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SESQUITERPENES, GUAIANOLIDES AND DITERPENES FROM *STEVIA MYRIADENIA**

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Key Word Index—*Stevia myriadenia*; Compositae; sesquiterpenes; sesquiterpene lactones; guaianolides; diterpenes; clerodane derivative; labdane derivative.

Abstract—The investigation of *Stevia myriadenia* afforded in addition to known compounds a new clerodane and a labdane derivative, two guaianolides, a bisabolene and a germacrene derivative. The structures were elucidated by spectroscopic methods. The chemotaxonomic situation is discussed briefly.

INTRODUCTION

From the large genus *Stevia* (Compositae, tribe Eupatorieae, subtribe Piqueriinae [1], more than 10 species have been investigated chemically. Some species afforded ent-kaurenoic acid derivatives [2–7], while others contain longipinene derivatives [7–9]. One species gave a pseudoguaianolide [10] and another a germacranolide [11], while some afforded guaianolides [7, 12, 13]. Also a few ent-labdane derivatives have been reported [14, 15]. The overall picture therefore is not very uniform and further investigations seem to be necessary to see how chemically heterogenous this large genus is. We have now studied a further species and the results are discussed in this paper.

RESULTS AND DISCUSSION

The roots of *Stevia myriadenia* Sch. Bip. ex Baker afforded germacrene D, bicyclogermacrene, caryophyllene and two polar sesquiterpenes, which could not be completely separated. The molecular formulae and the ^{1}H NMR spectral data (Table 1) showed, however, that the bisabolene derivative **14** and the hydroxyacetate **13**, derived from the known alcohol **12**, were present. Accordingly the ^{1}H NMR spectrum of **13** was similar to that of **12**. The relative position of the oxygen functions was further supported by the mass spectrum, which showed a fragment $[M - CH_2OAc]^+$. The structure of **14** followed from the mass spectrum, which showed elimination of water and angelic acid. The latter fragment was transformed by a typical McLafferty fragmentation to *m/z* 108 (C_7H_8O). Furthermore the nature of the side chain was indicated by the fragment *m/z* 69 (C_5H_9). The ^{1}H NMR spectrum was in part similar to those of related bisabolene derivatives. The position of the angelate residue followed from the chemical shift of H-15, while the orientation of the 2-hydroxy group could be deduced from the coupling $J_{1,6}$. Very similar bisabolene derivatives with oxygen functions at C-2 and C-15 were reported from *Stevia ovata* [8]. The aerial parts afforded germacrene D, bicyclogermacrene, lupeyl acetate and its Δ12-isomer, geranyl-linalol (**1**), 20 - hydroxygeranylnerol

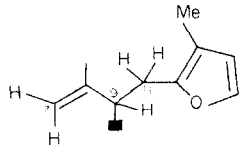
Table 1. ^{1}H NMR spectral data of compounds **13** and **14** (400 MHz, C_6D_6, TMS as internal standard)

	13	**14**
H-1	4.96 *brd*	3.90 *brd*
H-2	2.41 *dddd* 2.05 *m*	5.64 *brs*
H-5	4.91 *d*	—
H-6	5.40 *dd*	—
H-7	2.05 *m*	—
H-9	2.05 *m*	2.10 *brdt*
H-10	—	5.30 *tqq*
H-11	—	—
H-12	0.92 *d*	1.75 *brs*
H-13	0.85 *d*	1.64 *brs*
H-14	1.63 *brs*	0.78 *d*
H-15	4.04 *d*	4.57 *brs*
H-15′	3.76 *d*	
OAc	1.70 *s*	—
OAng	—	5.78 *qq* 2.06 *dq* 1.94 *dq*

J (Hz): Compound **13**: 1,2 = 12; 2,2′ = 13; 2,3 = 11; 2,3′ = 3; 5,6 = 15; 6,7 = 10; 11,12 = 11,13 = 7; 14,14′ = 11; compound **14**: 1,6 = 9; 7,14 = 8,9 = 9,10 = 7.

*Part 444 in the series "Naturally Occurring Terpene Derivatives". For Part 443 see Bohlmann, F. and Wallmeyer, M. (1982) *Phytochemistry* **21**, 2126.

