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## Pimelotides A and B, Diterpenoid Ketal-Lactone Orthoesters with an Unprecedented Skeleton from *Pimelea elongata*

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A detailed investigation of the minor phytochemical components of *Pimelea elongata* foliage led to the discovery of two new diterpenoid daphnane ketal-lactone orthoesters with an unprecedented skeleton, pimelotides A (**1**) and B (**2**). Their structures and relative configurations were elucidated by NMR spectroscopy.

The genus *Pimelea* (family Thymelaeaceae) comprises 108 species worldwide with about 90 of them endemic to Australia.[1] Plants of the Thymelaeaceae may be highly toxic to humans and livestock, and the toxicity is often associated with the presence of daphnane and/or tigliane diterpene esters,[2] well known to possess a wide range of significant biological activities including antineoplastic and neurotrophic effects.[3,4] Macrocyclic 1α-alkyldaphnane orthoesters seem to occur naturally only in the Thymelaeaceae family and possess an extra ring D due to the cyclization of the aliphatic orthoester chain from its penultimate (ω-1) carbon to C-1 of the diterpene skeleton.[4,5] All the 1α-alkyldaphnane orthoesters reported to date are the result of the cyclization of either a $C_{10}$ or $C_{16}$ chain and possess either *R* or *S* configuration at this ω-1 position in the macrocyclic ring.[4]

*Pimelea elongata* Threlfall[6] is one of three native *Pimelea* species (along with *P. trichostachya* and *P. simplex*) responsible for livestock poisoning in inland grazing areas of Australia (St. George disease),[7] but only simplexin, the main toxin of *P. simplex*, has previously been reported from *P. elongata*.[8] The present investigation of *P. elongata* has led to the discovery of two new, minor diterpenoid daphnane esters, with an unprecedented skeleton, pimelotides A and B (**1** and **2**, Figure 1). The structure and relative configuration of this new class of 1α-alkyldaphnane orthoesters were elucidated by spectroscopic (1D and 2D NMR experiments) and spectrometric (MS) methods.

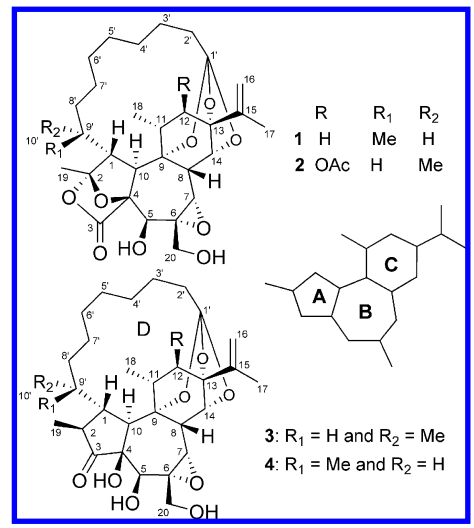


**Figure 1.** Structures of pimelotides A (**1**) and B (**2**) and known related $C_{30}$ 1α-alkyldaphnane-type orthoesters **3** and **4**.

Pimelotide A (**1**) provided an ion at *m/z* 569.2731 ($[M + Na]^+$), corresponding to a molecular formula of $C_{30}H_{42}O_9$ (calcd [M + Na], *m/z* 569.2726). Both the $^1H$ and $^{13}C$ NMR spectra presented many signals similar to those of a 1α-alkyldaphnane-type orthoester with a cyclized $C_{10}$ chain,[9] but there were some noticeable

