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CHARACTERIZATION AND FEEDING DETERRENT EFFECTS ON THE APHID, *SCHIZAPHIS GRAMINUM*, OF SOME DERIVATIVES OF THE SWEET COMPOUNDS, STEVIOSIDE AND REBAUDIOSIDE A[1]

N.P.D. NANAYAKKARA, J.A. KLOCKE,[2] C.M. COMPADRE,[3] R.A. HUSSAIN, J.M. PEZZUTO, and A.D. KINGHORN*

Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612

ABSTRACT.—The intensely sweet compounds, stevioside [**1**] and rebaudioside A [**2**], and 15 of their derivatives, were tested for feeding deterrent activity against the aphid, *Schizaphis graminum*. Included with these compounds was steviol [**4**], the aglycone of stevioside and rebaudioside A that was one of the most active compounds in this investigation. Loss of feeding deterrent activity of steviol was observed on acetylation or glycosylation of the C-13 tertiary hydroxy group or on methylation of the C-19 carboxylic acid substituent. In contrast, the antifeedant activity of steviol was not greatly affected by modification of either the C-16 exomethylene group or the C/D-ring junction stereochemistry. ^{13}C-nmr data have been obtained for 12 of the test compounds investigated.

Stevioside [**1**] and rebaudioside A [**2**] are the major sweet *ent*-kaurene glycoside constituents of the herb, *Stevia rebaudiana* (Bertoni) Bertoni (Compositae) (2). Products extracted from this plant are used commercially as sweetening agents in Japan and other countries. Considerable interest has been expressed in cultivating *S. rebaudiana* in a number of eastern and southeast Asian nations (2). Although stevioside and rebaudioside A were not mutagenic, we have found their enzymatically produced aglycone, steviol [**4**], to be mutagenic toward *Salmonella typhimurium* strain TM677 in the presence of a metabolic activating system derived from the livers of Aroclor-1254-pretreated rats (3). In previous studies in which several steviol derivatives were generated and tested for mutagenic activity, we have found that the tertiary hydroxy group at C-13 and the exomethylene functionality at C-16 are both required for the exhibition of mutagenicity by steviol in the test system employed (3,4).

Seventeen known diterpenes of the *ent*-kaurene and *ent*-bayerane types, including stevioside [**1**], rebaudioside A [**2**], steviol [**4**], and isosteviol [**17**], were utilized in the present investigation, and ^{13}C-nmr data have been determined for 11 of these compounds for the first time. Several derivatives in the study proved to exhibit significant feeding deterrent effects against the aphid, *Schizaphis graminum* Rondani (greenbug), when evaluated in a previously described bioassay (5). Structural requirements for these compounds as aphid feeding deterrents have been compared with those necessary to mediate mutagenic activity toward *S. typhimurium* strain TM677.

RESULTS AND DISCUSSION

The test compounds used in this study comprised three sweet glycoside constituents of *S. rebaudiana*, namely, stevioside [**1**], rebaudioside A [**2**], and steviolbioside [**3**], as well as steviol [**4**] and isosteviol [**17**], the compoounds produced when stevioside is hydrolyzed enzymatically or with mineral acids, respectively (2). The remaining derivatives, **5-16**, were produced by chemical modification of steviol. Many of

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[2]NPI-Plant Resources Institute, 417 Wakara Way, Salt Lake City, Utah 84108.

[3]Current address: Seaver Chemistry Laboratory, Pomona College, Claremont, California 91711.

