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SESQUITERPENE LACTONES OF *PIPTOTHRIX PUBENS*

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Key Word Index—*Piptothrix pubens*; Compositae; Eupatorieae; sesquiterpene lactones; germacrolides; guaianolides; heliangolides.

Abstract—Eleven sesquiterpene lactones, six of which are new, were identified from *Piptothrix pubens*. All but one of the compounds contained a C_{10}-diester attached at C-8. The taxonomic implications of these results are discussed.

INTRODUCTION

Piptothrix pubens Gray is the southernmost member of the species complex that includes *P. palmeri* and *P. sinaloae* [1]. *P. pubens* is found in Mexico from the State of Jalisco in the region around Guadalajara, north to the mountains bordering the State of Zacatecas, and west into the State of Aguascalientes. In many respects (e.g. floret number, head and floret size and fragility of the pappus) the three species are difficult to distinguish. Nevertheless, chemical differences have been found between *P. palmeri* and *P. sinaloae* (as defined by their degree of pubescence and geographic location).

Here the terpenoid chemistry of *P. pubens* from southern Zacatecas is reported. Eleven sesquiterpene lactones were isolated, ten of which contain C_{10} diester side chains. Six of these lactones are new.

RESULTS AND DISCUSSION

Compound **1** is the guaianolide 8β-4′-hydroxy-5′-(4″-hydroxytigloyloxy)-tigloyloxy-4β,15-dihydro-3-dehydrozaluzanin C, a compound recently reported from *Perityle vaseyi*. Difference and 2-D NOESY experiments confirmed that the terminal ester in this lactone is a 4″-hydroxytiglate rather than a 4″-hydroxyangelate [2].

A second compound (**2**), $C_{25}H_{30}O_8$ (CIMS), differed from **1** only in the nature of its ester function. The ^{1}H NMR spectrum of **2** (Table 1) also displayed signals for a 5′-esterified dihydroxytiglate moiety (δ7.02 *t*, 4.49 *br d*, 2H, 4.89 *d*, 2H), but unlike the spectrum for **1** it contained signals for a tigloyloxy group (δ6.78 *qq*, 1.89 *dd*, 3H, 1.80 *br s*, 3H) instead of those for a terminal 4″-hydroxy-tigloyloxy group. These data indicated that **2** was esterified with a 4′-hydroxy-5′-(tigloyloxy)-tigloyloxy function. The mass spectral peaks observed at *m/z* 359 [M + H − tiglic acid]$^+$, 245 [M + H − entire side chain + H]$^+$ and 83 [tiglate acylium]$^+$ were in accord with this conclusion.

The next six compounds differed from **1** and **2** in their skeletal structures. All six were heliangolides (i.e. germacra-1(10)*E*,4*Z*-dienolides) rather than guaianolides. The first compound in this series (**3**) was the known compound eupaformosanin [3]. The β-oriented ester function at C-8 in **3** contained only a single C_5 unit, a dihydroxytigloyloxy function.

The structure of the next heliangolide (**4**), $C_{27}H_{34}O_9$ (CIMS), followed easily from its ^{1}H NMR spectrum (Table 1), which was nearly identical to the spectrum of **3**. The two compounds both possessed the same 3α-acetoxy-8β-acyloxyheliang-6,12-olide skeleton, but differed in their C-8 ester functions. Additional signals representing a tiglate ester were present in the spectrum of **4**. Further more, the broad two-proton singlet for H-5′a and H-5′b was shifted downfield from δ4.38 in **3** to δ4.98 in **4**. These data together indicated that the tiglate was attached at C-5′ of the dihydroxytiglate ester at C-8. A mass spectral fragment at *m/z* 289 was observed for [M + H − C_{10} diester side chain + H]$^+$ fragment (in CIMS). This fragment in turn lost acetic acid to yield a prominent fragment at *m/z* 229 in agreement with the proposed attachment of the ester residues [i.e. the 5′-(tigloyloxy)-4′-hydroxytigloyloxy at C-8 with the acetoxy group at C-3]. Thus, **4** is 5′-tigloyloxyeupaformosanin.