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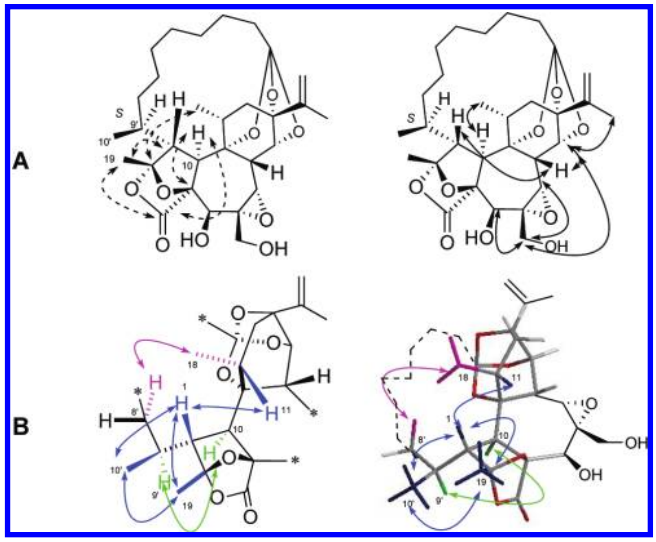


**Figure 2.** Significant 2D ROE and HMBC correlations for pimelotide A (**1**). HMBC correlations are shown as dashed arrows and ROE correlations as solid arrows in panel A. Panel B shows key ROE correlations in three dimensions that led to the assignment of the configuration at C-9′.

differences in chemical shifts for the protons and carbons of ring A, indicating structural differences in this ring. The molecular formula of $C_{30}H_{42}O_9$ for **1** was used to establish the presence of an extra oxygen atom and an additional unsaturation (or ring) in comparison to the previously reported[9] $C_{30}$ 1α-alkyldaphnane orthoesters **3** and **4** (Figure 1). In accord, the carbonyl signal at $\delta_C$ 174.1 in the $^{13}C$ NMR spectrum of **1** was consistent with a carboxylic acid or a lactone/ester group, rather than a ring A ketone as seen in orthoesters **3** and **4**.

A further unusual feature of the HMBC spectrum of **1** was a methyl singlet at $\delta_H$ 1.79 (H-19) attached to a quaternary carbon ($\delta_C$ 112.8, C-2). The chemical shift of $\delta_C$ 112.8 for C-2 established the presence of a ketal-type group at this position rather than the usual methine present in all previously reported diterpenoid daphnane esters such as **3** and **4**. Furthermore, cross-peaks in the HMBC spectrum between the $^1H$ NMR signal at $\delta_H$ 2.81 (H-10) and three quaternary carbon signals at $\delta_C$ 112.8 (C-2), 174.1 (C-3), and 86.5 (C-4) indicated the presence of a carbonyl group adjacent to C-4 of the skeleton. More precisely, it suggested a lactone linkage to C-2 and was supported by additional cross-peaks (HMBC) between H-19 ($\delta_H$ 1.79), C-2 ($\delta_C$ 112.8), and C-3 ($\delta_C$ 174.1) (Figure 2). The depicted ether linkage between C-2 and C-4 accounts for the additional unsaturation/ring required by the molecular formula and is supported by the chemical shifts of these carbons.

The presence of only two free hydroxy groups at C-5 and C-20 in **1** was verified by correlations between H-5 and H-20 and a 2H resonance at 1.56 ppm (2 × −OH) in the TOCSY spectrum, as well as by the observation of an additional coupling constant for the nonequivalent protons, H-20, with the corresponding hydroxy groups located at C-20. In addition, ROE correlations were observed (Figure 2A) that were consistent with the configuration of rings B, C, and D reported previously for other 1α-alkyldaphnane orthoesters.[4a] With these data, ketal-lactone structure **1** could be proposed for pimelotide A, without assignment of stereochemistry in the modified ring A.

Although the relative configuration at C-2 and C-4 is clearly constrained by the above results and the polycyclic framework of **1**, the absolute configuration at these centers has not been established unambiguously. The proposed assignment is based on the fact that all naturally occurring daphnane esters isolated to date are *R*-configured at C-4 and since all the 1α-alkyldaphnane orthoesters reported so far possess an *S*-configured methyl group (C-19) at C-2.[4a]