(**2**)[16], ent-kaurenic acid (**3**) and the corresponding 15-tigloyloxy (**4**) and senecioyloxy derivative (**5**), the diterpenic acids **6** and **7** as well as two further diterpenes, the acid **8**, isolated as its methyl ester **9**, and the furanolabdane **10**, which on acetylation gave the acetate **11**. The structure of **9** followed from the ^{1}H NMR spectrum (Table 2). The signals of the decalin part were similar to those of a clerodane from a *Symphyopappus* species[17]. However, the difference in the stereochemistry at C-2 clearly followed from the couplings observed, which showed that **9** had a 2β-acetoxy group. The nature of the side chain also followed from the corresponding ^{1}H NMR signals, which were nearly identical with those of the methyl ester of **7**. Accordingly the configuration of the 13,14-double bond was the same. This statement was further supported by the chemical shift of H-16, which is always more deshielded in isomeric esters. The structure of **10**, molecular formula $C_{20}H_{30}O_2$, followed from the ^{1}H NMR spectrum (Table 2) and from the mass spectrum as well as from the corresponding data of the acetate **11**. The spectrum of the latter in deuteriobenzene could be interpreted completely by first order analysis and by spin decoupling. The presence of an α,β-disubstituted furan followed from the chemical shifts of the lowfield signals (6.05 *d* and 7.13 *d*) and the nature of the substitution from the presence of small homoallylic coupling between the olefinic methyl group and an allylic methylene group. The chemical shift and the couplings of the latter required an α-position at the furan ring. Irradiation at δ 2.99 further showed that the corresponding proton was coupled with the olefinic methylene and with the α-furan methylene protons. Accordingly sequence **A** was established, which is most likely part of a labdane.



A

Table 2. ^{1}H NMR spectral data of compounds **9**–**11** (400 MHz, TMS as internal standard)

	9†	**10**	**11**	
	C_6D_6	$CDCl_3$	$CDCl_3$	C_6D_6
H-1α	1.63 *m*		1.75 *brd*	1.62 *brddd*
H-1β	1.96 *brd*		1.23 *m*	1.13 *ddd*
H-2α	5.50 *brdd*		1.55 *m*	1.46 *ddddd*
H-2β			1.45 *brd*	1.38 *brd*
H-3α	5.55 *brd*	*	1.23 *m*	1.16 *ddd*
H-3β			1.45 *m*	1.36 *brd*
H-5β	—		1.93 *m*	1.70 *dd*
H-6α	1.35 *m*		1.55 *m*	1.43 *ddd*
H-6β	0.95 *m*		1.93 *m*	1.97 *ddd*
H-7α	1.35 *m*	4.33 *dd*	5.39 *dd*	5.64 *dd*
H-8	1.70 *m*	—	—	—
H-9β	—	2.83 *brd*	2.76 *m*	2.99 *dddd*
H-11	1.60 *m*	2.77 *dd*		2.73 *brdd*
H-11′	1.47 *m*	2.58 *dd*	2.59 *dd*	2.60 *dd*
H-12	3.11 *ddd* 2.42 *ddd*	—	—	—
H-14	5.80 *brs*	6.10 *d*	6.12 *d*	6.05 *d*
H-15	—	7.17 *d*	7.17 *d*	7.13 *d*
H-16	1.66 *d*	1.97 *s*	1.97 *s*	1.89 *s*
H-17	1.00 *d*	4.97 *brs*	5.08 *brs*	5.20 *brs*
H-17′		4.69 *brs*	4.78 *brs*	4.90 *brs*
H-18	1.52 *brs*	0.89 *s*	0.86 *s*	0.87 *s*
H-19	0.82 *s*	0.82 *s*	0.83 *s*	0.75 *s*
H-20	0.67 *s*	0.74 *s*	0.77 *s*	0.70 *s*
OAc	1.80 *s*	—	2.03 *s*	1.71 *s*
OMe	3.47 *s*	—	—	—

*Overlapping multiplets.

†H-10 1.76 *d*.

J (Hz): Compound **9**: 1α,1β = 14; 1β,2 = 2,3 = 4; 1α,10 = 12; 8,17 = 7; 11,12 = 13; 11,12′ = 4; 11′,12 = 4; 11′,12′ = 13; 12,12′ = 13; compounds **10** and **11**: 1α,1β = 13; 1α,2α = 3.5; 1α,2β ~ 3; 1β,2α = 13; 1β,2β = 4; 2α,2β = 13.5; 2α,3α = 3; 2α,3β = 13; 2β,3α ~ 3; 2β,3β ~ 3; 3α,3β = 13; 5β,6α = 13; 5β,6β = 3; 6α,6β = 14; 6α,7α = 6β,7α = 3; 9β,17 ~ 1; 9β,11 = 4; 9β,11′ = 10; 11,11′ = 15; 11,16 ~ 1; 14,15 = 1.7; 17,17′ = 1.

