i> Robins.....	Vargas 4139 Peru, 1944	B	BB	BB
<i>S. hirsuta</i> DC. var. <i>hirsuta</i>	Hinton 2392 Mexico, 1932	B	B	B
<i>S. hirsuta</i> DC. var. <i>hirsuta</i>	Molina & Molina 26641 Guatemala, 1971	N	B	B
<i>S. hypomalaca</i> Robins.....	Pringle 9976 Mexico, 1902	B	B	BB
<i>S. hyptifolia</i> Gardn.....	Mexia 5540 Brazil, 1931	BB	BBB	—
<i>S. iltisiana</i> Grashoff.....	Fisher s.n. Mexico, 1924	BB	BB	BBB
<i>S. iltisiana</i> Grashoff.....	Pringle 11576 Mexico, 1903	BB	BB	BBB
<i>S. incognita</i> Grashoff.....	Ton 491 Mexico, 1966	B	B	B
<i>S. incognita</i> Grashoff.....	Williams 41681 Guatemala, 1972	B	BB	BB
<i>S. incognita</i> Grashoff.....	Molina & Molina 30047 Guatemala, 1974	B	B	B
<i>S. ialiscensis</i> Robins.....	No Collector Mexico, 1892	B	B	BB

TABLE 1. *Continued.*

Species	Voucher Specimen, Country, Collection Date	Unit tested (mm ²)		
		1-2	4-6	8-10
<i>S. jorullensis</i> HBK.....	Williams <i>et al.</i> 41438 Guatemala, 1972	B	B	BB
<i>S. jorullensis</i> HBK.....	Pringle 13085 Mexico, 1904	B	B	BB
<i>S. latifolia</i> Benth.....	Anderson & Laskowski 4432 Mexico, 1966	B	B	B
<i>S. latifolia</i> Benth.....	Smith 253a Mexico, 1894	N	B	B
<i>S. lehmannii</i> Hieron.....	Molina & Molina 26296 Guatemala, 1971	N	B	B
<i>S. lehmannii</i> Hieron.....	Purpus 3133 Mexico, 1908	N	B	B
<i>S. lemmonii</i> Hieron. var. <i>hispidula</i> Grashoff.....	Gentry 1414 Mexico, 1935	BBSS	BBSS	BBBS
<i>S. lemmonii</i> Hieron. var. <i>hispidula</i> Grashoff.....	Palmer 96 Mexico, 1906	B	B	B
<i>S. leptophylla</i> Sch.-Bip. ex Robins.....	Hassler 6617 Paraguay, 1900	N	B	B
<i>S. lucida</i> Lag.....	Killip 38078 Columbia, 1944	N	B	BB
<i>S. lucida</i> Lag.....	Wilbur 15405 Panama, 1971	N	B	B
<i>S. lucida</i> Lag.....	Steyermark 55645 Venezuela, 1944	N	B	B
<i>S. lucida</i> Lag. var. <i>lucida</i>	Morley 653 Mexico, 1946	N	B	BB
<i>S. lucida</i> Lag. var. <i>bipontini</i> Robins.....	Powell & Edmondson 665 Mexico, 1961	N	B	B
<i>S. lucida</i> Lag. var. <i>oaxacana</i> (DC.) Grashoff.....	Williams & Williams 21703 Mexico, 1962	B	B	B
<i>S. lundiana</i> DC.....	Hatschbach 12529 Brazil, 1965	N	N	B
<i>S. macbridei</i> Robins.....	Soukup 3002 Peru, 1946	B	B	BB
<i>S. macbridei</i> Robins.....	Isert 2064 Peru, 1863	B	B	BB
<i>S. mandonii</i> Sch.-Bip.....	Pennell 13372 Peru, 1925	BT	BT	BT
<i>S. mandonii</i> Sch.-Bip.....	Soukup 236 Peru, 1935	B	B	B
<i>S. melissaefolia</i> (Lamk.) Sch.-Bip.....	Macbride 5965 Peru, 1923	T	T	T
<i>S. melissaefolia</i> (Lamk.) Sch.-Bip.....	Cerrate 865 Peru, 1951	T	T	B
<i>S. menthaefolia</i> Sch.-Bip.....	Dusén 16948 Brazil, 1915	N	B	TB
<i>S. menthaefolia</i> Sch.-Bip.....	Martius 771 Brazil, ca. 1800	B	BB	BBB
<i>S. menthaefolia</i> Sch.-Bip.....	Buchtien s.n. Bolivia, 1914	N	B	B
<i>S. mercedensis</i> Hieron. ex Reiss.	Lossen 202 Argentina, 1925	B	B	—
<i>S. micradenia</i> Robins.....	Pringle 4543 Mexico, 1893	BBBSS	BBBSS	—
<i>S. micrantha</i> Lag.....	Sessé <i>et al.</i> 2589 Mexico, ca. 1800	N	N	N
<i>S. micrantha</i> Lag.....	Orcutt 4293 Mexico, 1910	N	B	B
<i>S. micrantha</i> Lag.....	Cronquist 10246 Mexico, 1965	N	B	B

