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PSEUDOGUAIANOLIDES RELATED TO CONFERTIN FROM *STEVIA ISOMECA*

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Key Word Index—*Stevia isomeca*, Compositae, sesquiterpene lactones, pseudoguaianolides

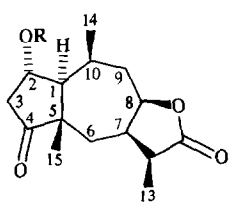
Abstract—The aerial parts of *Stevia isomeca* afforded, in addition to known compounds, four new sesquiterpene lactones, three pseudoguaianolides related to stevin and a xanthanolide The structures were elucidated by spectroscopic methods and a few chemical transformations

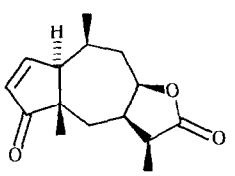
INTRODUCTION

The large, very variable genus *Stevia* (Compositae, tribe Eupatorieae) is placed in the *Piqueria* group [1] Chemically this genus is quite heterogeneous While parts of the genus gave diterpene glycosides [2], another group is characterized by the occurrence of longipinene derivatives [3] So far only a few sesquiterpene lactones have been reported [3–6], all being guaianolides except the pseudoguaianolide stevin [4] We now have investigated the aerial parts of *Stevia isomeca* Grashoff, the result of which is presented below

RESULTS AND DISCUSSION

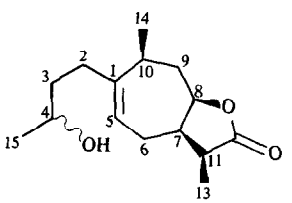
The aerial parts of *Stevia isomeca* afforded four sesquiterpene lactones, the pseudoguaianolides **1**, **2**, and **4** and the xanthanolide **6** The structure of the main component **2**, molecular formula $C_{17}H_{24}O_5$, followed from the ^{1}H NMR spectrum (Table 1) All signals could be assigned by spin decoupling leading to the sequences H-6 to H-10 (H-14) and H-1 (H-10) to H-3 The chemical shifts of H-2, H-7 (H-11–H-13) and H-8 required the presence of an 8,12-lactone with an acetoxy group at C-2 Furthermore these results required a pseudoguaianolide and the stereochemistry followed from the results of the NOE difference spectroscopy Thus irradiation of H-15 gave clear NOEs with H-2β, H-3β, H-6β, H-6α (weak) and H-14 Further NOEs were observed between H-14, H-2β and H-9β as well as between H-7 and H-8 The chemical shift and the coupling of H-13 was an indication of a β-methyl group at C-11 Also the ^{13}C NMR spectrum (see Experimental) was in good agreement with the proposed structure which was further supported by the transformation of **2** to the conjugated ketone **4** by mild alkali treatment of **2** Again the structure of **4** could be established by ^{1}H NMR spectroscopy (Table 1) All signals could be assigned by spin decoupling and NOE difference spectroscopy established the β-orientation of all three methyl groups Further alkali treatment of **4** gave the 1β-epimer **5** as followed from the ^{1}H NMR spectrum (Table 1) NOE difference spectroscopy again established the proposed configuration A clear NOE was obtained between H-15, H-1 and H-6α Furthermore, inspection of a model showed that the couplings observed agreed nicely with the stereochemistry Reaction of **2** in methanol with potassium carbonate gave in addition to **5** also the methoxy derivative **3** As the couplings of H-2 were changed and also a downfield shift of the H-14 signal could be observed, a 2β-methoxy derivative was most likely The spectral data of **1** (Table 1) indicated that the desacetyl derivative of **2** was present too Accordingly, especially the H-2 signal was shifted up field while the couplings were the same as those of **2**





1 R = H
2 R = Ac
3 R = Me (2αH)

4
5 1βH



6

The molecular formula of **6** was $C_{15}H_{24}O_3$ The nature of the oxygen functions already could be deduced from the IR spectrum which showed bands for hydroxyl and γ-lactone The ^{1}H NMR spectral data (Table 1) were in part close to those of 4H-tomentosin However, the absence of a methylene lactone was obvious A doublet quartet at δ2 80 which was coupled with a methyl doublet at δ1 13 indicated an 11α,13-dihydro derivative as the coupling

Table 1 ^{1}H NMR spectral data of 1–6 (400 MHz, $CDCl_3$, TMS as internal standard)

