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# BEYERENE DERIVATIVES AND OTHER TERPENOIDS FROM *STEVIA ARISTATA*

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**Key Word Index**—*Stevia aristata*; *S. andina*; Compositae; sesquiterpene lactones; germacranolides; diterpenes; beyerene derivatives; longipinene derivative.

**Abstract**—The aerial parts of *Stevia aristata* afforded in addition to known compounds two new germacranolides, a melampolide, a 4*Z*-melampolide and two beyerene derivatives. From the roots a new dihydrolongipinene derivative was isolated while the aerial parts of *Stevia andina* gave the *E*/*Z*-isomers of austroinulin and 6-desoxyaustroinulin. The absolute configuration of these typical diterpenes is discussed.

## INTRODUCTION

From the large genus *Stevia* (Compositae, tribe Eupatorieae, subtribe Piqueriinae) with nearly 200 species many interesting constituents have been isolated. In addition to germacranolides, similar to those of other genera of the tribe Eupatorieae, and labdane derivatives, longipinene derivatives are widespread [1, 2]. We now have investigated a further species from Paraguay, *S. aristata* D. Don. and *S. andina* B. L. Robinson from Peru. The results are discussed in this paper.

## RESULTS AND DISCUSSION

The aerial parts of *S. aristata* afforded as the main constituent austroinulin 7-*O*-acetate (**4c**) which was isolated from *S. berlandiera* [2] accompanied by the isomeric acetate **4b** [3, 4] and the triol **4a** [4, 5]. Furthermore the sesquiterpene lactones **1b** [1], **2b** [1] as well as the novel alcohols **1a**, **1c**, **2a** and **3** were present. In addition to beyerenic acid (**5a**) [6]* two hydroxy derivatives (**5b** and **5c**) also were isolated. The roots contain germacrene D and γ-humulene as well as the longipinene derivatives **7** [7], **8** [7] and the diangelate **6**. The structure of the latter followed from its $^1$H NMR spectrum (see Experimental) which was very similar to that of the corresponding diester **8** with one angelate group replaced by an acetate residue [8]. The nature of the ester groups could easily be deduced from the typical angelate signals.

The $^1$H NMR spectra of **1a**, **2a** and **3** (Table 1) were close to those of **1b**, **2b** and the acetate of **3** respectively, which were isolated recently from *Stevia amambayensis* [1]. The replacement of acetoxy by hydroxy groups caused, as expected, upfield shifts of H-4′ while the H-3′ signals were shifted downfield.

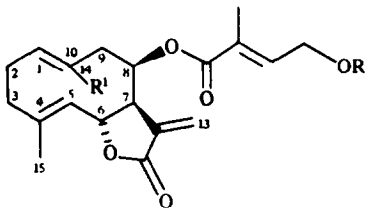
In the $^1$H NMR spectrum of **1c** (Table 1) all signals could be assigned by spin decoupling. They were in part similar to those of **1a**. An additional pair of broadened doublets and the upfield shift of the H-1 signal indicated the presence of the corresponding 14-hydroxy derivative. The configuration of the 1(10)-double bond followed

Table 1. $^1$H NMR spectral data of **1a**, **1c**, **2a** and **3** (400 MHz, $CDCl_3$, TMS as internal standard)