The main compound of the extract (**5**, $C_{27}H_{34}O_{10}$) was closely related to the previous heliangolide **4**. The ^{1}H NMR spectrum of **5** clearly indicated that the tigloyloxy group at C-5′ in **4** was replaced with a 4′-hydroxytigloyloxy function in **5**. This compound, 5′-(4″-hydroxytigloyloxy)-eupaformosanin (**5**), was recently reported from *Piptothrix areolare* [4].

The final three heliangolides could all be easily related to compound **5**. Two of these, **6** and **7**, differed in their C-8 acyloxy groups, while the last compound (**8**) possessed a modified skeletal structure. The ^{1}H NMR spectrum (Table 1) of the first of these compounds (**6**), $C_{29}H_{36}O_{11}$ (CIMS) exhibited an additional acetate methyl signal at δ2.09. Also, the two-proton signal (*br d*) for H-4″a and H-4″b was shifted downfield from δ4.34 in the spectrum of **5** to δ4.74 in **6**, indicating that C-4″ was the position of attachment for the new acetoxy group. The CIMS of **6** exhibited only a small M + 1 peak (*m/z* 561), but did display prominent fragments at *m/z* 501 and 229, corresponding to the loss of acetic acid and the entire C-8 side

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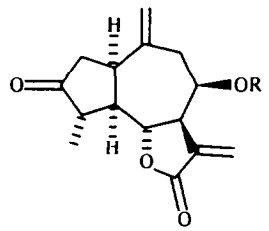

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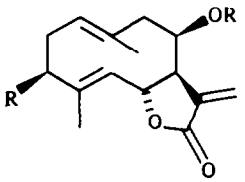
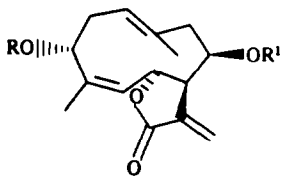
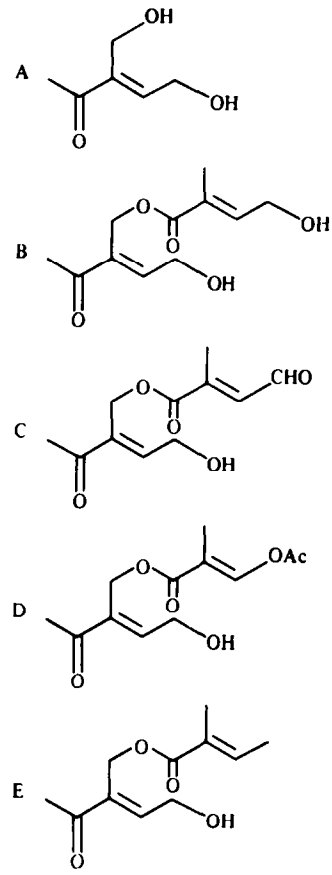


9 R = H, R^1 = B
10 R = OH, R^1 = D
11 R = OAc, R^1 = B



3 R = Ac, R^1 = A
4 R = Ac, R^1 = E
7 R = Ac, R^1 = C
5 R = Ac, R^1 = B
6 R = Ac, R^1 = D
8 R = H, R^1 = B



chain +H together with acetic acid, respectively. Therefore, compound **6** is 5'-(4'-acetoxytigloyloxy)-eupaformosanin. The ^{13}C NMR spectrum of **6** (see experimental) supported this conclusion. As expected the spectrum was similar to that of **5** [4] with major differences only in the signals of the terminal ester residue.

It was obvious from the ^1H NMR spectrum that the next compound (7), $C_{27}H_{32}O_9$ (CIMS), was also a simple derivative of **5**. Compound 7, which was first isolated from a population of *P. areolare* [5], is the 4"-oxo analogue of **5**.

The final heliangolide **8** ($C_{25}H_{32}O_9$) contained the same C_{10} diester function and carbon skeleton as compound **5**. Unlike the other heliangolides isolated from this population it lacked the acetate ester at C-3 and instead contained a hydroxyl group at this position. This was clear from the upfield position of the H-3 signal at δ4.62 relative to the position of this signal in the 3-acetoxy compounds (3–7) at *ca* δ5.58. The H-15 methyl was also shifted upfield *ca* 0.1 ppm in **8**, but most of the other signals in the ^1H NMR spectrum of **8** were similar to those exhibited by **5**. The molecular ion in the CIMS of **8** at *m/z* 477 $[M+1]^+$, as well as the additional fragments at 361 $[M+1-4''$-hydroxytiglic acid$]^+$, 247 $[M+1-$ side chain $+H]^+$ and 229 $[M+1-$ side chain $+H-H_2O]^+$ supported the conclusion that this compound is the 3-desacetyl analogue of **5**. That is, **8** is 3-desacetyl-5'-(4"-hydroxytigloyloxy)-eupaformosanin.