TABLE 1. *Continued.*

Species	Voucher, Specimen, Country, Collection Date	Unit tested (mm ²)		
		1-2	4-6	8-10
<i>S. microchaeta</i> Sch.-Bip.....	Botteri 407 Mexico, no date	N	N	N
<i>S. monardifolia</i> HBK.....	Pringle 11580 Mexico, 1903	B	BB	BB
<i>S. monardifolia</i> HBK.....	Balls & Gourlay 5288 Mexico, 1938	N	B	B
<i>S. multiaristata</i> Spreng.....	Cabrera 3341 Uruguay, 1935	N	N	T
<i>S. nelsonii</i> Robins.....	Leavenworth & Hoogstraal 1121 Mexico, 1941	N	S	S
<i>S. nepetifolia</i> HBK.....	Breedlove 14153 Mexico, 1965	N	N	B
<i>S. nepetifolia</i> HBK.....	Pringle 11581 Mexico, 1903	N	B	BB
<i>S. nepetifolia</i> HBK.....	Smith 1996 Colombia, 1900	N	B	B
<i>S. oligocephala</i> DC.....	Glaziou s.n. Brazil, 1907	SS	SSB	SSBB
<i>S. origanoides</i> HBK.....	Mexia 9043 Mexico, 1937	N	S	SB
<i>S. origanoides</i> HBK.....	Pringle 1780 Mexico, 1888	N	SB	SB
<i>S. origanoides</i> HBK.....	Shedon s.n. Mexico, 1892	SB	SB	SB
<i>S. ovalis</i> (Robins.) Robins.....	Pringle 4491 Mexico, 1893	N	B	SBB
<i>S. ovalis</i> (Robins.) Robins.....	Barnes & Land 187 Mexico, 1908	N	N	S
<i>S. ovata</i> Willd.....	Williams <i>et al.</i> 41694 Guatemala, 1972	N	N	N
<i>S. ovata</i> Willd.....	Stewart 1227 Mexico, 1941	N	N	B
<i>S. ovata</i> Willd. var. <i>ovata</i>	Molina & Molina 26935 Guatemala, 1971	N	B	B
<i>S. ovata</i> Willd. var. <i>reglensis</i> (Benth.) Grashoff.....	Pringle 6624 Mexico, 1896	B	B	BB
<i>S. oxylaena</i> DC.....	Hassler 12154 Paraguay, 1913	B	B	B
<i>S. oxylaena</i> DC.....	Lorentz 952 Uruguay, 1877	N	N	N
<i>S. pallida</i> (Sch.-Bip.) Hieron...	Killip & Smith 17269 Colombia, 1927	BBB	BBB	—
<i>S. palmeri</i> A. Gray var. <i>palmeri</i>	Standley 2475 Mexico, 1936	N	B	B
<i>S. palmeri</i> A. Gray var. <i>constricta</i> Grashoff.....	Ortega 7132 Mexico, 1933	N	B	BB
<i>S. pauciradiata</i> Bab.....	Glaziou 11025 Brazil (?)	N	N	N
<i>S. perfoliata</i> Cronq.....	Cronquist 11229 Mexico, 1974	S	SS	SSB
<i>S. phlebophylla</i> A. Gray.....	Pringle 2291 Mexico, 1889	BBBSS	BBBSS	—
<i>S. pilosa</i> Lag.....	Barkley <i>et al.</i> 2828 Mexico, 1947	BS	BBST	BB
<i>S. pilosa</i> Lag.....	Powell & Edmondson 735 Mexico, 1961	N	BS	BS
<i>S. plummerae</i> A. Gray var. <i>plummerae</i>	Gentry 1948 Mexico, 1935	N	B	B
<i>S. plummerae</i> A. Gray var. <i>plummerae</i>	LeSueur 961 Mexico, 1936	N	N	B
<i>S. pohliana</i> Baker.....	Dusén 16944 Brazil, 1915	B	BB	BB