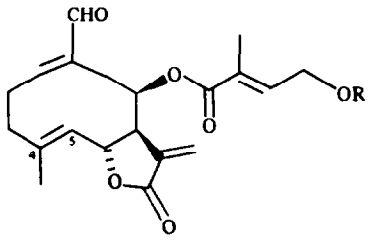
| | **1a** | **1c** | **2a** | **3** |
|---|---|---|---|---|
| H-1 | 5.90 *dd* | 5.02 *br dd* | 6.62 *ddd* | 6.64 *br dd* |
| H-2 | 3.50 *br dd* | 2.4–2.1 *m* | 2.30 *br ddd* | 2.78 *m* |
| H-2′ | 2.42 *m* | | 2.54 *m* | 2.70 *m* |
| H-3 | 2.36 *m* | | 2.41 *br dd* | 3.12 *ddd* |
| H-3′ | 2.30 *m* | | 2.12 *br dd* | 2.25 *m* |
| H-5 | 4.97 *br d* | 4.79 *br d* | 5.05 *br d* | 5.30 *br d* |
| H-6 | 5.05 *t* | 5.08 *t* | 5.10 *t* | 5.47 *dd* |
| H-7 | 2.89 *dddd* | 2.92 *dddd* | 2.50 *m* | 2.65 *m* |
| H-8 | 5.70 *m* | 5.78 *br d* | 6.45 *ddd* | 5.97 *ddd* |
| H-9 | 3.42 *br dd* | 3.30 *br dd* | 2.80 *ddd* | 3.00 *br dd* |
| H-9′ | 2.18 *br d* | 2.12 *br d* | 1.96 *ddd* | 2.56 *m* |
| H-13 | 6.25 *d* | 6.26 *d* | 6.21 *d* | 6.31 *d* |
| H-13′ | 5.59 *d* | 5.59 *d* | 5.58 *d* | 5.67 *d* |
| H-14 | — | 4.21 *br d* | 9.44 *d* | 9.41 *d* |
| H-14′ | | 3.72 *br d* | | |
| H-15 | 1.66 *br s* | 1.66 *d* | 1.82 *d* | 1.73 *d* |
| H-3′ | 6.76 *br t* | 6.68 *br d* | 6.75 *tq* | 6.72 *tq* |
| H-4′ | 4.26 *br dd* | 4.29 *br dd** | 4.35 *dq* | 4.33 *dq* |
| | 4.20 *br dd* | 4.24 *br dd** | | |
| H-5′ | 1.72 *br s* | 1.79 *br s* | 1.94 *br s* | 1.77 *d* |

*$J$ = 15 and 6 Hz; $J$ [Hz]: compound **1a**: 1,2 = 12; 1,2′ = 4; 2,2′ = 2,3′ = 13; 2,3 = 5; 5,6 = 6,7 = 9.5; 7,13 = 3.5; 7,13′ = 3; 8,9 = 5.5; 9,9′ = 15; compound **1c**: 1,2 = 12; 1,2′ = 5; 5,6 = 10; 6,7 = 8.5; 5,15 = 1; 7,13 = 3.5; 7.13′ = 3; 8,9 = 5; 9,9′ = 15; 14,14′ = 12; compound **2a**: 1,2 = 10; 1,2′ = 7; 1,9 = 2; 2,2′ = 13; 2,3 = 2; 2,3′ = 3,3′ = 12; 5,6 = 6,7 = 9; 7,8 = 1.5; 7,13 = 3.5; 7,13′ = 3; 8,9 = 1; 8,9′ = 10; 9,9′ = 14; 9,14 = 1; compound **3**: 1,2 ~ 8; 1,2′ ~ 7; 1,9 = 1,9′ ~ 1; 2,3 = 7; 2′,3 = 11; 2,3′ = 3; 2′,3′ = 7; 3,3′ = 14; 5,6 = 9; 6,7 = 4; 7,8 = 2; 7,13 = 3.5; 7,13′ = 3; 8,9 = 7; 8,9′ = 10; 9,9′ = 14; OCOR: 3′,4′ = 6; 4′,4′ = 14.

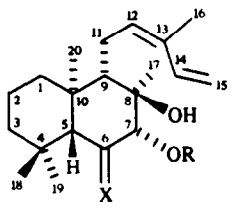
*Identical with compound **9** in Bohlmann, F. and Le Van, N. (1976) *Chem. Ber.* **109**, 1446 where erroneously the opposite configuration at C-4 and C-10 was presented.