The remaining three compounds isolated from this population were not heliangolides. All three differed in their skeletal configuration in having 4,5-*trans* double bonds; thus they are germacrolides (i.e. *trans*, *trans*-germacradienolides). The simplest of these compounds (**9**, $C_{25}H_{32}O_8$) was closely related to some monosubstituted costunolide derivatives reported from *Eupatoriastrum nelsonii* [6]. Lactone **9** contained only a β-oriented ester function at C-8. The ^1H NMR spectrum of **9** indicated this ester was identical to that attached to compounds **1**, **5** and **8**. The CIMS exhibited the expected M + 1 ion at *m/z* 461, as well as fragments representing the loss of the 4"-hydroxytiglic acid at *m/z* 345 and the C_{10} diester +H at *m/z* 231. Thus, compound **9** is 8β-4'-hydroxy-5'-(4"-hydroxytigloyloxy)-tigloyloxycostunolide.

Table 1. ^{1}H NMR data of compounds **2**, **4**, **6** (200 MHz) and **8**–**10** (360 MH$_3$). (δ, J values in Hz in parenthesis, $CDCl_3$, TMS)

H	2	4	6	8	9	10
1	3.10 *ddd* (1.8; 3.0; 9.8)	5.08 *br t* (7.8)	5.09 *br t* (8.0)	5.09 *br t* (7.9)	4.91 *br dd*[a]	4.94 *br dd*[a]
2α	2.58 *m*	2.11 m[a]	2.12 m[a]	2.12 *m*	2.08 –	2.31 *m*[a]
2β		2.64 *m*[b]	2.67 m	2.60 *m*		2.51 *ddd* (4.6; 6.1; 12)
3α					2.38 *m*	4.33 *dd* (6.1; 10.2)
3β		5.58 *dd* (5.0; 11.6)	5.58 *dd* (4.9; 12.0)	4.62 *dd* (5.3; 11.6)		
4	2.35 *m*					
5		5.25[c]	5.24[b]	5.17 *d* (10.6)	4.75 *br d* (9.7)	4.82 *br d* (9.7)
6	4.52 *t* (10.0)	5.25[c]	5.24[b]	5.28[a]	5.12 *dd* (8.8; 9.7)	5.15 *dd* (8.7, 9.7)
7α	3.32 *m*	3.00 *br s*	2.99 *br s*	2.99 *br s*	2.96 *m*	2.92 *m*
8α	5.78 *m*	5.25[c]	5.24[b]	5.27 *br s*[a]	5.87 *br d* (3.3)	5.82 *br d* (2.8)
9α	2.52 *dd* (4.2; 14.4)	2.40 *dd* (3.2; 14.2)	2.40 *dd* (3.1; 14.2)	2.39 *dd* (2.5; 14.6)	2.83 *br dd* (4.2; 14.7)	2.85 *br dd* (4.5; 14.9)
9β	2.76 *dd* (2.7; 14.4)	2.68 *dd*[b] (3.2; 14.2)	2.67 *dd* (3.1; 14.2)	2.75 *dd* (3.6; 14.6)	2.39 *br dd* (2.3; 14.7)	2.33 *m*[b]
13a	6.32 *d* (3.5)	6.38 *d* (2.3)	6.38 *d* (2.3)	6.39 *d* (2.4)	6.28 *d* (3.5)	6.28 *d* (3.4)
13b	5.67 *d* (3.0)	5.78 *d* (2.0)	5.79 *d* (1.9)	5.80 *d* (1.9)	5.63 *d* (3.0)	5.60 *d* (3.0)
14	4.81; 4.99 *br s*	1.80 *br s*	1.80 *br s*	1.79 *br s*	1.48 *br s*	1.49 *br s*
15	1.28 *d*	1.89 *br s*	1.89 *br s*	1.80 *br s*	1.78 *d* (1.0)	1.81 *d* (1.0)
3′	7.02 *t* (5.9)	6.99 *t* (5.8)	7.01 *t* (5.9)	7.11 *t* (5.9)	7.13 *t* (5.9)	7.08 *t* (5.8)
4′	4.49 *br d* (6.1)	4.49 *br t* (5.8)	4.49 *br d* (5.8)	4.47; 4.35 *ABX dd* (3.4; 15.2)	4.47; 4.56 *ABX dd* (6.0; 15.8)	4.48; 4.53 *ABX dd* (6.0; 15.8)
H-5′	4.89 *d* (6.1)	4.98 *br s*	4.98 *br s*	4.82; 4.99 *d* (12.2)	4.92; 4.96[a] *d* (12.2)	4.97 *br s*[a]
3″	6.78 *qq* (1.8; 6.5)	6.85 *qq* (1.9; 6.5)	6.72 *dt* (1.6; 6.1)	6.79 *dt* (1.1; 5.9)	6.67 *dt* (1.2; 5.9)	6.66 *dt* (1.3; 5.9)
4″	1.89 *dd* (1.1; 6.5)	1.78 *dd* (1.0; 6.5)	4.74 *dd* (1.6; 6.1)	4.34 *br d* (5.8)	4.25; 4.32 *ABX br dd* (7.0; 14.8)	4.73 *d* (6.2)
5″	1.87 *br s*	1.80 *br s*	1.86 *d* (1.6)	1.80 *br s*	1.81 *br s*	1.84 *s*
OAc		2.11 s[a]	2.09; 2.11 s[a]			2.10 *s*