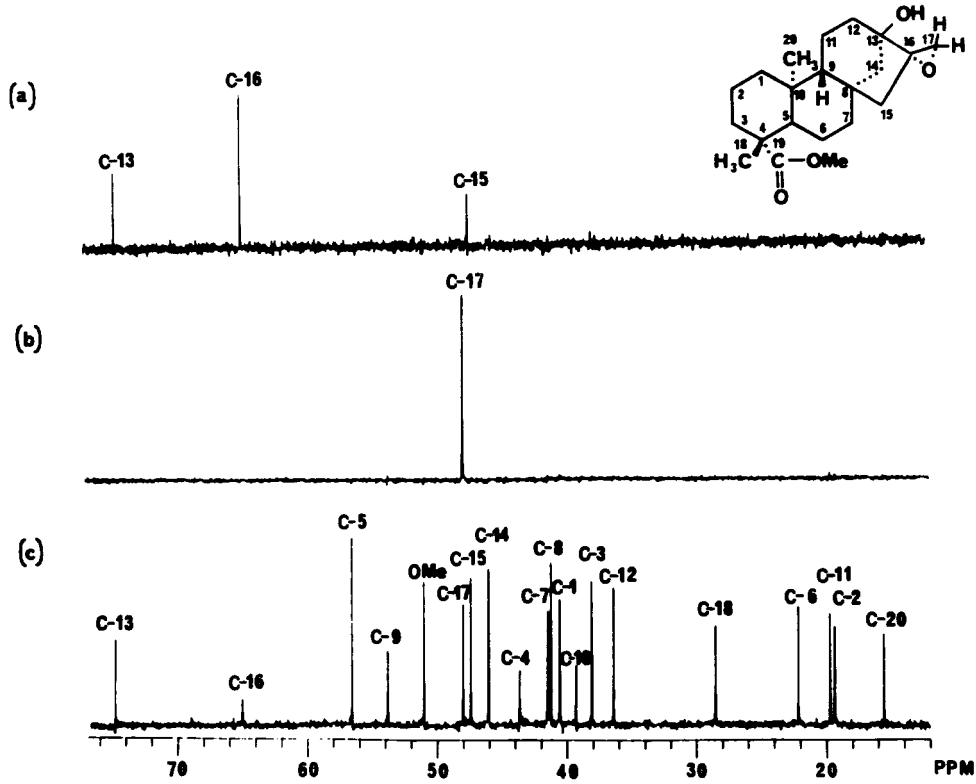


FIGURE 1. Assignment of the ^{13}C-nmr spectrum of compound **12**. (**a**) Selective INEPT spectrum obtained by irradiation of the 17 protons (2.50 ppm); (**b**) CSCM 1-D spectrum obtained by irradiation of the 17 protons (2.50 ppm); (**c**) Proton-noise-decoupled ^{13}C-nmr spectrum.

the test compounds employed were first produced in early investigations on the structural determination and synthesis of stevioside and/or steviol (6-8) and were incompletely characterized at the time. Thus, in addition to probing the activity of compounds **5-17** as aphid feeding deterrents as described herein, supportive spectroscopic data also were measured for these compounds as an integral part of this study.

Among the compounds studied in this investigation, ^{13}C-nmr data have appeared in the literature for the three sweet glycosides, stevioside [**1**], rebaudioside A [**2**], and steviolbioside [**3**] (9), in addition to steviol [**4**] (9,10), steviol methyl ester [**5**] (11), and 15α-hydroxysteviol [**13**] (10). Assignments of ^{13}C-nmr chemical shifts for the remaining compounds investigated are shown in Table 1 and were made mainly on the basis of chemical shift rules such as acetylation shifts and steric γ- and δ-effects, when compared with published data for steviol and other model compounds. In each case, chemical shifts were obtained from noise-decoupled spectra, and multiplicities were determined from SFORD and APT spectra. Thus, the resonances of steviol acetate [**6**] were readily assignable by direct comparison to chemical shifts of the parent compound, steviol [**4**]. However, signals for compounds **14-16** involved not only data comparison with **4** but also with those assigned to methyl 15-oxo-*ent*-kaur-16-en-19-oate (10), *ent*-kaur-17-nor-16-oxo-19-oic acid (12), and *ent*-kaur-16-en-19-ol (13), respectively. Analysis of the ^{13}C-nmr chemical shifts of the hydrogenated steviol derivatives dihydrosteviols A [**7**] and B [**9**] showed differences in the C/D rings when compared with steviol [**4**]. In both dihydrosteviol A [**7**] and its methyl ester [**8**] the C-16 β-methyl group resonated further upfield than in their epimeric counterparts with a C-16

