Daphnane- and Tigliane-Type Diterpenoid Esters and Orthoesters from *Pimelea elongata*

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Investigation of *Pimelea elongata* ("Lakebed Pimelea") afforded 18 tigliane- and daphnane-type diterpenes (**1**−**18**). Eight of these were new compounds: four (**1**−**3**, **5**) tigliane esters and four (**7**, **8**, **10**, **11**) daphnane orthoesters. The 10 known compounds were 12-*O*-decanoylphorbol-13-acetate (**4**), *P. simplex* subtoxin B (**6**), wikstroelide E (**9**), pimelotides A and B (**12**, **13**), gnidiglaucin (**14**), simplexin (**15**), huratoxin (**16**), kirkinine D (**17**), and 12-β-acetoxyhuratoxin (**18**). The structures and relative configurations of the new compounds were determined by 1D and 2D NMR spectroscopic studies in combination with MS analyses.

Pimelea poisoning of cattle, also known as St. George Disease, is unique to Australia and is characterized by subcutaneous edema, anemia, diarrhea, and heart failure. It affects cattle across inland grazing regions of Queensland, northern New South Wales, South Australia, and occasionally the Northern Territory, with serious economic impacts through production losses, stock deaths, and costs associated with accessing alternative pastures.[1] The native pasture plants responsible for this disease are *Pimelea trichostachya*, *P. simplex*, and *P. elongata* (Thymelaeaceae). The genus *Pimelea* comprises 108 species worldwide, with about 90 of them endemic to Australia.[2]

In 1979, Freeman et al.[3] isolated a diterpenoid orthoester, simplexin (**15**), from *P. simplex*, as the major active principle responsible for the plant's toxicity. A range of daphnane- and tigliane-type diterpene esters and orthoesters have been isolated and characterized from plants of the Thymelaeaceae and Euphorbiaceae families, and many of these compounds exhibit significant biological activities, including cytotoxic, antineoplastic, and neurotrophic effects.[4−7] The presence of simplexin (**15**) has also been confirmed in *Pimelea elongata* Threlfall, "Lakebed Pimelea" (then cited as *P. trichostachya* Form B),[3] and more recently in *P. trichostachya* and both subspecies of *P. simplex* (*P. simplex* subsp. *simplex* and *P. simplex* subsp. *continua*).[8]

Our preliminary work on *P. elongata* described the isolation and structure determination of two members of a new class of diterpenoid daphnane ketal-lactone orthoesters, pimelotides A (**12**) and B (**13**).[9] In the present work semipreparative RP-HPLC of the crude extract of *P. elongata* foliage and root furnished eight new diterpenoid esters/orthoesters, along with 10 known diterpenes (Figure 1). We report here the structures and stereochemistry of four new tigliane esters (**1**−**3**, **5**) and four new daphnane orthoesters (**7**, **8**, **10**, **11**), elucidated by 1D and 2D NMR spectroscopy in combination with mass spectrometry. Compounds **4**, **6**, and **14** were previously isolated from croton oil [12-*O*-decanoylphorbol-13-acetate (**4**)],[10] *P. simplex* [subtoxin B, (**6**)][3] and *Gnidia* sp. [gnidiglaucin (**14**)].[11] However, due to the lack of literature NMR data for these three compounds, full characterization has been carried out and is included in this work.

The remaining seven known compounds were all daphnane orthoesters. Compounds **12** and **13** corresponded to pimelotides A and B.[9] Compounds **9** and **15**−**18** were daphnane orthoesters and were identified by comparison of their NMR data with those reported in the literature. Their structures were identified as wikstroelide E (**9**) (also known as pimelea factor S_7),[12,13] simplexin (**15**) (also known as daphnopsis factor R_3; pimelea factor P_1; wikstrotoxin D),[3,14−18] huratoxin (**16**),[3,16,19,20] kirkinine D (**17**) (also known as peddia factor V_2; yuanhuagine),[21−23] and 12β-acetoxyhuratoxin (**18**) (also known as *P. simplex* subtoxin A, wikstroelide A).[3,6,20,23,24]

Of the 18 compounds isolated, six were diterpene esters (**1**−**6**) and 12 contained the more unusual orthoester linkage (**7**−**18**). The six diterpene esters all possessed a tigliane skeleton, with four (**1**−**4**) being phorbol esters and two (**5** and **6**) possessing a different oxygenation pattern (and generically referred to here as tigliane esters).

Compound **1** was isolated as a viscous oil and exhibited an ion (ESI-HRMS) at 579.2938 ($[M + Na]^+$) corresponding to the molecular formula $C_{32}H_{44}O_8$. The NMR spectra indicated the presence of six vinylic protons, at δ_H 7.57, 5.65, 5.39, 5.37, 5.33, and 5.27, with corresponding carbons at δ_C 160.5, 129.0, 129.7, 132.4, 127.3, and 126.7, respectively (see Table 1). The proton at δ_H 7.57 (H-1) presented HMBC correlations to the methyl signal at δ_C 10.0 (C-19) and to the ketone at δ_C 207.3 (C-3), whereas H-7 exhibited HMBC correlations to the hydroxymethyl at δ_C 67.9 (C-20) and a CH_2 (δ_C 38.8, C-5). Another cross-peak was observed between H-5 and C-3. The presence of methyl singlets at δ_H 1.21 and 1.19 (H-16 and H-17) and a doublet at δ_H 1.06 ($J = 5.0$ Hz, H-14) characteristic of a *gem*-dimethylcyclopropane moiety confirmed that **1** had a phorbol-type skeleton (I, Figure 1). The ^{13}C NMR spectrum revealed the presence of two further carbonyl groups at δ_C 172.5 and 173.7. HMBC correlations between the carbonyl at δ_C 173.7 (C-21) and the methyl singlet at δ_H 2.08 (H-22), as well as other cross-peaks between C-13 and H-22 and H-14, were explained by an acetate positioned at C-13. Other correlations, δ_C 172.5 (C-1′) to δ_H 5.39 (H-12) and 2.37 (H-2′), were due to a diunsaturated C_{10} ester chain attached to C-12 of the phorbol skeleton. COSY correlations between H-3′ (δ_H 2.43) and H-4′ (δ_H 5.33), H-5′ (δ_H 5.39), and H-6′ (δ_H 2.78) and between H-6′ and H-7′ (δ_H 5.27) confirmed the presence of double bonds at C-4′ in the ester chain. The carbon chemical shifts for all 10 carbons of the ester side chain of **1** were essentially identical to those reported for ethyl *Z,Z*-4,7-decadienoate,[25] indicating that both double bonds of the side chain were *cis*. Therefore the structure of **1** was established as 12-*O*-(4*Z*,7*Z*)-deca-4,7-dienoylphorbol-13-acetate.