The chemical shift of H-17 already indicated that the oxygen function was at C-7. The corresponding signal at δ 5.64 was a broadened triplet. The coupling indicated a β-orientation if the model was inspected. This was in agreement with the ^{1}H NMR spectral data of similar diterpenes. Spin decoupling allowed the assignment of all remaining methylene protons (H-1—H-3 and H-6) and of H-5. The assignment of the methyl singlets corresponded with that of similar diterpenes. The fragmentation pattern in the mass spectra of **10** and **11** also supported the proposed structure. Elimination of water and acetic acid respectively was followed by splitting of the 9,11-bond leading to the typical labdane fragment *m/z* 189 ($C_{14}H_{21}$); similarly the furan moiety gave the ion *m/z* 95 (C_6H_7O). Recently a 7-desoxy derivative of **10** was reported from *Pinus pumilis* [18] and named pumiloxide. The optical rotation of **11** indicates that an ent-labdane was more likely, if the rotation was compared with those of daniellic and lambertianic acid with known absolute configuration. However, a positive assignment was not possible since the influence of the oxygen function at C-7 is in

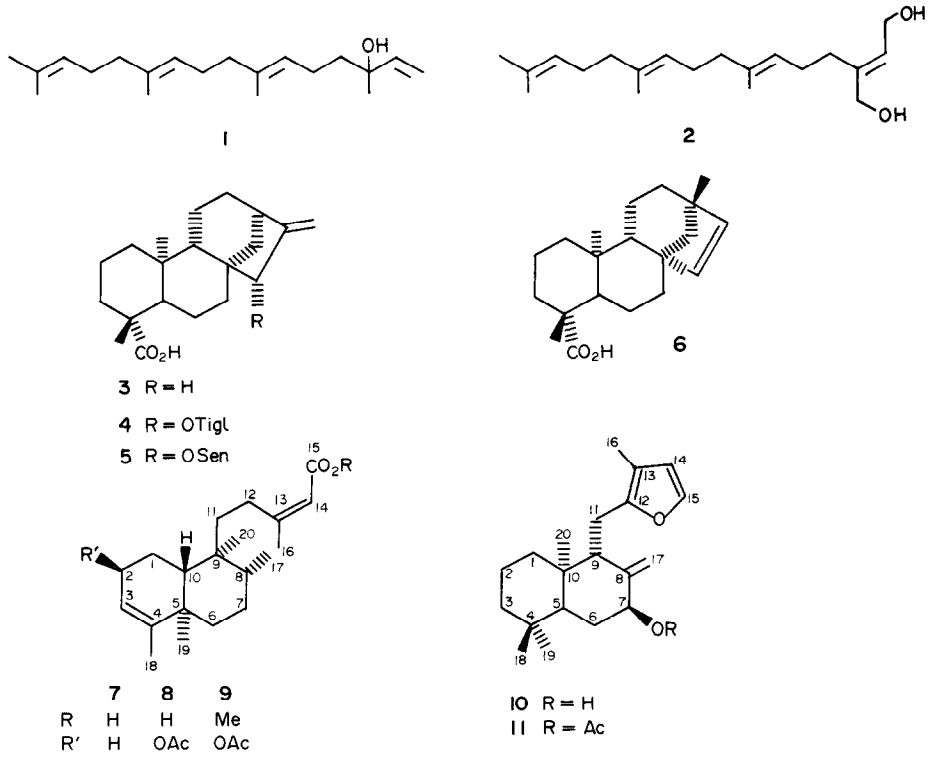
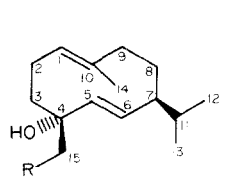


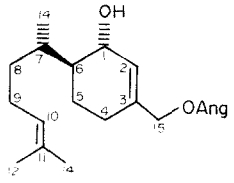
Table 3. ^{1}H NMR spectral data of compounds **15** and **16** (400 MHz, $CDCl_3$, TMS as internal standard)