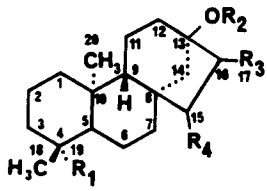
TABLE 1. ^{13}C-nmr Chemical Shifts of the Diterpene Derivatives **5-12** and **14-17**[a]

Carbon	Compounds											
	5[b]	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**14**	**15**	**16**	**17**
1	40.7	40.9	40.7	40.7	40.8	40.8	40.9	40.9	40.3	40.9	40.8	39.9
2	19.5	19.7	19.7	19.5	19.8	19.5	19.8[c]	19.4[d]	19.5	19.5[e]	18.7	19.4
3	38.2	38.5	38.3	38.2	38.7	38.2	38.5	38.1	38.4	38.3	36.1	38.3
4	43.8	43.8	43.2	43.3	42.9	43.8	43.8	43.7	43.7	43.6	36.1	43.5
5	56.7	56.8	56.9	56.8	57.0	56.8	56.8	56.6	56.2	56.4	56.9	56.8
6	22.3	22.4	22.8	22.6	23.2	22.8	22.5	22.1	21.1	21.2	20.5	22.2
7	41.6	41.4	42.3	42.5	40.8	40.8	41.8	41.5	33.6	40.6	42.4	41.5
8	41.6	42.7	43.8	43.8	41.1	42.8	41.4	41.3	55.2	40.4	41.8	38.1
9	54.0	53.7	54.7	55.5	54.6	54.5	54.1	53.9	50.9	52.8	55.2	54.5
10	39.4	39.6	39.8	39.4	39.7	39.4	39.7	39.3	40.5	39.6	39.2	39.4
11	20.7	20.2	20.2	20.1	20.4	20.3	19.9[c]	19.7[d]	20.8	19.2[e]	20.8	20.4
12	40.7	37.4	34.6	34.2	42.7	42.4	36.6	36.3	40.1	34.1	40.6	37.2
13	79.7	87.5	79.1	78.6	78.6	78.5	75.0	74.9	76.3	84.9	79.8	48.3
14	47.3	42.3	47.8	47.8	51.2	51.0	46.3	46.0	45.1	39.3	47.4	54.0
15	48.0	47.2	48.4	48.4	43.8	43.7	47.8	47.4	208.6	52.8	48.2	48.3
16	157.5	152.4	40.8	40.7	37.9	37.8	65.2	65.0	153.8	212.7	157.7	220.2
17	102.9	103.4	13.4	13.4	19.8	19.7	48.2	48.0	113.9	—	102.9	20.1
18	28.6	29.3	29.4	28.6	29.3	28.6	29.3	28.6	29.2	29.1	28.1	29.2
19	177.6	179.8	180.2	177.6	180.0	177.6	179.9	177.4	179.8	179.6	63.9	179.5
20	15.4	15.8	15.6	15.3	15.9	15.5	16.1	15.6	15.7	16.0	18.3	13.4
-OMe	51.1			51.0		51.0		51.1				
-OAc		169.2								169.0		
		22.0								20.2		

[a]90.8 MHz, C_5D_5N, δ scale, relative to TMS.

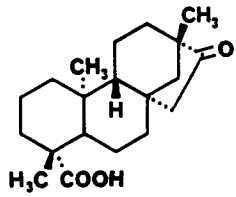
[b]^{13}C-nmr data for this compound has previously been measured in $CDCl_3$ but not in C_5D_5N (11).

[c-e]Interchangeable.