Compound **2** had the molecular formula $C_{32}H_{46}O_8$ (HRESIMS), indicating two additional protons in comparison to compound **1**. The ^1H and ^{13}C NMR data for **2** (Table 1) were nearly identical to those of **1**, except for the ester side chains, suggesting differences only in the number and/or location of the side-chain unsaturations. The ^1H and ^{13}C NMR spectra confirmed the presence of only one

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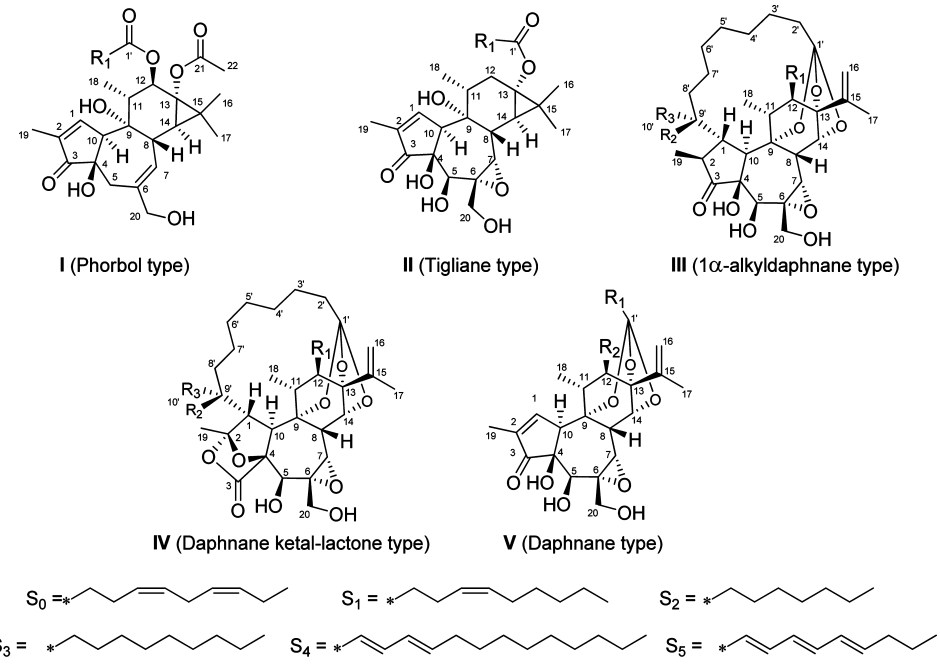

Cpd	Type	R_1	R_2	R_3	Cpd	Type	R_1	R_2	R_3
1	I	S_0			**10**	IV	H	H	Me
2	I	S_1			**11**	IV	OAc	Me	H
3	I	S_2			**12**	IV	H	Me	H
4	I	S_3			**13**	IV	OAc	H	Me
5	II	S_3			**14**	V	S_3	OAc	
6	II	S_4			**15**	V	S_3	H	
7	III	OAc	H	Me	**16**	V	S_4	H	
8	III	OAc	Me	H	**17**	V	S_5	OAc	
9	III	H	Me	H	**18**	V	S_4	OAc	

Figure 1. Structures of diterpenoid esters and orthoesters **1**−**18** from *P. elongata*.

double bond in the ester chain of **2**, located at C-4′ on the basis of COSY correlations between H-2′ (δ_H 2.35)/H-3′ (δ_H 2.01) and H-3′ (δ_H 2.01)/H-4′ (δ_H 5.40). Comparison of the chemical shifts of C-3′/C-6′ and H-4′/H-5′ in **2** with literature chemical shifts for (4*Z*)- and (4*E*)-decenoic acids[26] established the *cis*-configuration of the double bond in **2**. The shifts of C-3′ (δ_C 26.9) and C-6′ (δ_C 22.7) in **2**, for example, closely matched the corresponding carbons in (4*Z*)-decenoic acid (δ_C 27.2 and 22.9)[26] but differed significantly from those in (4*E*)-decenoic acid (δ_C 32.4 and 28.0).[26] Therefore, the structure of compound **2** was elucidated as 12-*O*-(4*Z*)-deca-4-enoylphorbol-13-acetate.

HRESIMS indicated that compound **3** had the molecular formula $C_{30}H_{44}O_8$. ^{1}H and ^{13}C NMR spectra of **3** contained resonances virtually identical to the phorbol skeleton shifts of **1** and **2**. Proton signals associated with the side chain were all located in the upfield region of the spectrum, indicating that **3** had a saturated side chain. This was consistent with the molecular formula and the nine degrees of unsaturation present in the phorbol core, C-13 acetate, and a saturated C_8 ester chain. Therefore **3** was identified as 12-*O*-octanoylphorbol-13-acetate. Although this compound has been synthesized (but not fully characterized),[27] it had not been reported from a natural source. Full NMR data are reported here for the first time (Table 1).

Compound **4** was an oil, and the positive ion HRESIMS provided an ion at 583.3241 ([M + Na]$^+$), corresponding to molecular formula $C_{32}H_{48}O_8$ ([M + Na]$^+$ calcd as 583.3247). The NMR spectra of **4** were almost identical to those of **3**, with the exception of two extra carbon signals in the upfield region of the ^{13}C NMR spectrum. This was also consistent with the molecular formula obtained by HRESIMS. The structure of **4** was therefore identified as 12-*O*-decanoylphorbol-13-acetate, previously isolated from croton oil.[11] Full characterization has been carried out in this work for the first time, and NMR data are presented in Table 1.