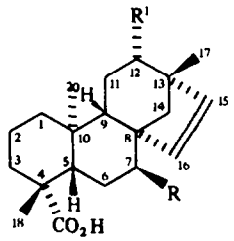


| | 1a | 1b | 1c |
|---|---|---|---|
| R | H | Ac | H |
| R¹ | $CO_2H$ | $CO_2H$ | $CH_2OH$ |



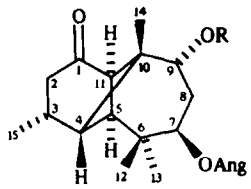
**2a** R = H
**2b** R = Ac
**3** R = H,4*Z*





| | 4a | 4b | 4c | 4d |
|---|---|---|---|---|
| R | H | H | Ac | Ac |
| X | βOH,H | βOAc,H | βOH,H | =O |

| | 5a | 5b | 5c |
|---|---|---|---|
| R | H | OH | H |
| R¹ | H | H | OH |



**6** R = Ang
**7** R = Ang,$\Delta^2$
**8** R = Ac

from the chemical shifts of H-1 and H-14 which agreed with those of similar germacranolides but differed from those of *E*-isomers [9] where the H-1 signal is shifted more downfield.

The $^1$H NMR spectrum of **5b** (Table 2) was similar to those of beyerene derivatives [6, 10]. Careful spin decoupling supported this assumption. However, the position of the hydroxy group could not be assigned. The couplings indicated an axial hydroxy group where the corresponding proton only had two neighbours. This would agree with a hydroxy at C-7 or C-12. A decision was possible by NOE difference spectroscopy which also established the stereochemistry of **5b**. Thus clear effects were observed between H-20, H-1α, H-6α and H-16, between H-18, H-5, H-6β and H-3β, between H-17 and H-15, between H-14α and H-7 as well as between H-7, H-14α and H-16.

The $^1$H NMR spectrum of the isomer **5c** (Table 2) differed from that of **5b** by the splitting of the signal of the proton under the hydroxy group and by the absence of a *w*-couplings of H-14. Furthermore H-11β and H-17 were more deshielded as in the spectrum of **5b**. These facts indicated the presence of a 12α-hydroxy group. NOEs between H-20 and H-15, between H-18 and H-5 and between H-12 and H-14β further supported this structure. The proposed absolute configuration could not be established but the positive optical rotation, which was observed also for beyerenol with known absolute configuration [11], supported this assumption.

The absolute configuration of **4a–c** so far is not established. We therefore transformed **4c** by oxidation with pyridine chlorochromate to **4d**. The CD spectrum showed a strong negative Cotton effect. Following the

Table 2. $^1$H NMR spectral data of **5b** and **5c** (400 MHz, $CDCl_3$, TMS as internal standard)

| | **5b** | **5c** |
|---|---|---|
| H-1α | 1.67 *br d* | 1.71 *br d* |
| H-1β | 0.98 *br dd* | 1.00 *m* |
| H-2α | 1.79 *m* | 1.80 *m* |
| H-2β | 1.42 *br d* | 1.43 *br d* |
| H-3α | 2.17 *br d* | 2.16 *br d* |
| H-3β | 1.08 *m* | 1.09 *m* |
| H-5 | 1.74 *dd* | 1.80 *m* |
| H-6α | 2.12 *dddd* | * |
| H-6β | 2.02 *br d* | |
| H-7 | 3.72 *t* | |
| H-11 | *ca* 1.60 *m* | 1.95 *m* |
| H-14α | 1.53 *dd* | 1.51 *d* |
| H-14β | 1.25 *d* | 1.00 *d* |
| H-15 | 5.58 *d* | 5.88 *d* |
| H-16 | 5.53 *d* | 5.58 *d* |
| H-17 | 1.05 *s* | 1.11 *s* |
| H-18 | 1.25 *s* | 1.25 *s* |
| H-20 | 0.68 *s* | 0.68 *s* |

*Overlapped multiplets: $J$ [Hz]: 1α,1β = 1β,2α = 2α,2β = 3α,3β = 5,6α = 6α,6β = 13; 5,6β = 2.3; 14α,14β = 9; 15,16 = 5.5 (compound **5b**: 6α,7 = 6β,7 = 2.5; 12,14α = 2; compound **5c**: 11α,12 = 10; 11β,12 = 5).

octant rule the presence of an *ent*-labdane seems to be likely as probably the 4α-methyl group is most important for the effect. Labdanes surely are precursors of **5a–c**. Therefore it is very likely that the same configuration should be proposed for both series.