	R_1	R_2	R_3	R_4
1	COO-β-glc	β-glc^2-β-glc	$=CH_2$	H_2
2	COO-β-glc	β-glc^2-β-glc ^{13} β-glc	$=CH_2$	H_2
3	COOH	β-glc^2-β-glc	$=CH_2$	H_2
4	COOH	H	$=CH_2$	H_2
5	$COOCH_3$	H	$=CH_2$	H_2
6	COOH	$COCH_3$	$=CH_2$	H_2
7	COOH	H	α-H, β-CH_3	H_2
8	$COOCH_3$	H	α-H, β-CH_3	H_2
9	COOH	H	α-CH_3, β-H	H_2
10	$COOCH_3$	H	α-CH_3, β-H	H_2
11	COOH	H	α-epoxy	H_2
12	$COOCH_3$	H	α-epoxy	H_2
13	COOH	H	$=CH_2$	α-OH, β-H
14	COOH	H	$=CH_2$	$=O$
15	COOH	$COCH_3$	$=O$	H_2
16	CH_2OH	H	$=CH_2$	H_2

glc=D-glucopyranosyl



17

α-methyl group, namely, dihydrosteviol B [**9**] and dihydrosteviol B methyl ester [**10**]. This observation is consistent with ^{13}C-nmr data obtained for some pairs of C_{20}-diterpenoid alkaloids epimeric at C-16 isolated previously (14). Assignments for the C-12, C-14, and C-15 carbons of dihydrosteviol A [**7**] were made as far as possible by comparison with chemical shifts assigned to a dihydrokaurene derivative with the same ring C/D stereochemistry generated from the diterpene alkaloid, cuauchichicine (15).

In steviol 16,17 α-epoxide [**11**] and its methyl ester [**12**], all ^{13}C-nmr signals were comparable with those of steviol [**4**] and steviol methyl ester [**5**], respectively, except for carbons C-12, C-14, C-15, C-16, and C-17. In the case of **12**, the quaternary carbon signal appearing at 65.0 ppm and the methylene carbon at 36.3 ppm were assigned in turn to C-16 and C-12. The observation in the ^{1}H-nmr spectrum of **12** of an assignable downfield AB quartet centered at 2.50 ppm for the H-17 protons, enabled the unambiguous assignment of the remaining carbons of this compound, using two modern nmr techniques (Figure 1). The first of these, CSCM-1D (16), allowed the correlation of H-17 protons with C-17 at 48.2 ppm by irradiation of the protons at 2.50 ppm. The second method, a selective INEPT experiment (17,18), again involved irradiation of H_2-17 at 2.50 ppm and provided selective enhancement of carbons two or three bonds distant, namely, C-13 (74.9 ppm), C-15 (47.4 ppm), and C-16 (65.0 ppm). In this

manner, the almost coincidental chemical shifts of C-15 and C-17 could be distinguished in the ^{13}C-nmr spectrum of steviol 16,17 α-epoxide methyl ester [**12**].

Isosteviol [**17**], a compound that may be obtained from steviol [**4**] by treatment with acid via a Meerwein rearrangement (8), is based on an *ent*-bayerane skeleton rather than on *ent*-kaurene. Accordingly, the ^{13}C-nmr chemical shifts of ring A of isosteviol were established by comparison with analogous data for steviol [**4**], while the remaining chemical shifts were assigned by recourse to published spectroscopic data for *ent*-bayerane (19) and [3,2,1] bicyclooct-6-one (20).