**Table 1.** $^1H$ and $^{13}C$ NMR ($CDCl_3$) Assignments ($\delta$ in ppm) of Pimelotides A (**1**) and B (**2**)

| | **1** | | **2** | |
|---|---|---|---|---|
| position | $\delta_H$ [*J* in (Hz)] | $\delta_C$ | $\delta_H$ [*J* in (Hz)] | $\delta_C$ |
| 1 | 2.50 dd 4.3, 10.2 | 55.4 | 2.35 dd 3.5, 8.0 | 53.9 |
| 2 | | 112.8 | | 112.4 |
| 3 | | 174.1 | | 172.4 |
| 4 | | 86.5 | | 85.9 |
| 5 | 4.86 brs | 68.7 | 4.87 brd 3.0 | 67.8 |
| 6 | | 59.3 | | 58.9 |
| 7 | 3.46 brs | 59.0 | 3.52 brs | 59.4 |
| 8 | 3.20 dd 1.4, 2.8 | 35.4 | 3.75 brd 2.6 | 34.1 |
| 9 | | 81.2 | | 79.8 |
| 10 | 2.81 d 4.3 | 54.5 | 3.03 d 8.0 | 48.4 |
| 11 | 1.98 m | 36.0 | 1.87 m | 45.1 |
| 12 | 1.70 d 14.0 | 36.6 | 5.00 s | 77.8 |
| | 2.08 dd 7.3, 14.0 | | | |
| 13 | | 83.3 | | 83.0 |
| 14 | 4.27 d 2.8 | 80.7 | 4.58 brd 2.6 | 78.9 |
| 15 | | 146.0 | | 142.7 |
| 16 | 4.87 brs | 111.1 | 4.91 brs | 112.7 |
| | 4.95 brs | | 4.97 brs | |
| 17 | 1.74 brs | 18.7 | 1.77 brs | 18.1 |
| 18 | 1.32 d 6.7 | 22.0 | 1.35 d 7.1 | 18.5 |
| 19 | 1.79 brs | 19.8 | 1.75 brs | 19.5 |
| 20 | 3.69 dd 4.7, 12.7 | 63.0 | 3.66 brd 12.4 | 63.4 |
| | 4.07 dd 6.7, 12.7 | | 4.10 brd 12.4 | |
| OAc | | | | 169.7 |
| Me | | | 1.98 s | 20.8 |
| 1′ | | 119.9 | | 120.0 |
| 2′ | 1.92 ddd 3.8, 7.3, 14.6 | 31.3 | 1.88 m | 33.6 |
| | 2.08 m | | | |
| 3′ | 1.56 m | 22.7 | 1.53 m | 19.9 |
| | 1.74 m | | 1.58 m | |
| 4′ | 1.27 m | 25.0 | 1.24 m | 28.8 |
| | 1.62 m | | | |
| 5′ | 1.17 m | 24.0 | 1.30 m | 23.9 |
| | 1.47 m | | 1.49 m | |
| 6′ | 1.09 m | 26.8 | 1.31 m | 24.2 |
| | 1.52 m | | | |
| 7′ | 1.22 m | 26.6 | 1.31 m | 25.1 |
| | 1.47 m | | | |
| 8′ | 0.77 m | 32.8 | 1.19 m | 25.6 |
| | 2.26 m | | 1.48 m | |
| 9′ | 1.13 m | 37.5 | 2.09 m | 29.1 |
| 10′ | 1.02 d 6.6 | 19.7 | 1.04 d 7.1 | 18.2 |

A 2D ROESY experiment allowed the determination of the configuration at C-9′ for **1** with the observation of several key ROE correlations: intense cross-peaks were observed between H-8′ and H-18, between H-1/H-19/H-10′, and finally between H-1/H-11 and H-9′/H-10 (see Figure 2). These correlations are consistent with an *S* configuration at position C-9′ of the macrocyclic ring D in **1**. Therefore the structure and relative configuration of pimelotide A were elucidated as **1**.