	15	**16**	$CDCl_3$–C_6D_6 (2:1)
H-2	3.09 *brd*	3.09 *brd*	2.92 *brd*
H-2′	2.98 *brd*	2.99 *brd*	2.82 *brd*
H-3	5.55 *brs*	5.58 *brs*	5.43 *brs*
H-5	3.36 *brd*	3.43 *brd*	3.18 *brd*
H-6	4.06 *dd*	4.11 *dd*	3.95 *dd*
H-7	2.89 *dddd*	3.08 *m*	2.70 *dddd*
H-8	4.45 *ddd*	5.66 *ddd*	5.43 *m*
H-9	2.53 *m*	2.66 *dd*	2.45 *dd*
H-9′		2.56 *brd*	2.27 *brd*
H-13	6.27 *d*	6.18 *d*	6.03 *d*
H-13′	5.53 *d*	5.45 *d*	5.24 *d*
H-14	1.76 *brs*	1.65 *brs*	1.49 *brs*
H-15	1.94 *brs*	1.96 *brs*	1.87 *brs*
OCOR	—	6.83 *t*	6.63 *t*
		4.43 *d*	4.10 *d*
		4.33 *s*	4.11 *s*

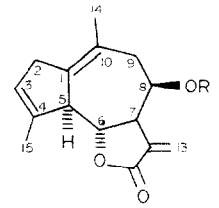
J (Hz): 2,2′ = 21; 5,6 = 6,7 = 10; 7,8 = 1.5; 7,13 = 3.5; 7,13′ = 3; 8,9 = 6; 8,9′ = 1.5; 9,9′ = 16.



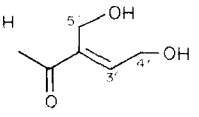
12 R = H
13 R = OAc



14



15 R = H
16 R =



question. So far no furanolabdane of type **10** has been reported from the Compositae. Furthermore the polar fractions of the aerial parts afforded two sesquiterpene lactones, the guaianolides **15** and **16**. The ^{1}H NMR spectra (Table 3) showed that **16** was an ester of **15**. The nature of the ester group clearly followed from the typical signals. In the spectrum of **15** all signals could be assigned by spin decoupling. A pair of broadened doublets at δ 3.09 and 2.98 were the signals of H-2. Accordingly these signals were altered on irradiation of the signals of the olefinic methyl groups and they were further coupled with an olefinic signal at δ 5.55. Furthermore irradiation at δ 4.06 collapsed the broadened doublet at 3.36 to a singlet and the fourfold doublet at 2.89 to a threefold doublet. As the latter was coupled with the exomethylene protons and with a proton, its signal being at 4.45, the protons H-5–H-8 could be assigned. The chemical shift of H-5, which was coupled too with the olefinic methyl at C-10 and with H-3, further supported the arrangement of the double bonds. The coupling $J_{7,8}$ clearly showed that the hydroxy group at C-8 was β-orientated. As usual in the spectrum of **16** some signals were slightly shifted, while the splitting pattern was the same. **15** is a 2-desoxo derivative of dehydroleucodin[19] or a 8-desacyl derivative of zuurbergenin[20], which, however, has an α-orientated oxygen function at C-8. The chemistry of this *Stevia* species differs in part from that of the species investigated previously. However, as mentioned before, guaianolides are present in three other species and different types of diterpenes have also been reported from several species. Also bisabolene derivatives similar to **14** were isolated from three other species. *Stevia setifera* [7] contains the Δ10,14-isomers of **15** and **16**, which may indicate a close relationship. The chemistry of other genera placed in the same subtribe should be studied. So far a similar compound has only been reported from a *Guevaria* species[21]. Further investigations are necessary to obtain a clearer picture of this large genus.

EXPERIMENTAL

The air-dried plant material, collected in north-eastern Brazil (voucher RMK 8887, deposited in the U.S. National Herbarium) was extracted with Et_2O–petrol (1:2) and the resulting extracts were separated first by CC (SiO_2) and further by TLC (SiO_2). Known compounds were identified by comparing the ^{1}H NMR spectra with those of authentic material. The roots (40 g) afforded 2 mg germacrene D, 4 mg bicyclogermacrene, 6 mg caryophyllene, 4 mg **13** and **14** (Et_2O–petrol, 1:1), which could not be separated completely. GC/MS showed that two compounds were present. ^{1}H NMR spectral data were taken from enriched mixtures. The aerial parts (270 g) gave 15 mg germacrene D, 7 mg bicyclogermacrene, 5 mg lupeyl acetate and 7 mg of its Δ12-isomer, 2 mg **1**, 5 mg **2**, 25 mg **3**, 20 mg **4**, 5 mg **5**, 3 mg **6**, 2 mg **7**, 2 mg **8** [isolated as its methyl ester **9** (Et_2O–petrol, 1:4)], 5 mg **10** (Et_2O–petrol, 1:3) 10 mg **15** and 3 mg **16** (both separated with Et_2O–petrol, 3:1).