When compounds **1-17** were compared in terms of their activity as aphid feeding deterrents (Table 2), the most active compounds were steviol [**4**], dihydrosteviols A [**7**] and B [**9**], steviol 16,17 α-epoxide [**11**], 15-oxosteviol [**14**], and isosteviol [**17**]. Modification of the C-13 tertiary hydroxy group of steviol by acetylation to produce **6** or glycosylation as in **3** resulted in reduced activity on *S. graminum*. The importance of a free carboxylic acid group at C-19 for the exhibition of feeding deterrent activity was evidenced by the inactivity of the methyl ester derivatives of compounds **7**, **9**, and **11**,

TABLE 2. Feeding Deterrent Effects of Stevioside and Rebaudioside A and Some Derivatives on the the Aphid, *Schizaphis graminum*

Test Compound	EC_{50} (ppm)[a]	Confidence Limits[b]
Stevioside [**1**]	650	520-813
Rebaudioside A [**2**]	[c]	
Steviolbioside [**3**]	470	236-935
Steviol [**4**]	150	111-202
Steviol methyl ester [**5**]	[c]	
Steviol acetate [**6**]	340	236-490
Dihydrosteviol A [**7**]	140	90-219
Dihydrosteviol A methyl ester [**8**]	[c]	
Dihydrosteviol B [**9**]	210	159-277
Dihydrosteviol B methyl ester [**10**]	[c]	
Steviol 16,17α-epoxide [**11**]	200	160-249
Steviol 16,17α-epoxide methyl ester [**12**]	[c]	
15α-Hydroxysteviol [**13**]	750	354-1590
15-Oxosteviol [**14**]	130	63-270
Steviol norketone acetate [**15**]	280	226-347
ent-Kaur-16-ene-13,19-diol [**16**]	[c]	
Isosteviol [**17**]	115	71-186

[a]EC_{50} is the effective concentration of additive necessary to reduce the percentage of aphids feeding to 50% of that of control.

[b]Confidence limits (95%) were determined by the method of Litchfield and Wilcoxon (27).

[c]No effect was observed at 650 ppm, the highest concentration normally used.

as well as of compound **16** in which the molecule of steviol was modified only in the substitution of its C-19 group by a hydroxymethyl unit. When both the C-13 and C-19 groups of steviol were glycosylated, as in stevioside [**1**] and rebaudioside A [**2**], diminished or no activity resulted (Table 2). Feeding deterrency of the parent substance steviol (EC_{50} 150 ppm) was not significantly affected by the introduction of a carbonyl group in ring D, as in 15-oxosteviol (**14**, EC_{50} 130 ppm) but was reduced when a 15 α-hydroxy group was present in **13** (EC_{50} 750 ppm). In contrast to the mutagenic activity of metabolically activated steviol when tested by forward mutation using *S. typhimurium* strain TM677 (3,4), the retention of the C-16 exomethylene group of steviol did not seem to be important for the maintenance of aphid feeding deterrency, inasmuch as compounds **7**, **9**, **11**, as well as **14**, were all active in the bioassay employ-

ed in the present investigation. Isosteviol [**17**], which retains the ring A/B structural characteristics of steviol, was not mutagenic (3) and was the most active substance tested for *S. graminum* feeding deterrency (EC_{50} 115 ppm).

S. graminum is a severe pest on winter wheat, sorghum, and other grains cultivated in the United States (21). A number of other substances have been tested against this organism using the present bioassay method, including groups of dihydrochalcones, flavanones, flavones, flavonols, miscellaneous phenolics, and labdane diterpene acids (21,22). The compounds tested in our investigation exhibited as great, or greater activity than the flavonoids and phenolics tested previously but were not as potent as several of the labdane diterpenes (21,22). It is interesting to note that among the dihydrochalcones tested by Dreyer and Jones (21) were three intensely sweet substances, namely, phlorizin, and naringin and neohesperidin dihydrochalcones (23). Stevioside [**1**] (EC_{50} 650 ppm) exhibited intermediate aphid feeding deterrency between phlorizin (EC_{50} 200 ppm) and naringin and neohesperidin dihydrochalcones (EC_{50} 1700 and 2000 ppm) (21).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Kofler hot-stage instrument and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. The uv spectra were obtained on a Beckman DB-G grating spectrophotometer, and the ir spectra were measured on a Nicolet MX-1 FT-ir interferometer. ^{1}H-nmr spectra were recorded in C_5D_5N, using TMS as internal standard, employing a Nicolet NT-360 instrument (360 MHz), except where indicated. ^{13}C-nmr spectra were recorded in C_5D_5N with a Nicolet NT-360 instrument operating at 90.8 MHz. Low-resolution mass spectra were obtained with a Varian MAT 112S instrument, operating at 70 eV.