The aerial parts of *S. andina* only gave known compounds including **4a** and 6-desoxy **4a** together with the 12*E*-isomers.

This investigation again showed that there are clear trends in the genus *Stevia*. In addition to constituents which indicated relationship to related genera there are others which may be characteristic for the genus or at least for parts of it. So far it cannot be decided whether large differences in concentration are important. As an example there are several species with high accumulations of longipinenes whereas in others these compounds are only present in traces. Accordingly, negative reports on these constituents may not be correct in all cases. Most likely all groups of natural compounds, which are present in *Stevia*, have to be taken into consideration for a possible subdivision of this large genus.

## EXPERIMENTAL

The air dried plant material was extracted with MeOH–$Et_2O$–petrol, 1:1:1, at room temperature and the extracts obtained were separated as reported previously [12]. The extract of 1.75 kg aerial parts of *S. aristata* (collected near San Antonia, Dept. Central, Paraguay, voucher Schmeda 723, deposited in the US National Herbarium) was separated by CC ($SiO_2$) into four fractions (1: $Et_2O$–petrol, 1:1, 2: $Et_2O$, 3: $Et_2O$–MeOH, 10:1 and 4: $Et_2O$–MeOH, 2:1). TLC of fraction 1 ($Et_2O$–petrol, 1:3) gave 50 mg taraxasteryl acetate and 10 mg beyerenic acid. Medium pressure chromatography ($SiO_2$, φ 30–60 μ, MPCC) of fraction 2 (80 25 ml fractions, $Et_2O$–petrol, 1:1, $Et_2O$) gave a mixture (2/1), 1.5 g **4c**, 500 mg **4b**, 100 mg **4a** and 100 mg **2b**. Fraction 2/1 was again separated by PTLC ($Et_2O$–petrol, 3:1) affording two bands (2/1/1 and 2/1/2). HPLC (RP 8, MeOH–$H_2O$, 17:3, 100 bar) of the less polar band (2/1/1) gave 4 mg **5b** ($R_t$ 1.4 min) and HPLC of 2/1/2 (same conditions) afforded 2 mg **5c** ($R_t$ 1.6 min). MPCC of fraction 3 ($Et_2O$–petrol, 1:1; $Et_2O$–MeOH, 20:1, 25 ml fractions) gave two polar fractions (3/1 and 3/2). HPLC (RP 8, MeOH–$H_2O$, 3:2) of 3/1 gave 50 mg **1a** ($R_t$ 1.6 min) and a mixture ($R_t$ 2.1 min) which by PTLC ($CHCl_3$–$C_6H_6$–$Et_2O$–MeOH, 30:30:30:1) gave 10 mg **1b** ($R_f$ 0.45) 2 mg **3** ($R_f$ 0.33), 20 mg **2a** ($R_f$ 0.30) and 10 mg **1a** ($R_f$ 0.20). HPLC of 3/2 (MeOH–$H_2O$, 3:2) gave 40 mg **1c** ($R_t$ 1.35 min).

The $^1$H NMR spectrum of fraction 4 showed the absence of acetates. Acetylation ($Ac_2O$, DMAP, $CHCl_3$, 20°) gave a mixture which afforded by PTLC ($CHCl_3$–$C_6H_6$–$Et_2O$, 1:1:1) 50 mg **1b** ($R_f$ 0.46).