Compound **5** was isolated as a white, amorphous solid, and HRESIMS provided a molecular formula of $C_{30}H_{46}O_8$. Methyl singlets at δ_H 1.05 (H-17) and 1.17 (H-16) corresponding to a *gem*-dimethylcyclopropane group and a proton doublet at δ_H 1.11 (H-14) suggested that **5** was a tigliane-type ester. Literature NMR data for the tigliane core of 6*R*,7*R*-epoxy-5β-hydroxy-12-deoxyphorbol-13-tetradecanoate[28] closely matched that observed for **5** (Table 2). Compound **5** was thus deduced to be a homologous ester with a saturated 10-carbon ester chain positioned at C-13. Therefore, **5** was identified as 6α,7α-epoxy-5β-hydroxy-12-deoxyphorbol-13-decanoate.

Compound **6** had the molecular formula $C_{34}H_{50}O_8$, and the ^{1}H and ^{13}C NMR spectra presented many similarities to those of **5** (Table 2). The almost identical chemical shifts suggested the same tigliane skeleton but a different ester chain at C-13. The ^{1}H NMR spectrum revealed that the aliphatic chain contained four vinylic protons at δ_H 5.74 (1H), 6.16 (2H), and 7.26 (1H). The carbonyl at δ_C 169.8 (C-1′) of **6** was shifted upfield by 3.5 ppm in comparison to **5**. HMBC correlations between C-1′ and the protons at δ_H 5.74

Table 1. ^{1}H and ^{13}C Data (δ in ppm) for the Phorbol-Type Compounds **1**−**4**

	1		**2**		**3**		**4**	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	7.57 brs	160.5	7.57 brs	160.9	7.57 brs	160.5	7.57 brs	160.5
2		132.6		132.6		132.9		132.9
3		207.3		207.3		208.8		208.8
4		73.6		73.7		73.7		73.7
5	2.46 d (18.5)	38.8	2.46 d (19.0)	38.5	2.46 d (18.0)	38.8	2.46 d (18.0)	38.8
6	2.51 d (18.5)	140.3	2.51 d (19.0)	140.4	2.51 d (18.0)	140.0	2.51 d (18.0)	140.0
7	5.65 d (5.6)	129.0	5.65 d (5.2)	129.4	5.65 d (5.0)	129.3	5.65 d (5.0)	129.3
8	3.21 dd (5.0, 5.6)	39.0	3.21 dd (5.2, 5.3)	39.1	3.20 dd (5.0, 5.3)	39.2	3.20 dd (5.0, 5.3)	39.2
9		78.0		78.4		78.1		78.1
10	3.23 brs	56.0	3.21 brs	56.1	3.23 brt (2.8)	56.3	3.23 brt (2.8)	56.3
11	2.12 dq (7.0, 10.2)	42.7	2.11 m	42.8	2.11 m	43.0	2.11 m	43.0
12	5.39 d (10.2)	76.9	5.40 d (10.3)	76.6	5.40 d (10.0)	76.8	5.40 d (10.0)	76.8
13		65.3		65.6		65.6		65.6
14	1.06 d (5.0)	36.3	1.06 d (5.3)	36.1	1.06 d (5.3)	36.2	1.06 d (5.3)	36.2
15		25.5		25.7		25.4		25.4
16	1.21 s	16.5	1.21 s	16.5	1.21 s	16.7	1.21 s	16.7
17	1.19 s	23.8	1.19 s	23.5	1.19 s	23.5	1.19 s	23.5
18	0.87 d (7.0)	14.4	0.87 d (6.4)	14.1	0.85 d (7.1)	14.2	0.85 d (7.1)	14.2
19	1.76 dd (1.3, 2.9)	10.0	1.76 brs	9.9	1.76 dd (1.1, 2.8)	10.1	1.76 dd (1.1, 2.8)	10.1
20	3.97 d (12.7)	67.9	3.97 d (13.2)	68.1	3.94 d (12.6)	67.9	3.94 d (12.6)	67.9
	4.02 d (12.7)		4.02 d (13.2)		4.02 d (12.6)		4.02 d (12.6)	
21		173.7		173.8		173.6		173.6
22	2.08 s	21.0	2.08 s	21.1	2.07 s	21.1	2.07 s	21.1
1′		172.5		173.1		173.5		173.5
2′	2.37 m	34.5	2.35 m	34.5	2.55 m	34.5	2.55 m	34.5
3′	2.43 m	23.0	2.01 m	26.9	1.61 m	25.0	1.61 m	25.0
4′	5.33 m	127.3	5.40 m	131.7	1.29 m	29.1	1.29 m	29.1
5′	5.39 m	129.7	5.30 m	127.0	1.29 m	29.1	1.29 m	29.1
6′	2.78 t (7.3)	25.5	2.37 m	22.7	1.25 m	31.6	1.29 m	29.1
7′	5.27 m	126.7	1.31 m	29.0	1.29 m	22.4	1.29 m	29.1
8′	5.37 m	132.4	1.26 m	31.3	0.87 t (7.2)	14.1	1.25 m	31.6
9′	2.04 dq (7.5, 7.6)	20.5	1.28 m	22.5			1.29 m	22.4
10′	0.94 t (7.6)	14.2	0.86 t (7.6)	14.2			0.87 t (7.2)	14.1

(H-2′) and 7.26 (H-3′) and COSY cross-peaks between all of the ethylenic protons indicated that the ester carbonyl and the two double bonds were conjugated. Chemical shifts of H-2′−H-5′ and C-2′−C-5′ in **6** closely matched literature chemical shifts for both methyl *E*,*E*-2,4-tetradecadienoate[29] and *E*,*E*-2,4-tetradecadienoic acid,[30] establishing that both double bonds were *trans* in **6**. Therefore the structure of **6** was established as 6α,7α-epoxy-4*β*,5*β*,9α,20-tetrahydroxy-13α-(2*E*,4*E*)-tetradeca-2,4-dienoyl-1-tiglien-3-one, identical to subtoxin B previously isolated from *P. simplex* (only partial ^{1}H NMR assignments were reported previously for **6**).[3]