	1	2 ($CDCl_3$)	C_6D_6	3*	4	5	6
H-1	2 05 *dd*	2 25 *m*	1 75 *m*	2 25 *m*	3 16 *br d*	2 43 *ddd*	—
H-2	4 57 *ddd*	5 35 *ddd*	5 22 *ddd*	4 03 *ddd*	7 51 *dd*	7 75 *dd*	2 28 *m* 1 97 *m*
H-3α	2 95 *dd*	3 14 *dd*	2 94 *dd*	2 94 *dd*	6 08 *dd*	6 15 *dd*	1 54 *m*
H-3β	2 18 *dd*	2 05 *dd*	1 87 *dd*	2 10 *dd*			1 43 *m*
H-4	—	—	—	—	—	—	3 77 *m*
H-5	—	—	—	—	—	—	5 50 *br d*
H-6α	2 39 *dd*	2 40 *dd*	2 22 *dd*	2 25 *m*	2 39 *dd*	2 10 *br d*	2 21 *m*
H-6β	1 18 *dd*	1 19 *dd*	0 71 *dd*	1 20 *dd*	1 27 *dd*	1 45 *dd*	1 87 *ddd*
H-7	2 59 *dddd*	2 58 *dddd*	1 59 *dddd*	2 25 *m*	2 72 *dddd*	2 12 *m*	2 67 *dddd*
H-8	4 64 *ddd*	4 64 *ddd*	3 84 *ddd*	4 69 *ddd*	4 67 *ddd*	4 36 *ddd*	4 62 *ddd*
H-9α	2 29 *ddd*	2 25 *m*	1 89 *ddd*	2 25 *m*	2 33 *ddd*	2 00 *br dd*	2 05 *m*
H-9β	1 68 *ddd*	1 67 *ddd*	1 23 *ddd*	1 70 *ddd*	1 85 *ddd*	1 88 *ddd*	
H-10	2 35 *m*	2 25 *m*	1 75 *m*	2 25 *m*	2 35 *m*	1 28 *m*	2 33 *m*
H-11	2 88 *dq*	2 85 *dq*	2 25 *dq*	2 88 *dq*	2 88 *dq*	2 75 *dq*	2 80 *dq*
H-13	1 19 *d*	1 17 *d*	1 05 *d*	1 29 *d*	1 19 *d*	1 23 *d*	1 15 *d*
H-14	1 18 *d*	1 09 *d*	0 79 *d*	1 14 *d*	1 13 *d*	1 20 *d*	1 13 *d*
H-15	1 06 *s*	1 07 *s*	0 49 *s*	1 03 *s*	1 28 *s*	1 13 *s*	1 20 *d*
OAc	—	2 08 *s*	1 73 *s*	—	—	—	—

*OMe 3 38 *s*,

J (Hz) 6α,6β = 15, 6α,7 = 2 5, 6β,7 = 14, 7,8 = 6, 7,11 = 9, 8,9α = 4, 8,9β = 11, 9α,9β = 13 5, 9β,10 = 13, 10,14 = 11,13 = 7, compounds **1–3** 1,2 = 10, 2,3α = 8, 2,3β = 7, 3α,3β = 19, compound **4** 1,2 ~ 3, 1,3 = 2 5, 2,3 = 6, 6α,7 = 4, compound **5** 1,2 = 3, 4,3 = 1 5, 1,10 = 10, 2,3 = 6, 6α,7 ~ 1 5, compound **6** 5,6β = 9, 6α,6β = 16, 6α,7 = 12, 6β,7 = 2 5, 7,8 = 6 5, 7,11 = 9, 8,9α = 6, 8,9β = 10, 10,14 = 11,13 = 7

$J_{11,13}$ was 8 Hz only Spin decoupling allowed the assignment of all signals though a few were overlapping The configuration at C-4 could not be determined **6** obviously is formed via fragmentation of a 4-hydroxy-8,12-guaianolide which also may be the precursor of the pseudoguaianolides

Stevia isomeca, according to Grashoff [7], is represented in collections by asexual plants only and he believes its relationship to be with *S jorullensis* HBK of the series *Corymbosae* Its chemistry differs from that of the species which so far have been studied The corresponding pseudoguaianolide stevin has been isolated [4] only from *Stevia rhombifolia* HBK, which according to Grashoff [8], is synonymous with, or exceedingly close to *S jorullensis* This, however, is a methylene lactone No further constituents have been reported from this species Clearly, more chemical investigations are necessary to get a more clear picture about the chemotaxonomy of the large genus

EXPERIMENTAL

The air dried aerial parts (160 g, voucher, Cofre de Perote, Mexico, Turner 15398, TEX, XAL) were extracted with MeOH–Et_2O–petrol, 1 1 1, at room temp and worked-up in the usual fashion [9] CC (SiO_2) fractions were as follows 1 (petrol), 2 (Et_2O–petrol, 1 9, and Et_2O–petrol, 1 3), 3 (Et_2O–petrol, 1 1) and 4 (Et_2O and Et_2O–MeOH, 9 1) Fraction 4 on standing at −20° gave 250 mg crystalline material which after crystallization from Et_2O–petrol gave pure **2** TLC (SiO_2, PF 254) of fraction 3 (Et_2O–petrol, 3 2) gave 3 mg **6** (R_f 0 22) and 7 mg **2** (R_f 0 3) TLC of the non-crystalline part of fraction 4 (Et_2O–C_6H_6–CH_2Cl_2, 1 4 4) gave a band which by repeated TLC (Et_2O–petrol, 3 2) gave 1 mg **1** (R_f 0 11) The second band (R_f 0 22) gave 1 8 mg **6** and the third one (R_f 0 47) afforded by HPLC (RP 8, MeOH–H_2O, 9 10) 10 mg **4** (R_t 16 5 min) and 5 mg **2** (R_t 17 7 min) Known compounds were identified by comparing the 400 MHz ^{1}H NMR spectra with those of authentic material and by co-TLC Compounds **1** and **6** were homogeneous by ^{1}H NMR and TLC in different solvent mixtures but could not be induced to crystallize