Methyl 2β-acetoxy-13,14-Z-kolavenoate (**9**). Colourless gum, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1725 *br* (OAc, CO_2R), 1640 (C=C); MS *m/z* (rel. int.): 376.261 [M]$^+$ (5) ($C_{23}H_{36}O_4$), 361 [M – Me]$^+$ (1), 344 [M – MeOH]$^+$ (9), 329 [344 – Me]$^+$ (42), 316 [M – HOAc]$^+$ (25), 301 [316 – Me]$^+$ (50), 274 [316 – MeOH]$^+$ (23), 259 [274 – Me]$^+$ (14), 189 [316 – $CH_2CH_2C(Me)$=$CHCO_2Me$]$^+$ (100).

6β-*Hydroxypumiloxide* (**10**). Colourless gum, MS *m/z* (rel. int.): 302.224 [M]$^+$ (18) ($C_{20}H_{30}O_2$), 284 [M – H_2O]$^+$ (11), 207 [M – C_6H_7O]$^+$ (10), 189 [207 – H_2O]$^+$ (15), 95 [methylpyrrylium ion]$^+$ (100). Acetylation (Ac_2O, 1 hr, 70°) afforded **11**, colourless gum, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1740, 1245 (OAc), 1650 (C=C); MS *m/z* (rel. int.): 344.235 [M]$^+$ (5) ($C_{22}H_{32}O_3$), 302 [M – ketene]$^+$ (10), 285 [M – OAc]$^+$ (22), 284 [M – HOAc]$^+$ (33), 269 [284 – Me]$^+$ (4), 189 [284 – methylpyrrylium]$^+$ (19), 95 [methylpyrrylium ion]$^+$ (100).

$$[\alpha]_{24°}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436\ \text{nm}}{-40 \quad -43 \quad -49 \quad -85} \ (CHCl_3;\ c0.3).$$

15 - *Acetoxy* - 4α - *hydroxygermacra* - 1E,5E - *diene* (**13**). Colourless oil, not free from **14**, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1740 (OAc); MS *m/z* (rel. int.): 280.203 [M]$^+$ (0.5) ($C_{17}H_{28}O_3$), 262 [M – H_2O]$^+$ (0.5), 207 [M – CH_2OAc]$^+$ (10), 81 [C_6H_9]$^+$ (100); CI (*iso*-butane): 281 [M + 1]$^+$ (0.5), 263 [281 – H_2O]$^+$ (100).

15 - *Angeloyloxy* - 2α - *hydroxy* - *bisabol* - 2,10 - *diene* (**14**). Colourless oil, not free from **13**, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1720 (C=CCO_2R); MS *m/z* (rel. int.): 320.235 [M]$^+$ (0.3) ($C_{20}H_{32}O_3$), 302 [M – H_2O]$^+$ (0.5), 220 [M – RCO_2H]$^+$ (3), 202 [220 – H_2O]$^+$ (8), 187 [202 – Me]$^+$ (7), 108 [C_7H_8O, McLafferty from 220]$^+$ (21), 83 [C_4H_7CO]$^+$ (100), 69 [C_5H_9]$^+$ (57); CI (*iso*-butane): 321 [M + 1]$^+$ (0.5), 303 [321 – H_2O]$^+$ (4), 203 [303 – AngOH]$^+$ (100).

8β-*Hydroxy-2-desoxodehydroleucodin* (**15**). Colourless gum, which could not be induced to crystallize, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600 (OH), 1775 (γ-lactone); MS *m/z* (rel. int.): 246.125 [M]$^+$ (27) ($C_{15}H_{18}O_3$), 228 [M – H_2O]$^+$ (11), 213 [228 – Me]$^+$ (11), 202 [M – CO_2]$^+$ (12), 153 (100), 135 (68), 121 (38), 107 (56).

$$[\alpha]_{24°}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436\ \text{nm}}{-32 \quad -32 \quad -36 \quad -66} \ (CHCl_3;\ c0.27).$$

8β - (4',5' - *Dihydroxytigloyloxy*) - 2 - *desoxodehydroleucodin* (**16**). Colourless gum, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3590, 3400 (OH), 1765 (γ-lactone), 1700 (C=CCO_2R); MS *m/z* (rel. int.): 342 [M – H_2O]$^+$ (0.5), 228.115 [M – RCO_2H]$^+$ (100) ($C_{15}H_{16}O_2$), 213 [228 – Me]$^+$ (32), 97 [RCO – H_2O]$^+$ (37), 69 [97 – CO]$^+$ (60).

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