The remaining compounds (**7**−**18**) were all daphnane-type orthoesters, and these constituted approximately 80% of all diterpenoids isolated from the foliage and 90% of those from roots. The roots displayed a much simpler phytochemical profile, with only **5**, **7**, **8**, and **15** being identified, while simplexin (**15**) and all of the other diterpenoids were present in the foliage. The predominant orthoester was simplexin (**15**) in both foliage and roots (approximately 50% by weight of all esters isolated from foliage; 95% by weight from roots). Given this, it is not surprising that the majority of the minor, more complex orthoesters identified appeared to derive from simplexin (**15**). Of the simple orthoesters (**14**−**18**), which differed in the identity of the orthoester side chain and/or the presence or absence of C-12 oxygenation, only **14** had not been previously characterized. Compound **14** was isolated as a colorless oil that exhibited a molecular ion (positive ion HRESIMS) at 613.2983 ($[M + Na]^+$) corresponding to the molecular formula $C_{32}H_{46}O_{10}$. This compound presented spectral characteristics very similar to simplexin (**15**),[14,15] and an increase of 58 in molecular weight in comparison to the latter suggested that **14** was an acetoxy analogue of **15**. The acetoxy group was confirmed by the presence of an extra quaternary signal at δ_C 169.4 and an extra methyl signal at δ_C 20.7 in the ^{13}C spectrum and a methyl singlet at δ_H 1.96 in the ^{1}H NMR spectrum. HMBC correlations between H-12 and C-11, δ_C 169.4 ($CH_3\underline{C}O$), and 142.8 (C-15) and ROESY cross-peaks between δ_H 4.92 (H-12)/δ_H 1.24 (H-18)/δ_H 1.50 (H-3′) and δ_H 1.96 ($C\underline{H}_3CO$)/δ_H 3.44 (H-8) indicated that **14** was acetylated at C-12 and that this acetoxy group was *β*-oriented. Therefore, compound **14** was 12*β*-acetoxysimplexin (see Table 3 for NMR data). The ^{1}H NMR data for this compound (also known as gnidiglaucin) agree with the partial data previously reported (no ^{13}C NMR data had been reported for **14**).[11]

Three 1-α-alkyldaphnanes structurally related to simplexin (**15**) were isolated with one, **9**, proving spectroscopically identical to the C-9′*S*-configured pimelea factor S_6, previously found in *P. simplex*[34] and also known as wikstroelide E (from *Wikstroemia retusa*).[13] Two stereoisomers (**7** and **8**) were isolated as an inseparable oily mixture in a 40/60 ratio (**7**/**8**). They both provided an ion at 613.2983 ($[M + Na]^+$) corresponding to the molecular formula $C_{32}H_{46}O_{10}$. The absence of the usual H-1 alkenyl proton at about δ_H 7.50, the presence of two sets of three methyl doublets for **7** and **8** (δ_H 0.94, 1.13, and 1.37 for **7** and δ_H 0.85, 1.06, and 1.45 for **8**), and a downfield shift of about 10 ppm for the ketone C-3 (δ_C 218.4 for **7** and δ_C 217.7 for **8**) indicated that these compounds were of the 1α-alkyldaphnane type (see Table 4). Only three carbon chemical shifts were significantly different between **7** and **8**. Carbons C-8′, C-9′, and C-10′ of the aliphatic chain of compound **7** (in comparison to **8**) exhibited an upfield shift of 9.8 ppm for C-8′ (δ_C 38.0 for **8** and δ_C 28.2 for **7**) and downfield shifts of 6.3 ppm for C-10′ (δ_C 12.3 for **8** and δ_C 19.1 for **7**) and of 3.4 ppm for C-9′ (δ_C 25.3 for **8** and δ_C 28.7 for **7**). Thus, these two compounds were epimeric at C-9′, consistent with literature reports of analogous epimeric pairs of 1α-alkyldaphnanes.[31]

The configuration of **7** and **8** at C-9′ was assigned on the basis of analysis of the 2D ROESY spectrum. Compound **7** showed intense cross-peaks between H-10′ (δ_H 0.94) and H-19 (δ_H 1.13) and between H-9′ (δ_H 2.37) and H-1 (δ_H 2.07) and H-18 (δ_H 1.37), whereas **8** exhibited a very strong NOE effect between H-10′ (δ_H

Table 2. ^{1}H and ^{13}C Data (δ in ppm) for the Tigliane-Type Compounds **5** and **6**

	5		**6**	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	7.68 brq (1.4)	164.1	7.69 brq (1.7)	164.5
2		134.1		133.9
3		210.1		209.0
4		72.4		72.3
5	4.23 s	71.7	4.24 brs	71.9
6		61.8		61.5
7	3.25 brs	65.7	3.25 brs	65.8
8	2.82 d (7.5)	36.2	2.82 d (7.5)	36.5
9		75.3		75.1
10	3.93 brs	49.6	3.93 brs	49.2
11	1.80 m	38.3	1.83 m	38.4
12	1.54 m	31.8	1.59 dd (9.4, 15.3)	32.1
13	2.02 dd (7.3, 15.0)	63.9	2.05 dd (7.4, 15.3)	63.3
14	1.11 d (7.4)	31.8	1.15 d (7.5)	32.0
15		23.9		23.6
16	1.17 s	22.8	1.19 s	22.9
17	1.05 s	15.7	1.06 s	15.8
18	0.90 d (6.6)	19.0	0.89 d (6.7)	19.1
19	1.75 dd (1.4, 2.9)	9.8	1.75 dd (1.4, 2.7)	9.9
20	3.88 brs	64.5	3.80 brs	64.9
1′		173.3		169.8
2′	2.28 t (7.4)	34.3	5.74 d (15.0)	118.2
3′	1.63 m	24.6	7.26 ddd (4.9, 10.2, 15.0)	146.7
4′	1.28 m	28.9	6.16 m	128.0
5′	1.25 m	29.0	6.16 m	146.1
6′	1.25 m	29.2	2.15 m	33.2
7′	1.24 m	29.4	1.41 m	28.5
8′	1.26 m	31.6	1.26 m	29.3
9′	1.27 m	22.6	1.26 m	29.3
10′	0.86 t (7.0)	14.1	1.26 m	29.3
11′			1.26 m	31.8
12′			1.26 m	29.1
13′			1.28 m	22.8
14′			0.86 t (6.8)	14.2