TABLE 1. *Continued.*

Species	Voucher Specimen, Country, Collection Date	Unit tested (mm ²)		
		1-2	4-6	8-10
<i>S. polycephala</i> Bertol. var. <i>polycephala</i>	Breedlove 7995 Mexico, 1964	B	BB	BB
<i>S. porphyrea</i> McVaugh.....	Waterfall 15446 Mexico, 1959	N	SB	SB
<i>S. porphyrea</i> McVaugh.....	Palmer 456 Mexico, 1896	B	BBS	BBBS
<i>S. puberula</i> Hook.....	Pennell 14367 Peru, 1925	B	BB	SBB
<i>S. puberula</i> Hook.....	Pennell 14336 Peru, 1925	N	B	BB
<i>S. purpusii</i> Robins.....	Purpus 1486 Mexico, 1905	N	N	B
<i>S. quitensis</i> HBK.....	Bonpland s.n. Ecuador, no date	N	B	SB
<i>S. rebaudiana</i> (Bertoni) Bertoni.	Gosling s.n. Paraguay, 1919	SSSS	—	—
<i>S. reticulata</i> Grashoff.....	No collector Mexico, 1893	N	N	S
<i>S. revoluta</i> Robins.....	Purpus 3842 Mexico, 1909	BB	BB	BBB
<i>S. rhombifolia</i> HBK.....	Williams 10629 Venezuela, 1938	B	BB	BB
<i>S. rhombifolia</i> HBK.....	Cuatrecasas 20845 Colombia, 1946	BB	BB	—
<i>S. rhombifolia</i> HBK.....	Holmgren 526 Ecuador, 1920	N	BB	—
<i>S. rhombifolia</i> HBK.....	Stork & Horton 10884 Peru, 1939	B	B	B
<i>S. rhombifolia</i> HBK.....	Dorantes <i>et al.</i> 767 Mexico, 1972	N	B	BB
<i>S. rhombifolia</i> HBK.....	Jiménez 1382 Costa Rica, 1963	N	N	N
<i>S. salicifolia</i> Cav. var. <i>salicifolia</i>	Cronquist & Fay 10823 Mexico, 1970	B	BB	BB
<i>S. salicifolia</i> Cav. var. <i>callodes</i> (Greenm.) Robins.....	Purpus 2541 Mexico, 1908	N	N	B
<i>S. salicifolia</i> Cav. var. <i>virgulifera</i> Robins.....	Palmer 931 Mexico, 1896	B	BB	BB
<i>S. sanguinea</i> Hieron.....	Fabris-Crisci 7385 Argentina, 1968	B	BB	BB
<i>S. satureifolia</i> (Lamk.) Sch.-Bip.....	Pastore 1146 Argentina, 1937	B	B	BB
<i>S. satureifolia</i> (Lamk.) Sch.-Bip.....	Herter s.n. Uruguay, 1925	N	N	N
<i>S. satureifolia</i> (Lamk.) Sch.-Bip.....	Cabrera 6839 Argentina, 1940	N	T	T
<i>S. seemannii</i> Sch.-Bip.....	Smith 254 Mexico, 1894	N	B	BB
<i>S. seleriana</i> Robins.....	Cronquist & Fay 10876 Mexico, 1970	N	B	B
<i>S. selloi</i> (Spreng.) Sch.-Bip.....	Hassler 3910 Paraguay, no date	N	B	B
<i>S. serrata</i> Cav.....	Gehriger 246 Venezuela, 1930	B	B	B
<i>S. serrata</i> Cav.....	Black 46-653 Colombia, 1946	N	N	B
<i>S. serrata</i> Cav. var. <i>serrata</i>	Williams <i>et al.</i> 41771 Guatemala, 1972	N	N	N
<i>S. serrata</i> Cav. var. <i>arguta</i> Robins.....	Powell & Edmondson 623 Mexico, 1961	B	BB	BB