Pimelotide B (**2**) provided an ion at *m/z* 627.2793 ($[M + Na]^+$), corresponding to a molecular formula of $C_{32}H_{44}O_{11}$ (calcd [M + Na], *m/z* 627.2781). This increase of 58 in molecular weight relative to **1** and similar patterns in the $^1H$ and $^{13}C$ NMR spectra for the two compounds suggested that **2** is an acetoxy-bearing derivative of pimelotide A (**1**) (see Table 1 for NMR data). The presence of an acetyl group was confirmed by the observation of an extra quaternary signal for **2** at $\delta_C$ 169.7 and an extra methyl signal at $\delta_C$ 20.8 in the $^{13}C$ NMR spectrum and a methyl singlet at $\delta_H$ 1.98 in the $^1H$ NMR spectrum. In the HMBC spectrum (Figure 3A), a cross-peak between the carboxyl group at $\delta_C$ 169.7 (OAc) and the proton at $\delta_H$ 5.00 (H-12) and cross-peaks between H-12 and C-11 ($\delta_C$ 45.1), C-13 ($\delta_C$ 83.0), C-14 ($\delta_C$ 78.9), and C-15 ($\delta_C$ 142.7) established that the acetate group is positioned at C-12. Furthermore a 2D ROESY correlation between H-12 and H-18 (more intense than between H-12 and H-11) indicated a 12$\beta$ configuration for the acetoxy group (Figure 3A). Additional ROE correlations were

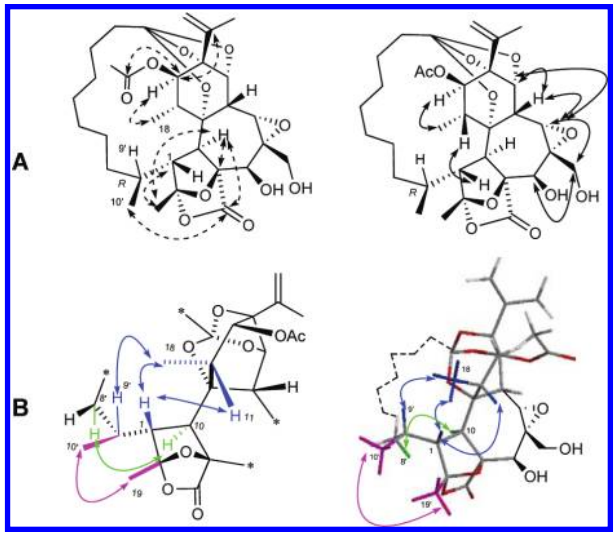


**Figure 3.** Significant 2D NOE correlations for pimelotide B (**2**). HMBC correlations are shown as dashed arrows and ROE correlations as solid arrows in panel A. Panel B shows key ROE correlations in three dimensions that led to the assignment of the configuration at C9′.

also observed (Figure 3A) that were consistent with the configuration of rings B, C, and D reported previously for other 1α-alkyldaphnane orthoesters.[4a]

The significant differences in the proton and carbon spectra between compounds **1** and **2**, apart from those arising from the presence of the acetoxy group in the ring at C-12 (ring C), were located at positions 8′ and 9′ of the macrocyclic D ring (see Table 1 for NMR data): a downfield shift was observed for C-8′ and C-9′ in **1** in comparison to **2**: C-8′ ($\delta_C$ 25.6) in **2** moved downfield by almost 7 ppm in **1** ($\delta_C$ 32.8), and similarly C-9′ gave a shift of about 8 ppm downfield in **1** ($\delta_C$ 37.5, C-9′) in comparison to the same carbon in **2** ($\delta_C$ 29.1, C-9′). Noticeable differences were also observed in the chemical shifts for protons H-8′ and H-9′, with a downfield shift for H-9′ by 1 ppm in **2** ($\delta_H$ 2.09) [in comparison to **1** ($\delta_H$ 1.13)] and a dramatic divergence in the shifts of H-8′ between the two molecules: $\delta_H$ 1.19/1.48 (**2**) to $\delta_H$ 0.77/2.26 (**1**). These shifts most likely reflect the highly constrained structure of the molecule. Similar, albeit smaller, effects are also seen at C-10/H-10, indicative of differing steric arrangements between positions 8′, 9′, and 10 in **1** and **2**. All these observations led to the conclusion that **1** and **2** are diastereomeric at position C-9′. (This hypothesis is supported by previous work on the isolation of other 1α-alkyldaphnane-type orthoesters where both diastereomers at C-9′ have been found.[3]) Although both compounds have similar ROE correlations, **2** showed intense cross-peaks between H-9′/H-18 and between H-8′ and H-10 not present in **1** (see Figure 3), in addition to the correlations between H-1/H-18, H-1/H-11, and H-10′/H-19 present in both molecules (Figures 2 and 3). Additionally, **2** lacked the ROE correlation between H-9′ and H-10. These correlations were consistent with C-9′ in **2** possessing an *R* configuration at this position in the macrocyclic ring D. Therefore the structure of pimelotide B was established as **2**.