Table 3. ^{1}H and ^{13}C Data (δ in ppm) for the Daphnane-Type Orthoester **14**

position	δ_H (J in Hz)	δ_C	position	δ_H (J in Hz)	δ_C
1	7.53 brs	160.2	CH_3CO		169.4
2		136.7	CH_3CO	1.96 s	20.7
3		209.3	1′		119.7
4		72.2	2′	1.90 m	34.7
5	4.23 s	71.7	3′	1.50 m	23.2
6		60.4	4′	1.24 m	31.9
7	3.51 s	64.5	5′	1.27 m	29.3
8	3.44 s	35.6	6′	1.27 m	29.5
9		80.0	7′	1.27 m	29.5
10	3.73 t (2.8)	47.0	8′	1.27 m	29.6
11	2.31 brq (7.0)	43.6	9′	1.27 m	22.7
12	4.92 brs	78.0	10′	0.85 t (7.5)	13.8
13		83.4			
14	4.68 d (2.6)	79.9			
15		142.8			
16	4.90 brs	112.6			
	4.97 brs				
17	1.78 brs	18.2			
18	1.24 d (7.4)	17.8			
19	1.77 dd (1.3, 2.9)	9.4			
20	3.78 dd (5.5, 13.0)	65.0			
	3.92 dd (5.7, 13.0)				

0.85) and H-10 (δ_H 3.30) and H-2 (δ_H 2.32), suggesting that **7** was *R* and **8** *S* configured at C-9′ of the macrocyclic ring (see Supporting Information).[32] This was corroborated by the upfield shift observed for H-10′ for **8** (δ_H 0.85) in comparison to H-10′ for **7** (δ_H 0.94), in agreement with an *S*-configured C-9′ in **8** based on previous literature reports.[31,33] The ^{1}H and ^{13}C spectra revealed one acetate group in both **7** and **8**. Strong ROESY correlations between the protons at δ_H 4.98 (H-12) and 1.37 (H-18) for **7** and δ_H 4.94 (H-12) and 1.45 (H-18) for **8** determined the 12β orientation of this acetate group in both compounds.

Therefore, the structures of **7** and **8** were established as C-12 acetoxy analogues of the 1α-alkyldaphnanes C-9′*S* pimelea factor S_6 and C-9′*R* pimelea factor S_7 previously seen in *P. simplex*.[34] Literature NMR data for these non-acetoxy-bearing compounds are in good agreement with those reported here for acetates **7** and **8**.

Four pimelotides (**10**−**12**), ketal lactones structurally derived from 1α-alkyldaphnanes, were also isolated. Pimelotide A (**12**) and pimelotide B (**13**) were reported earlier from this plant.[9] The other two (**10** and **11**) were isolated as an inseparable oily mixture in a 1:2 ratio (**10**:**11**). Compound **10** provided an ion at 569.2739 ([M + Na]$^+$) corresponding to the molecular formula $C_{30}H_{42}O_9$. In addition, **10** had a molecular formula identical to that of pimelotide A (**12**) and chemical shifts similar to both **12** and pimelotide B (**13**) (an acetoxy derivative of the C9′-epimer of **12**),[9] indicating that **10** had the same daphnane ketal-lactone skeleton as **12** and **13**. The proton NMR spectrum of **10** exhibited signals characteristic of a daphnane ketal-lactone skeleton: two methyl doublets at δ_H 1.08 (H-10′) and 1.26 (H-18), two methyl singlets at δ_H 1.74 (H-17) and 1.75 (H-19), as well as HMBC correlations between H-19 (δ_H 1.75) and a quaternary carbon (δ_C 112.8, C-2), establishing the presence of a ketal-type group at this position. Further cross-peaks in the HMBC spectrum between the ^{1}H NMR signal at δ_H 3.03 (H-10) and three quaternary carbon signals at δ_C 112.8 (C-2), 172.5 (C-3), and 86.5 (C-4) indicated the presence of a carbonyl group adjacent to C-4. Comparison of NMR data for the macrocyclic D ring of **10** with resonances of **12** and **13**[9] indicated significant shift differences at positions 8′ and 9′ between **10** and **12**, whereas virtually identical shifts were observed between **10** and **13**, leading us to the conclusion that **10** was the C-9′ diastereoisomer of **12**, with the same C-9′ configuration as in acetate **13**.

This was confirmed by the 2D ROESY spectrum. **10** exhibited ROE correlations similar to those seen for **12**: intense cross -peaks between H-9′ (δ_H 2.15)/H-18 (δ_H 1.26) and between H-8′ (δ_H 1.21/1.50) and H-10 (δ_H 3.03). Additional correlations were present between H-1 (δ_H 2.45)/H-18 (δ_H 1.26), H-1(δ_H 2.45)/H-11 (δ_H 2.02), and H-10′ (δ_H 1.08)/H-19 (δ_H 1.75), but no ROE correlations were observed between H-9′ (δ_H 2.15) and H-10 (δ_H 3.03) (see Supporting Information). These correlations were consistent with C-9′ in **10** possessing the *R* configuration in the macrocyclic ring D.[32] Therefore, the structure of pimelotide C was established as **10**.