TEST COMPOUNDS.—Stevioside [**1**] and rebaudioside A [**2**] were isolated from *S. rebaudiana* using droplet counter-current chromatography (24). Steviolbioside [**3**], steviol [**4**], and isosteviol [**17**] were prepared from **1** by alkaline, enzymatic, and acid hydrolysis, respectively (3,24). Steviol acetate [**6**], dihydrosteviols A [**7**] and B [**9**], and steviol 16,17 α-epoxide [**11**] were prepared from steviol [**4**], as described previously (3,4). Compounds **1-4** exhibited closely comparable ^{13}C-nmr data to those published previously (9,10) while the ^{13}C-nmr chemical shifts obtained in this investigation for compounds **6**, **7**, **9**, **11**, and **17** are shown in Table 1.

The known compounds steviol methyl ester [**5**], dihydrosteviol A methyl ester [**8**], and dihydrosteviol B methyl ester [**10**] were generated by the overnight methylation of **4**, **7**, and **9**, respectively, using CH_2N_2 (Diazald, Aldrich, Milwaukee, Wisconsin). The methyl ester of steviol 16,17 α-epoxide [**12**], also a known compound, was obtained from **5** by the method of Mori *et al.* (8). These methyl esters were crystallized from MeOH, and, in turn, exhibited the following data: steviol methyl ester [**5**], mp 108-110° [lit. 111-112° (6)]; $[\alpha]^{25}D-67.5°$ (*c* 0.4, MeOH); uv λ max (MeOH) 208 nm (log ε 2.39); ir ν max (KBr) 3496, 3428, 2945, 1728, 1691, 1238, 1166 cm^{-1}; ^{1}H nmr δ 0.87 (3H, s, 20-CH_3), 1.16 (3H, s, 18-CH_3), 3.62 (3H, s, -$COOCH_3$), 4.83 (1H, br s, 17-H_2), and 5.02 (1H, br s, 17-H_2); ms *m/z* 332 (M^+, 24%), 314 (10), 274 (11), 273 (11), 254 (12), 146 (22), 135 (15), 121 (100), 107 (31), 105 (19), 95 (26), 93 (23), 91 (28), 81 (34), 67 (27), 55 (41); dihydrosteviol A methyl ester [**8**], mp 112-114°, $[\alpha]^{25}D-58.1°$ (*c* 0.26, MeOH) [lit. mp 116-119°, $[\alpha]^{20}D-69.7°$, *c* 0.78, MeOH (7)]; uv λmax (MeOH) 206 nm (log ε 2.23); ir ν max (KBr) 3476, 2955, 2936, 1724, 1707, 1235, 1176 cm^{-1}; ^{1}H nmr δ 0.87 (3H, s, 20-CH_3), 1.17 (3H, s, 18-CH_3), 1.18 (3H, d, J=7 Hz, 17-CH_3), 3.61 (3H, s, -$COOCH_3$); ms *m/z* 334 (M^+, 28%), 316 (3), 291 (100), 275 (14), 231 (10), 123 (48), 121 (20), 109 (19), 95 (13), 81 (16), 67 (12), 55 (16); dihydrosteviol B methyl ester [**10**], mp 128-130°, $[\alpha]^{25}D-98.8°$ (*c* 0.25, MeOH) [mp 132-134°, $[\alpha]^{20}D-100.5\pm2°$ (*c* 1.0, MeOH) (7)]; uv λ max (MeOH) 205 nm (log ε 2.21); ir ν max (KBr) 3500, 2956, 2924, 1725, 1701, 1241, 1160 cm^{-1}; ^{1}H nmr δ 0.87 (3H, s, 20-CH_3), 1.17 (3H, s, 18-CH_3), 1.22 (3H, d, J=7 Hz, 17-CH_3), 3.60 (3H, s, -$COOCH_3$); ms *m/z* 334 (M^+, 17%), 316 (17), 291 (100), 275 (15), 231 (24), 123 (74), 121 (34), 109 (30), 107 (20), 95 (22), 93 (18), 91 (16), 81 (30), 79 (23), 67 (26), 55 (38); steviol, 16,17 α-epoxide methyl ester [**12**], mp 144-145°; $[\alpha]^{25}D-113.6°$ (*c* 0.35, MeOH), exhibited mp, ir, and ^{1}H-nmr data consistent with literature values (8), and uv, λ max (MeOH) 225 (sh) nm (log ε 2.47); ms *m/z* 348 (M^+, 4%), 330 (6), 291 (30), 231 (16), 181 (21), 168 (15), 161 (17), 150 (20), 147 (20), 135 (26), 123 (56), 121 (100), 109 (55), 107 (52), 95 (45), 81 (55), 55 (67). The ^{13}C-nmr data of compounds **5**, **8**, **10**, and **12** are shown in Table 1.