The extract of 120 g roots gave by CC two fractions (1: petrol and 2: $Et_2O$). PTLC of fraction 1 gave 2 mg γ-humulene and 2 mg germacrene D. PTLC of fraction 2 ($Et_2O$–petrol, 1:1) gave 20 mg **6** ($R_f$ 0.6) and a mixture which gave by HPLC (RP 8, MeOH–$H_2O$, 4:1) 5 mg **8** ($R_t$ 2.9 min), 100 mg **7** ($R_t$ 3.7 min) and 10 mg **6** ($R_t$ 5.1 min).

The aerial parts of *S. andina* (70 g, voucher RMK 9279, collected in Peru) gave by CC and PTLC 55 mg **1a** (12-*E* and 12-*Z ca* 1:1) and 65 mg 6-desoxy **1a** (12-*E* and 12-*Z ca* 1:1) as well as 30 mg germacrene D, 40 mg taraxasteryl acetate, 20 mg lupeyl acetate and 230 mg *ent*-kaurenic acid. Known compounds were identified by comparing the 400 MHz $^1$H NMR spectra with those of authentic material.

*Grazielic acid*-[*4-hydroxytiglate*] (**1a**). Colourless oil; IR $\nu_{max}^{CHCl_3}$ cm$^{-1}$: 3420 (OH), 1760 (γ-lactone), 1720 (C=CCO$_2$R); MS *m/z* (rel. int.): 376.152 [M]$^+$ (0.2) (calc. for $C_{20}H_{24}O_7$: 376.152), 358 [M − $H_2O$]$^+$ (2.5), 261 [M − $RCO_2$]$^+$ (50), 99 [RCO]$^+$ (64), 98 [$RCO_2H$ − $H_2O$]$^+$ (100), 71 [99 − CO]$^+$ (47); $[\alpha]_D^{24°}$ = −107 ($CHCl_3$; *c* 6.6).

*14-Hydroxy-8β-[4-hydroxytigloyloxy]-costunolide* (**1c**). Colourless oil; IR $\nu_{max}^{CHCl_3}$ cm$^{-1}$: 3600, 3500, 3400 (OH), 1760 (γ-lactone), 1715 (C=CCO$_2$R); MS *m/z* (rel. int.): 344.162 [M − $H_2O$]$^+$ (0.1) (calc. for $C_{20}H_{24}O_5$: 344.162), 246 [M − $RCO_2H$]$^+$ (4), 228 [246 − $H_2O$]$^+$ (10), 99 [RCO]$^+$ (100), 71 [99 − CO]$^+$ (78); $[\alpha]_D^{24°}$ = +23 ($CHCl_3$; *c* 3.9).

*8β-[4-Hydroxytigloyloxy]-14-oxo-acanthospermolide* (**2a**). Colourless oil; IR $\nu_{max}^{CHCl_3}$ cm$^{-1}$: 3620, 3500 (OH), 1770 (γ-lactone), 1720 (C=CCO$_2$R), 2720, 1690, 1635 (C=CCHO); MS *m/z* (rel. int.): 360.157 [M]$^+$ (0.2) (calc. for $C_{20}H_{24}O_4$: 360.157), 244 [M − $RCO_2H$]$^+$ (12), 99 [RCO]$^+$ (100); $[\alpha]_D^{24}$ = −13 ($CHCl_3$; *c* 0.87).

*8β-[4-Hydroxytigloyloxy]-14-oxo-4Z-acanthospermolide* (**3**). Colourless oil; IR $\nu_{max}^{CHCl_3}$ cm$^{-1}$: 3600 (OH), 1760 (γ-lactone), 1720 (C=CCO$_2$R); MS m/z (rel. int.): 360.157 [M]$^+$ (0.3) (calc. for $C_{20}H_{24}O_6$: 360.157), 244 [M − $RCO_2H$]$^+$ (15), 99 [RCO]$^+$ (100), 71 [99 − CO]$^+$ (56).