Compound **11** ($C_{32}H_{44}O_{11}$) had an increase of 58 in molecular weight relative to **10**, and similar patterns in the NMR spectra for the two compounds suggested that **11** had a skeleton similar to **10** but with an extra acetoxy group (Table 4). Virtually identical NMR data were observed for **11** and pimelotide A (**12**) for the macrocyclic D ring, and differences at positions 8′ and 9′ of the same ring between **11** and **13** suggested that **11** was a diastereoisomer of **13** and had the same C-9′ configuration as the desacetoxy derivative **12**. A 2D ROESY experiment confirmed the configuration at C-9′ for **11** with the key ROE cross-peaks observed between H-8′ (δ_H 2.21)/H-18 (δ_H 1.77), H-1 (δ_H 2.40)/H-19 (δ_H 1.77), H-19 (δ_H 1.77)/H-10′ (δ_H 1.02), and H-1 (δ_H 2.40)/H-11 (δ_H 1.84), and finally a correlation between H-9′ (δ_H 1.12)/H-10 (δ_H 2.81) not present in **10**. These correlations were consistent with an *S* configuration at C-9′ of the macrocyclic ring D in **11**. An acetoxy group was also confirmed by an extra quaternary signal for **11** at δ_C 169.8, an extra methyl signal at δ_C 21.2, and a methyl singlet at δ_H 1.97. In the HMBC spectrum, a cross-peak between the carboxyl group at δ_C 169.8 (OAc) and the proton at δ_H 5.00 (H-12) and cross peaks between H-12 and C-11 (δ_C 44.7), C-13 (δ_C 82.9), C-14 (δ_C 79.3), and C-15 (δ_C 143.7) established that the acetate group was at C-12. A 2D ROESY correlation between H-12 and H-18 (more intense than between H-12 and H-11) indicated the 12β orientation of the

Table 4. ^{1}H and ^{13}C Data (δ in ppm) for 1α-Alkyldaphnane Orthoesters **7** and **8** and Pimelotides C (**10**) and D (**11**)

	7		**8**		**10**		**11**	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	2.07 m	49.6	2.08 m	48.9	2.45 dd (3.3, 7.6)	54.3	2.40 dd (4.4, 10.2)	55.6
2	2.27 dd (5.8, 11.6)	43.9	2.32 dd (6.5, 10.9)	42.5		112.8		111.9
3		218.4		217.7		172.5		174.4
4		75.4		75.6		86.3		86.6
5	4.01 brs	71.4	4.03 brs	71.5	4.89 brs	68.1	4.84 brs	68.7
6		60.5		60.9		59.0		59.3
7	3.46 brs	64.4	3.48 brs	64.4	3.43 brs	59.8	3.55 brs	59.4
8	3.50 d (2.8)	35.8	3.42 d (2.5)	35.3	3.10 brd (2.7)	35.9	3.86 dd (1.3, 3.0)	34.3
9		79.8		79.4		81.2		80.3
10	3.11 d (12.0)	43.6	3.30 d (12.0)	42.8	3.03 d (7.6)	49.0	2.81 d (4.5)	54.6
11	2.40 q (7.2)	43.9	2.52 q (7.0)	43.4	2.02 m	37.0	1.84 q (6.6)	44.7
12	4.98 brs	78.6	4.94 brs	78.3	1.72 d (13.5)	36.9	5.00 s	77.9
					2.14 dd (7.3, 13.5)			
13		79.8		79.4		83.8		82.9
14	4.56 d (2.8)	80.3	4.63 d (2.5)	80.9	4.25 d (2.7)	81.1	4.60 d (3.0)	79.3
15		143.2		143.1		146.2		143.7
16	4.90 brs	112.8	4.91 brs	112.8	4.87 brs	112.2	4.91 brs	113.1
	4.96 brs		4.96 brs		4.97 brs		4.96 brs	
17	1.77 brs	18.5	1.78 brs	18.5	1.74 brs	18.8	1.77 brs	18.5
18	1.37 d (7.0)	18.9	1.45 d (7.0)	18.9	1.26 d (6.4)	21.4	1.40 d (6.6)	19.8
19	1.13 d (6.5)	13.9	1.06 d (6.5)	13.3	1.75 brs	19.8	1.77 brs	19.5
20	3.83 d (13.1)	65.3	3.83 d (13.1)	65.3	3.62 d (12.6)	63.9	3.72 d (12.4)	63.1
	3.92 d (13.1)		3.90 d (13.1)		4.07 d (12.6)		4.10 d (12.4)	
CH_3CO		169.7		169.7				169.8
CH_3CO	1.97 s	21.2	1.98 s	21.2			1.97 s	21.2
1′		120.3		120.2		119.5		120.7
2′	1.85 m	33.6	1.78 d (14.1)	33.6	1.86 m	33.7	1.87 m	31.6
	1.90 m		1.98 dd (11.8, 14.1)		1.92 m		2.05 m	
3′	1.54 m	19.4	1.59 m	20.1	1.54 m	20.3	1.53 m	22.3
	1.67 m		1.67 m		1.92 m		1.75 m	
4′	1.24 m	27.6	1.25 m	28.0	1.28 m	28.8	1.15 m	24.2
	1.30 m		1.36 m				1.44 m	
5′	1.31 m	28.8[a]	1.30 m	28.4[a]	1.33 m	24.2	1.24 m	26.7
			1.35 m		1.53 m		1.47 m	
6′	1.24 m	29.3[a]	1.29 m	29.1[a]	1.21 m	24.4	1.25 m	25.0
					1.33 m		1.61 m	
7′	1.32	29.5[a]	1.23 m	29.6[a]	1.31 m	25.4	1.10 m	26.9
							1.47 m	
8′	1.25 m	28.2	1.29 m	38.0	1.21 m	25.9	0.75 m	32.9
	1.36 m		1.38 m		1.50 m		2.21 m	
9′	2.37 m	28.7	2.68 m	25.3	2.15 m	29.4	1.12 m	37.6
10′	0.94 d (6.5)	19.1	0.85 d (6.4)	12.3	1.08 d (6.5)	18.6	1.02 d (6.5)	20.0

[a] Signals can be interchanged.

acetoxy group. Therefore, the structure of pimelotide D was elucidated as **11**.