[a–c]Overlapping signals.

The next compound (**10**, $C_{27}H_{34}O_{10}$) had a ^{1}H NMR spectrum that was similar to the one for 3β-hydroxyliacylindrolide. However, the two compounds clearly had different side chains at C-8. Apart from the signals for the skeletal protons, the remaining resonances of **10** closely matched those of the side chain ester found in compound **6**. In accord with these observations, the CIMS displayed a molecular ion (i.e. M + 1) at *m/z* 519 and contained appropriate fragments for the loss of acetic acid, 4″-acetoxytiglic acid and the entire side chain + H at *m/z* 459, 361 and 247, respectively. Compound **10** must therefore be 3β-hydroxy-8β-4′-hydroxy-5′-(4″-acetoxy-tigloyloxy)-tigloyloxy-costunolide.

The final compound isolated (**11**, $C_{27}H_{34}O_{10}$) was an isomer of both **10** and the heliangolide **5**. Compound **11** possessed the same substitutional pattern as the latter, but with the stereochemistry of **10**. It was evident from the ^{1}H NMR spectrum that **11** was 3β-acetoxy-8β-4′hydroxy-5′-(4″-hydroxytigloyloxy)-tigloyloxycostunolide, a compound reported from *P. areolare* [4].

The chemistry of yet another element in the *P. palmeri–sinaloae–pubens* complex appears to be distinct. This collection produced a number of C_{10}-diester sesquiterpene lactones. Although *P. palmeri* yielded a single heliangolide with the same substitution pattern [1], the compound differed in both the nature of its side chain (a

simple acetate ester) and in the β-configuraton of its oxygen function at C-3. On the other hand, *P. sinaloae* contained lactones of a different skeletal type with simple tiglate esters [8]. Further, thymol esters are not major constituents in *P. pubens*, unlike *P. sinaloae* and *P. palmeri*. Thus, the chemistries of these three taxa do not intergrade as smoothly as do their morphology.

The chemistry of *P. pubens* demonstrates a connection to some elements of *Ageratina*. At least three species in the subgenus *Neogreenella*, *A. tristis* [9], *A. cronquistii* [1] and *A. tomentella* [10] produce sesquiterpene lactones with C_{10}-diester side chain. However, other compounds with similar ester groups have also been isolated from a few other elements of the tribe [1].