*Oxidation of* **4a**. **4a** (10 mg) in 2 ml $CHCl_3$ were stirred for 12 hr with 20 mg pyridine chlorochromate. TLC ($Et_2O$–petrol, 3:1) gave 2 mg **4d**, colourless oil; IR $\nu_{max}^{CCl_4}$ cm$^{-1}$: 3400 (OH), 1735 (C=O, OAc); MS *m/z* (rel. int.): 362.246 [M]$^+$ (0.5) (calc. for $C_{22}H_{34}O_4$: 362.246), 344 [M − $H_2O$]$^+$ (5), 302 [M − HOAc]$^+$ (24), 221 [302 − side chain]$^+$ (20), 81 [$C_6H_9$]$^+$ (100); $^1$H NMR ($CDCl_3$): δ 2.31 (*s*, H-5), 5.11 (*s*, H-7), 5.51 (*br t*, H-12), 6.86 (*dd*, H-14), 5.24 (*d*, H-15t), 5.13 (*d*, H-15c), 1.82 (*br s*, H-16), 1.22 (*s*, H-17), 1.15 (*s*, H-18), 0.93 (*s*, H-19), 0.83 (*s*, H-20); ($J$ [Hz]: 11,12 = 7; 14,15t = 17; 14,15c = 11); CD (MeCN): $\Delta\varepsilon_{295}$ = −1.5.

*7β-Hydroxybeyerenic acid* (**5b**). Colourless crystals, mp 240°; IR $\nu_{max}^{CCl_4}$ cm$^{-1}$: 3600 (OH), 3500–2600, 1695 ($CO_2H$); MS *m/z* (rel. int.): 318.220 $[M]^+$ (9) (calc. for $C_{20}H_{30}O_3$: 318.220), 300 $[M-H_2O]^+$ (19), 255 $[300-CO_2H]^+$ (7), 197 (100), 147 (79), 107 (41); $[\alpha]_D^{24°} = +10$ ($CHCl_3$; *c* 0.4).

*12α-Hydroxybeyerenic acid* (**5c**). Colourless oil; IR $\nu_{max}^{CCl_4}$ cm$^{-1}$: 3600 (OH), 3500–2600, 1695 ($CO_2H$); MS *m/z* (rel. int.): 318.220 $[M]^+$ (18) (calc. for $C_{20}H_{30}O_3$: 318.220), 300 $[M-H_2O]^+$ (24), 285 $[300-Me]^+$ (7), 255 $[300-CO_2H]^+$ (6), 107 (100), 81 (67).

*8-Desacyloxyrastevione angelate* (**6**). Colourless oil; IR $\nu_{max}^{CCl_4}$ cm$^{-1}$: 1720 (C=O, C=CCO$_2$R); MS *m/z* (rel. int.): 416.256 $[M]^+$ (0.2) (calc. for $C_{25}H_{36}O_5$: 416.256), 316 $[M-RCO_2H]^+$ (1), 217 $[316-RCO_2]^+$ (12), 83 $[RCO]^+$ (100); $^1$H NMR ($CDCl_3$): $\delta$ = 2.15 and 2.56 (*dd*, H-2), 2.35 (*ddq*, H-3), 2.17 (*d*, H-4), 1.60 (*s*, H-5), 5.10 (*dd*, H-7), 2.03 and 2.24 (*ddd*, H-8), 5.11 (*t*, H-9), 3.03 (*d*, H-11), 1.03 (*s*, H-12), 0.90 (*s*, H-13), 0.94 (*s*, H-14, 1.11 (*d*, H-15); (*J* [Hz]: 2,2′ = 19; 2,3 = 9; 2′,3 = 6; 3,15 = 7; 4,11 = 5; 7,8 = 12; 7,8′ = 1.5; 8,8′ = 15; 8,9 = 8,9′ = 3; $[\alpha]_D^{24°} = -23$ ($CHCl_3$; *c* 2.35).

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