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# SESQUITERPENE LACTONES AND OTHER CONSTITUENTS FROM *PIPTOTHRIX AREOLARE*

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**Key Word Index**—*Piptothrix areolare*; Eupatorieae; Compositae; sesquiterpene lactones; new $C_{10}$ diester side chains; germacranolides; heliangolides; thymol derivative.

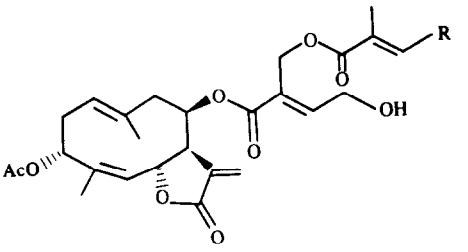
**Abstract**—The investigation of *Piptothrix areolare* afforded, in addition to 7-acetoxytinifoline and *cis*-*p*-hydroxycinnamic acid, two known and two new sesquiterpene lactones sharing unusual $C_{10}$ diester side chains. These data support morphological evidence which indicates *Piptothrix* is closely related to the genus *Ageratina*. The structures and stereochemistry were determined by spectroscopic methods.

## INTRODUCTION

As part of our continuing phytochemical study of the tribe Eupatorieae (Compositae), we describe here the isolation and identification of two new sesquiterpene lactones 3-*epi*-4″-oxo-5″-desoxyprovincialin (**1**) and 4′-[4″-oxotigloxy]-eupaformosanin (**2**) and four known compounds: 5″-desoxy-3-*epi*-4″-hydroxyprovincialin (**3**), 3-acetoxy-5′-desoxy-4′,4″-dihydroxyliacylindrolide (**4**), 7-acetoxytinifoline (**5**) [1] and *cis*-*p*-hydroxycinnamic acid (**6**), from *Piptothrix areolare* (DC) King and H. Robinson. Compound **4** has been isolated from *Piptothrix pubens* [2], and **3** and **5** were obtained from another population of *Piptothrix areolare* [3].

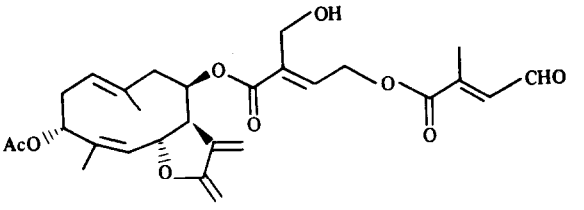
## RESULTS AND DISCUSSION

The CIMS of **1**, a new heliangolide, exhibited a strong $[M+1]^+$ ion at *m/z* 517 (27%) suggesting a molecular weight of 516 and a $C_{27}H_{32}O_{10}$ formula. Spectral data established the presence of an α-methylene-γ-lactone moiety (IR: 1744, 1662 $cm^{-1}$; $^{13}C$ NMR: δ124.2, C-13 and 170.1, C-12; $^1H$ NMR: δ6.37, 1H, *d*, *J* = 2.2 Hz, H-13a and 5.79, 1H, *d*, *J* = 2.2 Hz, H-13b). Comparison of $^1H$ NMR, $^{13}C$ NMR and mass spectral data indicated that **1** and eupaformosanin (**7**) [4] differed only in the nature of the side chain (Tables 1–3). The presence of an aldehyde function in **1** was evident from the IR ($\nu_{C=O(CHO)}$ 1704 $cm^{-1}$ and $\nu_{C-H(CHO)}$ 2840 $cm^{-1}$), $^{13}C$ NMR and $^1H$ NMR spectral data (aldehydic carbon signal at δ191.6 and aldehydic proton signal at δ10.17) [5]. The base peak in the CI-MS of **1** was at *m/z* 229 $[228+1]^+$ as expected for the fragment $C_{15}H_{16}O_2$ formed by the parent skeleton after loss of its ester substituents. This fragment (*m/z* 228) was also prominent in the EIMS. Another strong fragment (57%) representing $C_{25}H_{28}O_8$ (*m/z* 457), formed by the loss of acetic acid, appeared in the CIMS. These observations, in conjunction with $^1H$ NMR evidence (see below), indicated that the other side chain had the formula $C_{10}H_{12}O_6$. That a single $C_{10}$ side chain was present was supported by a side chain acylium ion at *m/z* 211, and a fragment at *m/z* 289, $[M+1-(C_{10}$ side chain + H)$]^+$. Moreover, the side chain fragment at *m/z* 97 ($C_5H_5O_2$) appeared both in the CIMS (40%) and EIMS (70%) in accord with a terminal oxoangelate or oxotiglate group as part of a $C_{10}$ diester. The position of the aldehyde function at C-4″ of the terminal ester was established by the appearance of the aldehydic proton signal as a doublet. Since this signal in **1** appeared downfield at δ10.17 and the side chain methyl group signal was observed at δ2.30, the stereochemistry of the C-2″/3″ double bond was assigned an *E*-configuration [5]. Therefore, the terminal ester is a