EXPERIMENTAL

Plant material. Leaves and heads of *Piptothrix pubens* (2 kg) were collected on 15 November 1984 in Mexico in the state of Zacatecas (near the Jalisco border) 33 km W of Monte Escobedo on the road to Mesquitic (municipio Monte Escobedo), 22–20′ N, 103–35′ W, elevation 2300 m. At the time of collection most of the plants were past flowering. A voucher specimen, Barrie and Gage no. 1114, is deposited in the University of Texas Herbarium.

Extraction and isolation of the compounds. Unground plant material (450 g) was extracted with CH_2Cl_2 for 20 min. The extract was concd to a syrup, then the concentrate was taken up in MeOH. The resulting soln was filtered and then the filtrate was diluted with H_2O until an 80% MeOH soln was obtained. This aq. soln was then partitioned against hexane (×4), concd until only H_2O remained, and then partitioned against CH_2Cl_2 (×2). The combined CH_2Cl_2 extract was dried with dry $MgSO_4$ and then concd to a golden yellow syrup (16 g). The syrup was dissolved in a minimum volume of MeOH–CH_2Cl_2 (3:1) and an aliquot equivalent to 6 g was chromatographed over Sephadex LH-20 packed in the same solvent. Sixty 10 ml fractions were collected and monitored by TLC (CH_2Cl_2–C_6H_6–EtOAc, 3:3:1 and 2:2:1) Fractions were combined accordingly. Each group was redissolved in cyclohexane–CH_2Cl_2–EtOH (7:4:1) and chromatographed over a second Sephadex LH-20 column packed in the same solvent; 4 ml fractions were collected. Compounds were purified further by prep. TLC (silica gel, 2 mm layer, CH_2Cl_2–C_6H_6–EtOAc, 2:2:1 and 1:1:1, double development).

8β,4′-Hydroxy-5′-(tigloyloxy)-tigloyloxy-4β,15-dihydro-3-dehydrozaluzanin C (**2**). Colourless gum (20 mg). IR ν_{max}^{KBr} cm^{-1}: 3400 *br*, 1750 *br*, 1720, 1705, 1240. CIMS (CH_4, 0.5 torr, direct probe) *m/z* (rel. int.): 459 $[M+1]^+$ (7), 359 $[M+1-C_5H_8O_2]^+$ (10.4), 263 (22.9), 245 ($[M+1-$side chain $+H]^+$ (22), 101 [tiglic acid $+H]^+$ (100), 83 [tiglate]$^+$ (23).

5′-Tigloyloxyeupaformosanin (**14**). Colourless gum (8 mg). IR ν_{max}^{KBr} cm^{-1}: 3400 br, 1755, 1740, 1725, 1660, 1255 br. CIMS (CH_4, 0.5 torr, direct probe) *m/z* (rel. int.): 503 $[M+1]^+$ (0.5), 485 $[M+1-H_2O]^+$ (0.7), 443 $[M+1-AcOH]^+$ (3), 289 $[M+1-$side chain $+H]^+$ (3.7), 257 (3.4), 229 $[M+1-$side chain $-AcOH+H]^+$ (50.8), 101 [tiglic acid $+H]^+$ (100), 83 [tiglate]$^+$ (22).

5′-(4″-Acetoxytigloyloxy)-eupaformosanin (**6**). Colourless gum (44 mg). IR ν_{max}^{KBr} cm^{-1}: 3440, 1760, 1740, 1705, 1655, 1270, 1240. CIMS (CH_4, 0.5 torr, direct probe) *m/z* (% rel. int): 561 $[M+1]^+$ (0.5), 501 $[M+1-AcOH]^+$ (4), 289 $[M+1-$side chain $+H]^+$ (7.1), 229 $[M+1-$side chain $-AcOH+H]^+$ (76), 99 (100). ^{13}C NMR ($CDCl_3$, TMS, 22.6 MHz): δ170.8 (*s*, 4″-acetoxy carbonyl), 170.2 (*s*, C-12), 169.4 (*s*, 3-acetoxy carbonyl), 167.2 (*s*, C-1″), 165.3 (*s*, C-1′), 148.2 (*d*, C-3′), 137.4 (*d*, C-3″), 137.4 (*s*, C-4), 135.9 (*s*, C-11), 130.3 (*s*, C-2″), 126.9 (*s*, C-2′), 125.5 (*d*, C-1), 124.8 (*t*, C-13), 124.5 (*d*, C-5), 80.0 (*d*, C-3), 74.3 (*d*, C-6), 70.8 (*d*, C-8), 61.1 (*t*, C-4″), 59.5 (*t*, C-4′), 58.6 (*t*, C-5′), 48.7 (*d*, C-7), 43.2 (*t*, C-9), 30.6 (*t*, C-2), 21.1 (*q*, 4″-acetoxy Me), 20.7 (*q*, 3-acetoxy Me), 18.6 (*q*, C-14), 18.0 (*q*, C-15), 12.8 (*q*, C-5″).