TABLE 1. *Continued.*

Species	Voucher Specimen, Country, Collection Date	United tested (mm ²)		
		1-2	4-6	8-10
<i>S. soratensis</i> Hieron.....	Pennell 13536 Peru, 1925	N	N	B
<i>S. soratensis</i> Hieron.....	Weberbauer 6920 Peru, 1909-1914	N	N	N
<i>S. stenophylla</i> A. Gray.....	Johnston 8907 Mexico, 1941	B	BB	BB
<i>S. subpubescens</i> Lag.....	Cronquist & Sousa 10417 Mexico, 1965	N	B	B
<i>S. subpubescens</i> Lag. var. <i>intermedia</i> Grashoff.....	Anderson & Laskowski 4430 Mexico, 1966	N	N	N
<i>S. subpubescens</i> Lag. var. <i>opaca</i> (Sch.-Bip.) Robins.....	Mexia 1615 Mexico, 1927	N	B	B
<i>S. subpubescens</i> Lag. var. <i>subpubescens</i>	Haenke 1703 Mexico, 1791	N	N	N
<i>S. subpubescens</i> Lag. var. <i>subpubescens</i>	Cronquist & Fay 10906 Mexico, 1970	B	B	BB
<i>S. tapacariensis</i> Hieron.....	Eyerdam 24784 Bolivia, 1939	N	N	N
<i>S. tephra</i> Robins.....	Pringle 7965 Mexico, 1899	B	BB	BB
<i>S. tephra</i> Robins.....	Purpus 4830 Mexico, 1910	N	B	B
<i>S. tephrophylla</i> Blake.....	Breedlove & Raven 13643 Mexico, 1965	BB	BB	BB
<i>S. tomentosa</i> HBK.....	Purpus 2550 Mexico, 1907	BB	BB	BB
<i>S. tomentosa</i> HBK.....	Mueller 2397 Mexico, 1935	B	BB	—
<i>S. trifida</i> Lag.....	Rzedowski 27054 Mexico, 1970	N	B	B
<i>S. triflora</i> DC.....	Pringle 11835 Mexico, 1935	BB	BBB	—
<i>S. triflora</i> DC.....	Williams & Molina 42299 Nicaragua, 1973	B	B	BB
<i>S. urticaefolia</i> Billb. in Thunb..	Williams & Assis 7238 Mexico, 1945	B	BB	—
<i>S. vaga</i> Griseb.....	Cabrera & Fabris 19930 Argentina, 1969	N	N	S
<i>S. vaga</i> Griseb.....	Lossen 238 Argentina, 1925	B	B	BB
<i>S. venosa</i> A. Gray.....	Gentry 1948 Mexico, 1935	N	B	B
<i>S. vernicosa</i> Greenm.....	Pringle 10349 Mexico, 1907	B	BB	—
<i>S. veronicae</i> DC.....	Herter 1971 Uruguay, 1947	T	TT	—
<i>S. villaregalis</i> McVaugh.....	Pringle 2486 Mexico, 1889	B	BB	—
<i>S. villaregalis</i> McVaugh.....	Barnes & Land 198A Mexico, 1908	N	B	—
<i>S. viscida</i> HBK.....	LeSueur 347 Mexico, 1935	BBS	BBS	BBS
<i>S. viscida</i> HBK.....	Molina & Molina 26559 Guatemala, 1971	N	SB	SB
<i>S. wagneri</i> Hieron.....	Vogl 505 Venezuela	N	N	S
<i>S. weberbaueri</i> Hieron.....	Stafford 438 Peru, 1937	T	TT	TT
<i>S. yaconensis</i> Hieron.....	Cabrera 3032 Argentina, 1933	N	N	N