**1** R = CHO

**3** R = $CH_2OH$




**2**

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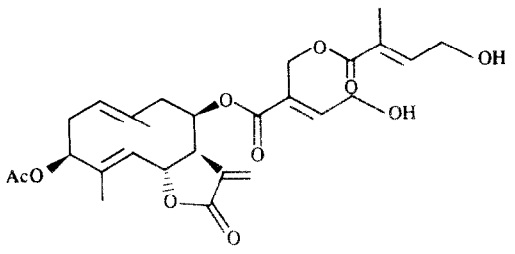

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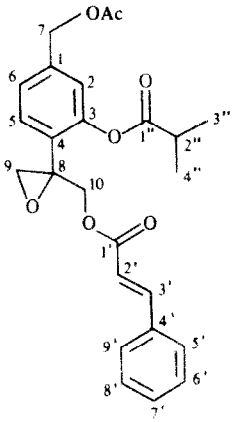

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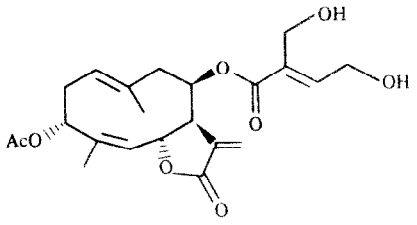

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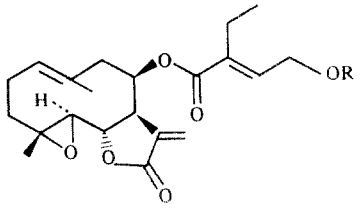

**8a** R = H

**8b** R = Ac

Table 1. $^1$H NMR spectral data for compounds **1** and **2***

| H | 1 | 2 |
|---|---|---|
| 1 | 5.09 m | 5.09 m |
| 2a | 2.70 *m* | 2.70 *m* |
| 2b | 2.30 *m* | 2.30 *m* |
| 3 | 5.58 *br dd* ($J$ = 6.3, 10.5) | 5.60 *br dd* ($J$ = 6.3, 10.5) |
| 5 | 5.20 *m* | 5.20 *m* |
| 6 | 5.25 obscured | 5.25 obscured |
| 7 | 3.00 *m* | 3.00 *m* |
| 8 | 5.23 *m* | 5.23 *m* |
| 9a | 2.76 *dd* ($J$ = 3.2, 12.8) | 2.76 *dd* ($J$ = 3.2, 12.8) |
| 9b | 2.41 *dd* ($J$ = 3.2, 12.8) | 2.41 *dd* ($J$ = 3.2, 12.8) |
| 13a | 6.37 *d* ($J$ = 2.2) | 6.37 *d* ($J$ = 2.2) |
| 13b | 5.79 *d* ($J$ = 2.2) | 5.79 *d* ($J$ = 2.2) |
| 14 | 1.79 *br s* | 1.80 *br s* |
| 15 | 1.88 *br s* | 1.88 *br s* |
| 3′ | 7.07 *t* ($J$ = 6.2) | 6.80 *t* ($J$ = 6.2) |
| 4′ | 4.50 *d* ($J$ = 6.2) | 5.04 *d* ($J$ = 6.2) |
| 5′a | 4.98 *d* ($J$ = 12) | 4.40 *br s* (H—5′a,b) |
| 5′b | 5.05 *d* ($J$ = 12) | |
| 3″ | 6.74 *dq* ($J$ = 6.8, 1.4) | 6.86 *dq* ($J$ = 6.8, 1.4) |
| 4″ | 10.17 *d* ($J$ = 6.8) | 10.19 *d* ($J$ = 6.8) |
| 5″ | 2.30 *d* ($J$ = 1.4) | 2.35 *d* ($J$ = 1.4) |
| 2‴ (OAc) | 2.11 *s* | 2.10 *s* |

*Run at 360 MHz in $CDCl_3$ with TMS as internal standard. Coupling constants in Hz.