3-Desacetyl-5′-(4″-hydroxytigloyloxy)-eupaformosanin (**8**). Colourless gum (6 mg). IR ν_{max}^{KBr} cm^{-1}: 3450 br, 1758, 1705, 1650, 1245, CIMS (CH_4, 0.5 torr, direct probe) *m/z* (rel. int.) 477 $[M+1]^+$ (0.25), 459 $[M+1-H_2O]^+$ (1), 361 $[M+1-C_5H_8O_3]^+$ (0.6), 343 $[M+1-C_5H_8O_3-H_2O]^+$ (1), 247 $[M+1-$side chain $+H]^+$ (13.5), 229 $[M+1-$side chain $-H_2O+H]^+$ (29.7), 213 [side chain acylium]$^+$ (2.9), 117 [4-hydroxytiglic acid $+H]^+$ (50.5), 99 [4-hydroxytiglate]$^+$ (100).

8β-4′-Hydroxy-5′-(4″-hydroxytigloyloxy)-tigloyloxy-costunolide (**9**). Colourless gum (18 mg). IR ν_{max}^{KBr} cm^{-1}: 3450, 1760, 1710, 1655, 1270, 1250, 1230, CIMS (CH_4, 0.5 torr, direct probe) *m/z* (rel. int.): 461 $[M+1]^+$ (1.4), 345 $[M+1-C_5H_8O_3]^+$ (4.3), 231 $[M+1-C_{10}H_{14}O_6]^+$ (64), 213 [side chain acylium]$^+$ (7.9), 117 (30.3), 99 [4-hydroxytiglate]$^+$ (100).

3β-Hydroxy-8β-4′-hydroxy-5′-(4″-acetoxytigloyloxy)-tigloyl oxycostunolide (**10**). Colourless gum (14 mg). IR ν_{max}^{KBr} cm^{-1}: 3450 br, 1760, 1735, 1705, 1650, 1265, 1240. CIMS (CH_4, 0.5 torr, direct probe) *m/z* (rel. int.): 519 $[M+1]^+$ (1.3), 459 $[M+1-HOAc]^+$ (1.9), 361 $[M+1-4$-acetoxytiglic acid$]^+$ (2), 359 (3.6), 247 $[M+1-$side chain $+H]^+$ (6), 99 (100).

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REFERENCES

1. Gage, D. A. (1986) Ph.D. Dissertation. University of Texas at Austin.
2. Pfeil, R. M., Gage, D. A., Lee, E. F., Miski, M., Mabry, T. J. and Powell, A. M. (1987) *Phytochemistry* **26**, 199.
3. Lee, K. H., Kimura, T., Haruna, M., McPhail, A. T. and Onan, K. D. (1977) *Phytochemistry* **16**, 1068.
4. Gage, D. A., Miski, M. and Mabry, T. J. (1987) *Phytochemistry* (submitted).
5. Fang, N. B., Gage, D. A. and Mabry, T. J. (1987) *Phytochemistry* (in press).
6. Bohlmann, F., Zdero, C. and Turner, B. L. (1984) *Phytochemistry* **23**, 1055.
7. Bohlmann, F. and Dutta, L. N. (1979) *Phytochemistry* **18**, 847.
8. Miski, M., Gage, D. A. and Mabry, T. J. (1987) *Phytochemistry* (in press).
9. Bohlmann, F., Banerjee, S., Wolfrum, C., Jakupovic, J., King, R. M. and Robinson, H. (1985) *Phytochemistry* **24**, 1319.
10. Fang, N. B. and Mabry, T. J. (1987) *Phytochemistry* (in press).