15α-Hydroxysteviol [**13**] was prepared by dissolving steviol (**4**, 2 g) in recently distilled dioxane (20 ml) and was then treated with selenium dioxide (1.2 g) for 6 h at room temperature (25). After removal of

solvent, the yellow residue was purified by column chromatography over Si gel (120 g), using a mixture of petroleum ether-Me_2CO (85:15) as eluent, to give 700 mg of **13**. After recrystallization from MeOH/$CHCl_3$, 15 α-hydroxysteviol [**13**] exhibited mp 292-295°; $[\alpha]^{25}D$ −101.0° (*c* 0.2, MeOH); uv λ max (MeOH) 207 nm (log ε 2.76); ir ν max (KBr) 3469 (sh), 3386 (br), 2946, 1696, 1432, 1386, 1175, 997, 907 cm^{-1}; ^1H nmr (200 MHz) δ 1.26 (3H, s, 20-CH_3), 1.37 (3H, s, 18-CH_3), 4.28 (1H, br s, 15-βH), 5.66 (1H, br s, 17-H_2), 5.74 (1H, br s, 17-H_2); ms *m/z* 334 (M^+, 30%), 316 (27), 298 (6), 237 (26), 235 (24), 221 (20), 161 (20), 148 (39), 137 (93), 123 (37), 100 (53), 91 (44), 81 (57), 67 (45), 55 (73), 43 (86), 41 (100). 15 α-Hydroxysteviol [**13**] exhibited closely comparable ^{13}C-nmr data to published values (10).

15-Oxosteviol [**14**] was produced by oxidation of 15α-hydroxysteviol (**13**, 500 mg) in $CHCl_3$ (300 ml), by addition to pyridinium chlorochromate (464 mg) in $CHCl_3$ (100 ml). After 6 h 200 ml of dry Et_2O were added, the supernatant decanted, and the residue washed with additional quantities of Et_2O. The pooled Et_2O fractions were dried at room temperature, and the solid residue was purified by column chromatography on Si gel (100 g), using petroleum ether-Me_2CO (85:15) as solvent, to afford 80 mg of pure 15-oxosteviol [**14**], which exhibited mp 216-218°; $[\alpha]^{25}D$ −230° (*c* 0.11, MeOH); uv λ max (MeOH) 238 nm (log ε 2.95); ir ν max (KBr) 3444 (br), 2953, 2870, 1727, 1697, 1437, 1275 cm^{-1}; ^1H nmr δ 0.98 (3H, s, 20-CH_3), 1.25 (3H, s, 18-CH_3), 5.44 (1H, s, 17-H_2), 6.04 (1H, s, 17-H_2); ^{13}C nmr, see Table 1; ms *m/z* 332 (M^+, 40%), 314 (13), 304 (24), 286 (10), 268 (13), 165 (26), 161 (31), 148 (46), 136 (60), 123 (78), 121 (74), 109 (67), 95 (47), 83 (91), 77 (56), 67 (65), 55 (97), 41 (100).