4″-oxotiglate. The attachment of this ester to the 5′ position of a dihydroxytiglate residue was indicated by the similarity of the $^1$H NMR signals for H-3′, H-4′ (2H) and H-5′a,b to those of the other compounds containing related $C_{10}$-diester residues [2, 3, 6–8]. Decoupling experiments confirmed the structure of the ester residue in **1**. Irradiation at $\delta$10.17, the frequency of the aldehydic proton, collapsed a double quartet at $\delta$6.74 (H-3″) to a narrow quartet. Reciprocally, when the signal at $\delta$6.74 (H-3″) was irradiated both the aldehydic proton doublet at $\delta$10.17 ($J$ = 6.8 Hz) and the narrow doublet for the methyl group at $\delta$2.30 (H-5″, $J$ = 1.4 Hz) appeared as singlets. In addition, irradiation at $\delta$7.07 (H-3′) converted a doublet at $\delta$4.50 (H-4′, 2H, $J$ = 6.2 Hz) into a singlet confirming that the dihydroxytiglate group must be esterified at the 5′-position. Thus, **1** can be formulated as 3-*epi*-4″-oxo-5′-desoxyprovincialin.

The molecular formula of the second new compound **2** ([M + 1]$^+$ at $m/z$ 517 (45%)) indicated that it was an isomer of **1**. Spin decoupling experiments were undertaken to assign the signals at $\delta$6.80 and 5.04 to the H-3′ and H-4′, respectively. Irradiation at $\delta$6.80, the frequency of the H-3′ signal, collapsed a doublet at $\delta$5.04 (H-4′) to a broad singlet. In addition, irradiation at $\delta$5.04 simplified the triplet at $\delta$6.80 to a singlet. Comparison of $^1$H NMR and $^{13}$C NMR data (Tables 1 and 2) indicated that the only difference between **1** and **2** was the position of carbon ester attachment of oxotiglate to the dihydroxytiglate. The 0.54 ppm downfield shift of H-4′ (from $\delta$4.50 for **1** to 5.04 for **2**) and 0.27 ppm upfield shift of H-3′ (from 7.07 for **1** to 6.80 for **2**) indicated that the oxotiglate moiety must be at the C-4′ position of the dihydroxytiglate group as shown in structure **2**. These observations are in accord with $^1$H NMR data for H-3′ and H-4′ of eupassopilin (**8a**) and its 4′-monoacetate (**8b**) [9]. When eupassopilin (**8a**) was

Table 2. $^{13}$C NMR spectral data for compounds **1** and **2**.*

| C | 1 | 2 |
|---|---|---|
| 1 | 124.3[a] | 124.2 |
| 2 | 29.6 | 29.8 |
| 3 | 74.1 | 74.1 |
| 4 | 137.2 | 137.2 |
| 5 | 125.2 | 125.2 |
| 6 | 80.1 | 80.1 |
| 7 | 48.9 | 48.9 |
| 8 | 70.4 | 70.3 |
| 9 | 43.2 | 43.2 |
| 10 | 135.3 | 135.3 |
| 11 | 133.8 | 134.0 |
| 12 | 170.1 | 170.2 |
| 13 | 124.2[a] | 124.2 |
| 14 | 17.9[b] | 18.0[a] |
| 15 | 18.4[b] | 18.4[a] |
| 1′ | 165.0 | 164.8 |
| 2′ | 126.2 | 138.3 |
| 3′ | 158.3 | 145.0 |
| 4′ | 59.6 | 57.2 |
| 5′ | 59.3 | 61.3 |
| 1″ | 167.1 | 165.6 |
| 2″ | 136.0 | 136.0 |
| 3″ | 148.2 | 148.3 |
| 4″ | 191.6 | 191.7 |
| 5″ | 13.1 | 13.1 |
| 1‴ (OAc) | 169.2 | 169.4 |
| 2‴ (OAc) | 21.0 | 21.0 |

*Run at 90.8 MHz in $CDCl_3$ with TMS as internal standard.