*B, slightly bitter; BB, moderately bitter; BBB, strongly bitter; N, neutral (not bitter and not sweet); T, salty; S, slightly sweet; SS, moderately sweet; SSS, strongly sweet. Where mixed taste sensations are recorded (SB, etc.), tastes are coded in the order in which they were experienced. See text for details of the test method.

A taste considered sweet was evident in 24 samples, representing 18 *Stevia* species and varieties, namely: *S. caracasana* DC.; *S. lemmonii* Hieron. var. *hispidula* Grashoff (sample collected in Mexico in 1935); *S. micradenia* Robins.; *S. nelsonii* Robins.; *S. oligocephala* DC., *S. origanoides* HBK. (samples collected in Mexico in 1888, 1892 and 1937); *S. ovalis* (Robins.) Robins. (samples collected in Mexico in 1893 and 1908); *S. perfoliata* Cronq.; *S. phlebophylla* A. Gray; *S. pilosa* Lag. (samples collected in Mexico in 1947 and 1961); *S. porphyrea* McVaugh (samples collected in Mexico in 1899 and 1959); *S. puberula* Hook.; *S. quitensis* HBK.; *S. rebaudiana* (Bertoni) Bertoni; *S. reticulata* Grashoff; *S. vaga* Griseb. (sample collected in Argentina in 1969); *S. viscida* HBK. (samples collected in Mexico in 1935 and Guatemala in 1971), and *S. wagneri* Hieron. Samples of *S. lemmonii* var. *hispidula*, collected in Mexico in 1936, and *S. vaga*, collected in Argentina in 1925, were not found to be sweet.

In table 1 an attempt has been made to grade the intensities of the sweet responses experienced. The herbarium samples of *S. lemmonii* var. *hispidula*, *S. micradenia*, *S. oligocephala*, *S. perfoliata* and *S. phlebophylla* leaves exhibited the strongest sensations of sweetness, excluding the *S. rebaudiana* sample, of the 18 species and varieties listed above. Since none of these samples produced the intensity and duration of taste response exhibited by the *S. rebaudiana* specimen, it may be inferred that if *ent*-kaurene glycosides are responsible for their sweet effects, the concentrations of these compounds are in all probability much lower than in *S. rebaudiana*. It may be seen from table 1 that the sweet taste of these samples was in many cases accompanied by bitterness. This may be due to the masking of the taste of *ent*-kaurene glycosides by sesquiterpene lactones. Sesquiterpene lactones are bitter (18,19) and have been isolated previously from species in the genus *Stevia* (20-24).

About three-quarters of the 18 sweet species and varieties listed in table 1 are native to North and Central America, with only *S. oligocephala*, *S. quitensis*, *S. rebaudiana*, *S. vaga* and *S. wagneri* being South American species. When the sweet *Stevia* species of North and Central American origin are classified according to Grashoff's recent scheme (3), it may be seen that taxonomically they are not closely related, since they represent all three generic subdivisions (*Podoccephalae*, *Corymbosae*, *Fruticosae*) of this geographic section of the genus. Of the South American species mentioned above, *S. rebaudiana* belongs to the Series *Multiaristatae* (25), but the exact taxonomic affinities of the other four are not clear at present (26-28).

The exact treatment undergone by each specimen used for the present study is not known. However, the fact that the sample of *S. rebaudiana* which was collected in Paraguay in 1919 remained intensely sweet demonstrates that any treatment to which the specimens have been exposed appears not to affect this property. Thus it is evident that the *ent*-kaurene glycosides have remained stable in the *S. rebaudiana* sample, and that such constituents of the other herbarium samples of *Stevia* would be expected to behave similarly and appear sweet if present. In support of this notion, stevioside is known to be stable at 100° for at least 24 hr at acid and neutral pH's (11,14).