In this study 18 diterpene esters and orthoesters, including eight new compounds containing daphnane- or tigliane-type skeletons, have been isolated and characterized from *P. elongata*, representing the first detailed phytochemical profile of this plant. Compounds **10**−**13** (pimelotides A−D) are the first known examples of daphnane ketal-lactone-type diterpenoid orthoesters and therefore could be used as chemotaxonomic markers for *P. elongata*.

With such a detailed phytochemical profile of *P. elongata* we proposed a biosynthetic pathway to the various compounds (see Supporting Information). For the daphnane-derived diterpenes, a parent diterpenoid precursor would likely undergo condensation with a fatty acid to produce the orthoesters **15**, **16**, and the previously reported peddia factor V_1 (**19**) (Figure 2).[21] Hydroxylation at C-12 and acetylation would then yield the other simple orthoesters, **14**, **17**, and **18**. Alternatively, the major orthoester simplexin (**15**) could undergo cyclization of the orthoester side chain onto the cyclopentenone to yield **9** and presumably its C-9′ epimer **20** (pimelea factor S6; see Figure 2).[34] Although **20** was not found in this work, it has been reported to co-occur with **9** in *P. simplex*.[34] Hydroxylation and acetylation at C-12 in **9** and **20** would then yield **7** and **8**; alternatively cyclization of **14** could directly give **7** and **8**. The 1α-alkyldaphnane orthoesters **9** and **20** are also likely precursors of **12** and **10**, respectively. Initial C-2 hydroxylation followed by a Baeyer−Villiger reaction and subsequent spontaneous cyclization could form the ketal seen in **10**−**13**. Baeyer−Villiger reactions are well precedented and have for example previously been reported to be catalyzed by flavin monooxygenases from bacteria (e.g., *Pseudomonas putida*)[35] and from plants, where they are catalyzed by cytochromes P450, which are responsible for brassinolide (a plant steroid hormone) biosynthesis.[36,37] Again, either C-12 hydroxylation and acetylation of **10** and **12** could yield **13** and **11**, respectively, or **13** and **11** could be derived directly via a Baeyer−Villiger reaction from **7** and **8**, respectively.

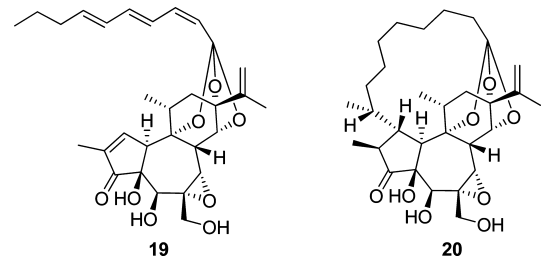

Figure 2. Structures of peddia factor V_1 (**19**) and pimelea factor S6 (**20**).[21,34] Although neither were isolated in this work, they may be biosynthetically related to the compounds reported.

The origins of the minor tigliane esters (**1**−**6**) are similarly explained. A 12-deoxyphorbol precursor is processed via C-12 oxidation and acylation with a fatty acid to yield **1**−**4** or alternatively via C-5 hydroxylation, C-6−C-7 epoxidation, and acylation to yield **5** and **6**. Given the similarity of the proposed transformations leading to compounds, e.g., C-12 and C-5 oxidation in both the daphane and tigliane series, it may be that these are carried out by the same enzyme systems that possess a somewhat relaxed substrate specificity.

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 spectra were recorded at 500 or 750 MHz with the residual ^{1}H signal in the $CDCl_3$ solvent (δ 7.24 ppm) as internal standard. ^{13}C NMR spectra were recorded at 125 or 188 MHz with the central peak of the $CDCl_3$ triplet (δ 77.0 ppm) as internal standard. *J* values are reported in Hz. High-resolution mass spectra were recorded on a Bruker MicrOTof-Q spectrometer equipped with a DIONEX UltiMate 3000 micro LC system (ESI mode). Low-resolution mass spectra were recorded on a Bruker Esquire HCT 3D ion trap spectrometer (ESI mode). HPLC was carried on a LC-10AT Shimadzu liquid chromatograph system, equipped with a photodiarray detector and/or low-temperature evaporative light scattering detector (LT-ELSD). HPLC method A: A reversed-phase semipreparative column (Phenomonex Luna 5 μm C-18(2) 100 Å 250 × 15 mm) was used at ambient temperature; 95% MeOH was run isocratically at a flow rate of 3.7 mL/min for 50 min. The LT-ELSD was conditioned at 42 °C with a pressure of 200 kPa. HPLC method B: A reversed-phase column (Phenomonex Luna 5 μ C-18(2) 100 Å 250 × 4.6 mm) at 52 °C was used with a flow rate of 1 mL/min. A gradient of MeCN/H_2O was set up, running from 20% MeCN to 100% in 50 min and maintained at 100% for 20 min. The LT-ELSD was conditioned at 52 °C with a pressure of 200 kPa.

Extraction and Isolation. Compounds **1**−**18** were isolated from a collection of *P. elongata* (AQ751686) from a site near Bollon Queensland, but processed in three slightly different manners as follows:

