

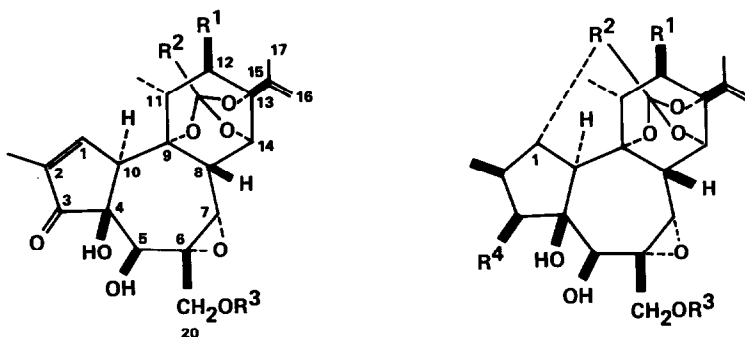
PISCICIDAL CONSTITUENTS OF PIMELEA SPECIES

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Two fish toxins, *linifolin a* and *linifolin b*, which are both diterpene orthoesters, have been isolated from *Pimelea linifolia*.

*Pimelea linifolia* (Thymelaeaceae) and *Pimelea ligustrina* grow along the East coast of Australia. *Pimelea* species,<sup>1</sup> including *P. linifolia*<sup>2</sup> and *P. ligustrina*,<sup>2</sup> have been found to possess anti-cancer activity. In the search for compounds responsible for this activity, two piscicidal compounds have been isolated. Column chromatography of a chloroform extract of *P. linifolia* on silica gel followed by Sephadex LH<sub>20</sub> yielded a mixture of two compounds which was lethal to fish.<sup>3</sup> The two compounds, linifolins a and b, were separated and purified by high pressure liquid chromatography on a reverse phase C<sub>18</sub> column.



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2

1: R<sup>2</sup> = C<sub>6</sub>H<sub>5</sub>, R<sup>3</sup> = H; 1a: R<sup>1</sup> = H; 1b: R<sup>1</sup> = OCOCH=CHCH=CHC<sub>6</sub>H<sub>5</sub>; 1c: R<sup>1</sup> = OH

2: (C-1)-R<sup>2</sup> = (C-1)-CH(CH<sub>3</sub>)(CH<sub>2</sub>)<sub>7</sub>, R<sup>3</sup> = H, R<sup>4</sup> = OCOC<sub>6</sub>H<sub>5</sub>; 2a: R<sup>1</sup> = OCOCH<sub>3</sub>; 2b: R<sup>1</sup> = H

*Linifolin a* (2a): (8 x 10<sup>-4</sup>% of dry weight of plant); cims M<sup>+</sup>+H 697.3569 (C<sub>39</sub>H<sub>53</sub>O<sub>11</sub> requires 697.3587), m/e 696, 678, 665, 647, 636, 105; ir (thin film) 3450 (OH), 1740, 1720 (CO), 1650 cm<sup>-1</sup> (C=C); λ<sub>max</sub><sup>MeOH</sup> 279, 267, 229 nm (log ε 3.25, 3.28 and 3.88); nmr (CDCl<sub>3</sub>) δ, 5 aromatic H: 8.00(m) and 7.49(m); 3-H : 5.04 (d, J=6Hz); 12-H: 4.93(s); 16-H<sub>2</sub> : 4.96(s) and 4.88 (s); 14-H : 4.60 (d, J=2Hz); 5-H: 4.10(s); 20-H<sub>2</sub>: 3.84 (d, J=6Hz); 8-H : 3.48 (d, J=2Hz); 7-H : 3.43(s); 10-H : 3.11 (d, J=12Hz); acetate CH<sub>3</sub>: 2.30(s); 17-CH<sub>3</sub> : 1.77(s) and 1.79(s); side chain CH<sub>3</sub> : 1.52 (d, J= 6Hz); side chain-H<sub>14</sub>: 1.23; 19-H<sub>3</sub> : 1.03 (d, J=6Hz); 18-H<sub>3</sub>: 0.84(d, J=6Hz); 2.81 (broad s); 2.61(m) and 2.57(m).

*Linifolin b* (2b): (8x10<sup>-4</sup>% of plant) was identical, according to our more detailed spectral results, with *Pimelea* factor P<sub>2</sub> isolated by Hecker *et al.*<sup>4</sup> from another *Pimelea* species, ms M<sup>+</sup>

638.3509(C<sub>37</sub>H<sub>50</sub>O<sub>9</sub> requires 638.3453) m/e 607, 589, 105;  $\lambda_{\text{max}}^{\text{MeOH}}$  279, 229 (log  $\epsilon$  3.16, 4.00); nmr (CDCl<sub>3</sub>)  $\delta$ , 5 aromatic H : 8.03(m) and 7.49(m); 3-H : 5.05 (d, J=4.5Hz); 16-H<sub>2</sub> : 4.94(s) and 4.85(s); 14-H : 4.26(d, J=2Hz); 5-H : 4.14(s); 20-H<sub>2</sub> : 3.85 (d, J=9Hz); 7-H : 3.33(s); 10-H : 3.08 (d, J=11Hz); 8-H : 2.87 (d, J=2Hz); 17-H<sub>3</sub> : 1.71(s); 19-H<sub>3</sub> : 1.03(d, J=6Hz); 18-H<sub>3</sub> : 0.84 (d, J=6Hz); H envelope : 1.23; side chain CH<sub>3</sub> 1.42 (d, J=7Hz) and 2.06(s), 2.35(m) and 2.55(m). The spectral results indicate that linifolin a is an acetoxy derivative of linifolin b. Analysis of the nmr spectrum of linifolin a and comparison with those of linifolin b, daphnetoxin<sup>5</sup> (1a), mezerein<sup>6</sup> (1b) and 12-hydroxydaphnetoxin<sup>7</sup> (1c) indicates that linifolin a is the 12 $\beta$ -acetoxy derivative (2a) of linifolin b. Linifolin b has also been isolated by us from *P. ligustrina* (27x10<sup>-4</sup>% of plant). Although structurally related to gnidimacrin,<sup>8</sup> which possesses potent anti-leukaemic activity, these two compounds did not show this activity.<sup>2</sup>

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#### References and Notes

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2. Anti-cancer activity was assayed by the U.S. National Cancer Institute according to the procedures of R.I. Geran, N.H. Greenberg, M.M. McDonald, A.M. Schumacher and B.J. Abbott, Cancer Chemother. Repts., (3), 3, 1 (1972) : Gnidimacrin T/C 180 at dose 12-16  $\mu\text{g kg}^{-1}$  mouse;<sup>8</sup> linifolin a T/C 107 at 6-100  $\mu\text{g kg}^{-1}$ ; linifolin b T/C 116 at 100  $\mu\text{g kg}^{-1}$ ; *P. linifolia* ethanol extract T/C 159 at 25 mg  $\text{kg}^{-1}$ ; *P. ligustrina* ethanol extract T/C 163 at 50 mg  $\text{kg}^{-1}$ . T/C  $\geq$  130 is the activity criterion.
3. Piscicidal assay (S.M. Kupchan, Y. Shizuri, W.C. Summer, H.R. Haynes, A.P. Leighton and B.R. Sickles, J. Org. Chem., 41, 3850 (1976)), using *Gambusia affinis* fish: linifolin a had a minimum lethal concentration of 60  $\mu\text{g l}^{-1}$ ; linifolin b, 6  $\mu\text{g l}^{-1}$ .
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