2α-Hydroxy-11α,13-dihydroconfertin (**1**) Colourless oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3610 (OH), 1775 (γ-lactone), 1745 (C=O), MS *m/z* (rel int) 266 152 [M]$^+$ (9) (calc for $C_{15}H_{22}O_4$ 266 152), 248 [M − H_2O]$^+$ (3), 233 [248 − Me]$^+$ (3), 220 [248 − CO]$^+$ (4), 55 (100), $[\alpha]_D$ −12 ($CHCl_3$, *c* 0 05)

2α-Acetoxy-11α,13-dihydroconfertin (**2**) Colourless crystals, mp 158°, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1780 (γ-lactone), 1745 (OAc, C=O), MS *m/z* (rel int) 308 [M]$^+$ (1), 266 152 [M − ketene]$^+$ (35) (calc for $C_{15}H_{22}O_4$ 266 152), 248 [M − HOAc]$^+$ (75), 233 [248 − Me]$^+$ (16), 220 [248 − CO]$^+$ (100), 55 (85), $[\alpha]_D$ +141 ($CHCl_3$, *c* 1 3), ^{13}C NMR ($CDCl_3$) 38 9 *d*, 69 1 *d*, 42 5 *t*, 213 7 *s*, 51 7 *s*, 36 2 *t*, 48 2 *d*, 79 5 *d*, 28 4 *t*, 26 4 *d*, 38 3 *d*, 178 0 *s*, 10 3 *q*, 21 3 *q*, 17 4 *q* (170 6 *s* and 20 8 *q*, OAc)

To 5 mg **2** in 2 ml MeOH 0 5 ml 2 N KOH was added After 2 hr standing at room temp TLC (C_6H_6–CH_2Cl_2–Et_2O, 4 4 1) gave 3 mg **5**, colourless oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1770 (γ-lactone), 1710 (C=CC=O), MS *m/z* (rel int) 248 141 [M]$^+$ (15) (calc for $C_{15}H_{20}O_3$ 248 141), 233 [M − Me]$^+$ (25), 220 [M − CO]$^+$ (3), 205 [220 − Me]$^+$ (3), 124 (100), 55 (72), $[\alpha]_D$ +36 ($CHCl_3$, *c* 0 21)

To 5 mg **2** in 2 ml MeOH 0 5 ml 2 N K_2CO_3 was added After 30 min standing at room temp TLC (C_6H_6–CH_2Cl_2–Et_2O, 4 4 1) gave a band (R_f 0 55) which by HPLC (RP 8, MeOH–H_2O, 9 10, *ca* 100 bar, flow rate 3 ml/min) gave 0 5 mg **4** (R_t 11 7 min) and 2 mg **3** (R_t 13 0 min), colourless oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1770 (γ-lactone), 1740 (C=O), MS *m/z* (rel int)

280 $[M]^+$ (78), 248 $[M-MeOH]^+$ (45), 220 $[248-CO]^+$ (12), 121 (100)

To 5 mg **2** in 2 ml dioxane 0 5 ml 2 N K_2CO_3 was added After 1 hr standing at room temp 3 mg **4** were obtained by TLC (Et_2O–C_6H_6–CH_2Cl_2, 1 4 4, R_f 0 55), identical with the natural compound

2,3-*Dehydro*-11α,13-*dihydroconfertin* (**4**). Colourless crystals, mp 167°, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1770 (γ-lactone), 1715 (C=CC=O), MS *m/z* (rel int) 248 141 $[M]^+$ (45) (calc for $C_{15}H_{20}O_3$ 248 141), 233 $[M-Me]^+$ (48), 220 $[M-CO]^+$ (13), 148 (72), 55 (100); $[\alpha]_D$ −12 ($CHCl_3$, *c* 0 12)

2-*Desacetoxy*-11α,13-*dihydroxanthuminol* (**6**) Colourless oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3610 (OH), 1775 (γ-lactone); MS *m/z* (rel int) 252 173 $[M]^+$ (3) (calc for $C_{15}H_{24}O_3$ 252 173), 234 $[M-H_2O]^+$ (13), 219 $[234-Me]^+$ (6), 208 $[M-C_2H_4O]^+$ (15), 121 (82), 55 (100), $[\alpha]_D$ +18 ($CHCl_3$, *c* 0 36)

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