[a,b]These assignments in each column may be interchanged.

acetylated at C-4′, the downfield shift of H-4′ is 0.41 ppm (from $\delta$4.30 for **8a** to 4.71 for **8b**) and the upfield shift of H-3′ is 0.22 ppm (from 6.88 for **8a** to 6.66 for **8b**) [9]. Moreover, a two-proton broad singlet at $\delta$4.40 for H-5′ supports this conjecture [4]. These spectral findings established **2** to be 4′-[4″-oxotigloxy]-eupaformosanin (**2**).

## EXPERIMENTAL

Leaves of *P. areolare* were collected at two sites in Mexico (state of Nayarit, Barrie and Gage 1236 and state of Zacatecas, Barrie and Gage 1137, voucher is on deposit in the Plant Resources Center of the University of Texas at Austin). Collections from the two locations had identical TLC patterns and were combined (200 g). The 30 min $CH_2Cl_2$ extract of plant material was evapd at low temp. (40°) until the extract was dry (6.7 g). The residue was dissolved in MeOH, and after standing for 2 hr the soln was filtered. After filtering, $H_2O$ was added to the filtrate until the soln reached approx. 80% aq. MeOH. The soln was then extracted × 3 with hexane and then the aq. soln was conc. to dryness (2 g) at low temp. (40°). The resulting concentrate was dissolved in a minimum amount of hexane–$CH_2Cl_2$–MeOH (7:4:1) and applied to a Sephadex LH-20 (Pharmacia) column packed in the same solvent. The column was then also eluted isocratically. Seventy-seven (30 ml) fractions were collected followed by a final fraction of 100 ml. All fractions were checked on silica gel plates using hexane–EtOAc (3:7). The $^1$H NMR spectrum of the residue from combined fractions 40–50 showed a mixture of aldehydic sesquiterpene lactones. When the material from fractions 40–50 were subjected to prep. TLC (silica gel 1.5 mm, EtOAc–hexane, 4:6 for two runs, 4.5:5.5 for two runs and 5:5 for two additional runs) the two new compounds **1** and **2** were obtained. The known compounds were identified by comparing the 360 MHz $^1$H NMR spectra with those of authentic material and by co-TLC.

*Compound* **1**. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3440 (OH), 2840, 1704 (aldehyde C=O), 1744 ($\gamma$-lactone), 1718, 1662 (C=CCO$_2$R). CIMS (methane, 0.5 torr, probe) *m/z* (rel. int.): 517 (27) $[M+1]^+$ $C_{27}H_{32}O_{10}$, 457 (57) $[M+1-HOAc]^+$, 403 (10) $[M+1-$oxotiglic acid$]^+$, 289 (14) $[M+1-(C_{10}$ diester side chain + H$)]^+$, 229 (100) $[M+1-HOAc-(C_{10}$ diester side chain + 1$)]^+$, 211 (27) $[C_{10}$ diester side chain acylium ion$]^+$, 97 (40) [oxotiglate acylium ion]$^+$.

*Compound* **2**. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3440 (OH), 2850, 1711 (aldehyde C=O), 1752 ($\gamma$-lactone), 1725, 1670 (C=CCO$_2$R). CIMS (methane, 0.5 torr, probe) *m/z* (rel. int.): 517 (45) $[M+1]^+$ $C_{27}H_{32}O_{10}$, 457 (98) $[M+1-HOAc]^+$, 403 (10) $[M+1-$oxotiglic acid$]^+$, 289 (11) $[M+1-(C_{10}$ diester side chain + H$)]^+$, 229 (100) $[M+1-HOAc-(C_{10}$ diester side chain + H$)]^+$, 211 (19) $[C_{10}$ diester side chain acylium ion$]^+$, 97 (70) [oxotiglate acylium ion]$^+$.

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