Steviol norketone acetate [**15**] was prepared by oxidation of steviol acetate (**6**, 0.5 g), in THF-H_2O (1:1, 10 ml), using OsO_4 (0.25 mg in 1 ml H_2O) (26). After 10 min, $NaIO_4$ (1.05 g) was added slowly with stirring, and the reaction mixture was kept overnight at room temperature, diluted with H_2O (10 ml), and extracted with EtOAc (4×15 ml). On drying, the residue from the EtOAc layer was chromatographed over Si gel (100 g) with EtOAc-hexane (35:65) as solvent. After crystallization from Me_2CO/H_2O, pure steviol norketone acetate (**15**, 300 mg) was found to exhibit mp 229-230° [lit. 221-223° (25)]; $[\alpha]^{25}D$ −97.4° (*c* 0.35, MeOH); uv λ max (MeOH) 205 (sh) nm (log ε 2.55); ir ν max (KBr) 3400-2900, 1761, 1735, 1693, 1259, 1244, 1230 cm^{-1}; ^1H nmr δ 1.16 (3H, s, 20-CH_3), 1.36 (3H, s, 18-CH_3), 2.05 (3H, s, -$COCH_3$); ^{13}C nmr, see Table 1; ms *m/z* 362 (M^+, 10%), 320 (13), 302 (6), 277 (47), 259 (7), 231 (9), 123 (16), 121 (21), 109 (22), 95 (21), 81 (31), 67 (28), 55 (57), 43 (100).

ent-Kaur-16-ene-13,19-diol [**16**] was obtained using a literature method (8), in which steviol methyl ester (**5**, 100 mg) was refluxed with $LiAlH_4$ (50 mg) in THF (10 ml) for 4 h. The reaction product was worked up by addition of H_2O, was poured into dilute HCl (5%, 25 ml), and was extracted into Et_2O. The organic layer was washed with H_2O and dried, and the residue was crystallized from MeOH to afford *ent*-kaur-16-ene-13,19-diol (**16**, 60 mg) as white needles, mp 238-240°; $[\alpha]^{25}D$ −59.7° (*c* 0.23, MeOH), that exhibited mp, ir, and ^1H-nmr data consistent with published values (8), and uv λ max (MeOH) 211 nm (log ε 2.17); ^{13}C nmr, see Table 1; ms *m/z* 304 (M^+, 16%), 286 (18), 274 (100), 121 (84), 109 (40).

ACTIVITY ON INSECTS.—A previously described feeding deterrency bioassay with the aphid, *S. graminum* (5) was employed as the primary test system in this study. The pure test compounds **1-17** were dissolved or dispensed (sonicated) into the aqueous aphid diet. Diets were inoculated with aphids (all ages) of biotypes C and E, maintained in continuous light on a greenbug-susceptible sorghum line. Feeding deterrency was determined after 24 h as the percentage of aphids feeding on the test diets compared to the percentage (usually>95%) feeding on the appropriate controls. Feeding deterrency was reported for each test compound as an EC_{50} value, the effective concentration of each necessary to reduce the percentage feeding to 50% of that of the control (Table 2). EC_{50} values were derived from log-dose probit lines, fitted by eye.

Steviol [**4**] exhibited no activity when tested against larvae of *Heliothis virescens* Fabr. (tobacco budworm) and *Aedes aegypti* L. (yellow fever mosquito), at concenteration levels up to 500 ppm and 40 ppm, respectively (J.A. Klocke, unpublished results).

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