It is thus of some interest to correlate the results of this study on dried herbarium *Stevia* leaf samples with mention of the presence or absence of sweetness possessed by fresh *Stevia* leaves as recorded in the literature. Evidence for the sweet properties of herbarium samples of *S. caracasana* and *S. lemmonii* var. *hispidula* noted in table 1 confirms the results obtained when fresh leaves of these taxa were tested by members of our group in the field in Mexico (1). However, organoleptic data for two herbarium samples of *S. monardifolia* HBK. (table 1) are not in agreement with the sensation of sweetness recorded when a fresh leaf of this Mexican species was tested in the field (1). Herbarium specimens of *S. aristata* D. Don, collected in Paraguay in 1905 and Argentina in 1954, were

not found in the present study to exhibit a sweet taste (table 1). These observations disagree with the claim that this woody species is sweet (29). Negative data expressed in table 1 in regard to the sweetness of *S. ovata* Willd. and *S. serrata* Cav. herbarium samples are in accordance with the absence of sweetness exhibited by fresh leaves of these species (6,7,17,30).

In a preliminary study our group has reviewed certain ethnobotanical literature on *Stevia* species, with the objective of detecting additional species with sweet properties (1). While such reports indicate that in the genus only *S. rebaudiana* has a pronounced sweet taste, three other species, *S. collina* Gardn., *S. satuireifolia* (Lamk.) Sch.-Bip. and *S. punensis* Robins. may also be sweet (1). However, while no herbarium sample of *S. punensis* was available for study, herbarium leaf samples of *S. collina* and *S. satuireifolia* were found to be devoid of sweetness (table 1). The absence of sweetness exhibited by a fresh sample of *S. satuireifolia* has been noted previously (31).

We have expressed concern elsewhere at the alarming rate at which the natural area of growth of *S. rebaudiana* in the Sierra of Amambay region of Paraguay is diminishing, due to the seemingly indiscriminate destruction of its flora for commercial ventures (1). It is evident that other members of the genus are also sensitive to disturbances to their natural habitats by mankind and livestock. For example, it is possible that *S. phlebophylla*, one of the few species determined as being sweet in this study (table 1), may now be extinct (3). The specimen investigated by us, collected by Pringle in 1889 in the region of Guadalajara, Mexico, represents one of three existing collections of this species (3). Therefore, with only about one-half of all known *Stevia* species having been evaluated at this time, there is an urgent need for the performance of organoleptic and phytochemical studies on the remaining members of the genus to determine if any other species, apart from *S. rebaudiana* and the other 17 taxa identified as being sweet in table 1, may prove to possess sweet *ent*-kaurene glycoside constituents.

EXPERIMENTAL

PLANT MATERIAL.—*Stevia* leaf samples (one to three leaves) were procured from herbarium specimens housed at the John G. Searle Herbarium, Field Museum of Natural History, Chicago, Illinois. The binomial, voucher specimen number, country and date of collection for each specimen sampled are recorded in table 1. Herbarium specimens were stored in metal cases, at room temperature, with suitable air-conditioning. Open trays of naphthalene or *p*-dichlorobenzene were used as contact insecticides during storage.

PREPARATION OF LEAF MATERIAL FOR TASTE-TESTING.—*Stevia* leaves (whole or fragments) were cleaned by successive immersion in 70% ethanol for 10–30 sec, water for 30 sec, and in a second vessel of water for 20–60 sec. Samples were dried on absorbent paper, and prior to testing were trimmed to various sizes (1–2, 4–6, and 8–10 sq. mm), as far as quantities of leaf materials permitted.

ORGANOLEPTIC TASTE-TESTING OF STEVIA LEAVES.—Fragments of each cleansed leaf (specimen) were placed at the tip of the tongue, chewed, and tasted by one of us (D.D.S.). The mouth was rinsed with generous quantities of water after each leaf fragment was tasted. Tests were accomplished in small batches of 5–10.

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