Therefore, two minor phytochemical constituents (**1** and **2**) with an unusual carbon skeleton have been isolated from *P. elongata* foliage. These represent the first known example of daphnane ketal-lactone-type diterpenoid orthoesters from a natural source and as such have interesting biosynthetic implications as well as possibly being chemotaxonomic markers for *P. elongata*. Given the very low abundance of these compounds in *P. elongata* it is likely that confirmation of their absolute configuration and exploration of their biological properties will require development of synthetic routes to these compounds.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured at 25 °C on a JASCO P-2000 polarimeter. NMR spectra were recorded on Bruker AV500 or AV750 MHz spectrometers. $^1$H and $^{13}$C spectra were recorded with the residual protonated signal in the $CDCl_3$ solvent ($\delta_H$ 7.24) or the central peak of the $CDCl_3$ triplet ($\delta_C$ 77.00) as internal standard. *J* values are reported in Hz. HRESIMS were recorded on a Bruker MicrOTof-Q spectrometer (Dionex UltiMate 3000 micro LC system, ESI mode). ESIMS were recorded on a Bruker Esquire HCT 3D ion trap spectrometer (ESI mode). HPLC purification was carried on a LC-10AT Shimadzu HPLC system, equipped with an ELSD detector.

**Plant Material.** The bulk mature seeding *P. elongata* samples were collected in June 2007 from a site west of Bollon in Queensland (Australia). A voucher specimen (AQ751686) is deposited at the Queensland Herbarium, Brisbane, and was identified by Wayne Harris.

**Extraction and Isolation.** Extraction of the plant material (500 g) with 90% MeOH/$H_2O$ was followed by concentration and eventual partition between acetonitrile and hexane. The concentrated acetonitrile extract was further purified by flash chromatography (silica gel, hexane/ethyl acetate). The combined fractions containing the novel pimelotides were then subjected to RP-HPLC [LC-10AT Shimadzu liquid chromatograph (acetonitrile/water gradient from 20% to 100% of acetonitrile in 50 min, then 100% acetonitrile for 20 min, flow rate of 1 mL/min) using a ELSD-LT Shimadzu detector (52 °C, *P* 200 kPa) and a Phenomenex HPLC column (Luna 5$\mu$ $C_{18}$(2), 250 × 4.6 mm)] to give **1** (0.7 mg, $t_R$ 44.76 min) and **2** (1.9 mg, $t_R$ 42.33 min) as colorless oils.

**Pimelotide A (1):** colorless oil; $[\alpha]_D$ +2.1 (*c* 0.07, $CHCl_3$); $^1$H and $^{13}$C NMR data, see Table 1; ESIMS 569 $[M + Na]^+$, 585 $[M + K]^+$; HRESIMS *m/z* 569.2793 $[M + Na]^+$ (calcd for $C_{30}H_{42}O_9Na$, 569.2726).

**Pimelotide B (2):** colorless oil; $[\alpha]_D$ +1.4 (*c* 0.2, $CHCl_3$); $^1$H and $^{13}$C NMR data, see Table 1; ESIMS 627 $[M + Na]^+$, 643 $[M + K]^+$; HRESIMS *m/z* 627.2795 $[M + Na]^+$ (calcd for $C_{32}H_{44}O_{11}Na$, 627.2781).

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**Supporting Information Available:** Experimental procedures, 1D and 2D NMR spectra, and Chem 3D c3d files for compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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