Procedure A: Isolation of Compounds 4, 6, 9−**15, and 18.** Air-dried and milled *P. elongata* foliage (500 g) was soaked with 90% MeOH (2.5 L) for 1 day. The solution was decanted, and the plant material was re-extracted with MeOH (1 L) for another day, followed by vacuum filtration of the mixture. The MeOH extracts were combined and concentrated under reduced pressure. The crude extract (300 mL) was transferred to a separatory funnel containing 40 mL of brine solution and extracted with CH_2Cl_2 (3 × 450 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in hexane (400 mL) and partitioned with MeCN (4 × 200 mL). The MeCN layers were combined and concentrated to 400 mL. The extract was then filtered through a plug of glass wool to remove any precipitate, followed by a wash with hexane (200 mL). MeCN was removed under reduced pressure to yield a dark green gum (5.27 g). A portion of this material (4 g) was subjected to vacuum-assisted Si gel column chromatography (CC) (column i.d. 8.5 cm, wet-packed with hexane to a height of 7 in.) and eluted with EtOAc/hexane (15:85, 600 mL; 20:80, 400 mL; 40:60, 400 mL; 35:65, 400 mL; 45:55, 400 mL; 50:50, 400 mL) to yield six fractions. Fractions 4 and 5 contained simplexin by TLC (1:1 EtOAc/hexane, R_f 0−0.5, visualization by ammonium metavanadate[3]) and were subsequently combined and dried under reduced pressure to give a green oil (680 mg). The combined material was purified further with a second column (column i.d. 4 cm; wet packed with hexane to 7 in.) eluted with EtOAc/hexane (15:85, 600 mL; 20:80, 200 mL; 25:75, 200 mL; 30:70, 200 mL; 35:65, 200 mL; 50:50, 200 mL) and combined into six fractions based on TLC. HPLC purification of fraction 5 (R_f 0.017−0.46, using HPLC method A) afforded **15** (12.6 mg, >95% pure). Separation of fraction 6 using HPLC method A provided **14** (0.4 mg), **6** (2.9 mg), **9** (5.0 mg, >85% pure), and **18** (1.7 mg). Other less well-resolved peaks on the HPLC were collected as mixtures and repurified by HPLC method B to provide **4** (0.5 mg), **10** and **11** [1.7 mg as a mixture in a 1:2 ratio (**10**:**11**)], **12** (0.7 mg), **13** (1.9 mg), and **15** (4.5 mg). A further 3.9 mg of **15** was isolated from fraction 4 by HPLC method A, giving a total of 21 mg of simplexin (**15**) from 4 g of the crude extract.

Procedure B: Isolation of Compounds 1−**3, 14, 16, and 17.** Air-dried and milled *P. elongata* foliage (40 g) was extracted on the basis of the procedure described above. The crude residue obtained after the liquid−liquid partition process was directly subjected to HPLC purification (HPLC method A). From this separation, additional **1** (0.3 mg), **2** (0.4 mg), **3** (0.8 mg), **14** (0.9 mg), **16** (<0.1 mg), and **17** (0.6 mg) were isolated as pure compounds.

Procedure C: Isolation of Compounds 5, 7, and 8. Air-dried and milled *P. elongata* root (AQ751686, 100 g) was extracted with 90% MeOH according to the procedure described above. After the liquid−liquid partition, the crude residue was dissolved in a minimum of CH_2Cl_2 and loaded evenly onto three Si-SPE cartridges (Phenomenex Strata SI-1 silica 2 g, pretreated with CH_2Cl_2). The cartridge was eluted gravitationally with CH_2Cl_2, followed by 0.5% MeOH/CH_2Cl_2. The elution was followed by TLC (1:1 EtOAc/hexane), and fractions with R_f 0.1−0.45 were combined. Upon solvent evaporation, the residue was subjected to HPLC purification (using HPLC method A) to yield **15** (19.1 mg), **5** (0.7 mg), and **7** and **8** [0.4 mg, inseparable mixture in a 40:60 ratio (**7**:**8**)].

12-*O*-(4*Z*,7*Z*)-Deca-4,7-dienoylphorbol-13-acetate (1): viscous oil; $[\alpha]_D$ +33.3 (*c* 0.04, $CHCl_3$); ^{1}H ($CDCl_3$, 750 MHz) and ^{13}C NMR ($CDCl_3$, 188 MHz), Table 1; ESIMS 579 $[M + Na]^+$; HRESIMS *m*/*z* 579.2938 (calcd for $C_{32}H_{44}O_8Na$, 579.2934).

12-*O*-(4*Z*)-Deca-4-enoylphorbol-13-acetate (2): viscous oil; $[\alpha]_D$ +27.6 (*c* 0.1, $CHCl_3$); ^{1}H ($CDCl_3$, 750 MHz) and ^{13}C NMR ($CDCl_3$, 188 MHz), see Table 1; ESIMS 581 $[M + Na]^+$; HRESIMS *m*/*z* 581.3085 (calcd for $C_{32}H_{46}O_8Na$, 581.3090).

12-*O*-Octanoylphorbol-13-acetate (3): oil; $[\alpha]_D$ +7.4 (*c* 0.08, $CHCl_3$); ^{1}H ($CDCl_3$, 750 MHz) and ^{13}C NMR ($CDCl_3$, 188 MHz), see Table 1; ESIMS 555 $[M + Na]^+$; HRESIMS *m*/*z* 555.2928 (calcd for $C_{30}H_{44}O_8Na$, 555.2934).

12-*O*-Decanoylphorbol-13-acetate (4): oil; $[\alpha]_D$ +7.1 (*c* 0.05, $CHCl_3$); ^{1}H ($CDCl_3$, 750 MHz) and ^{13}C NMR ($CDCl_3$, 188 MHz), see Table 1; HRESIMS *m*/*z* 583.3241 (calcd for $C_{32}H_{48}O_8Na$, 583.3247).

6α,7α-Epoxy-5*β*-hydroxy-12-deoxyphorbol-13-decanoate (5): amorphous solid; $[\alpha]_D$ −10.9 (*c* 0.06, $CHCl_3$); ^{1}H ($CDCl_3$, 750 MHz) and ^{13}C NMR ($CDCl_3$, 188 MHz), see Table 2; ESIMS 557 $[M + Na]^+$, 573 $[M + K]^+$; HRESIMS *m*/*z* 557.3085 (calcd for $C_{30}H_{46}O_8$+Na, 557.3090).

6α,7α-Epoxy-4*β*,5*β*,9α,20-tetrahydroxy-13α-(2*E*,4*E*)-tetradeca-2,4-dienoyl-1-tiglien-3-one (*P. simplex* subtoxin B) (6): oil, $[\alpha]_D$ +13.2 (*c* 0.09, $CHCl_3$); ^{1}H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), Table 2; ESIMS 609 $[M + Na]^+$, 625 $[M + K]^+$; HRESIMS *m*/*z* 609.3398 (calcd for $C_{34}H_{50}O_8Na$, 609.3403).

1,2-Dihydro-5-hydroxy-9′-methyl-6α,7α-epoxy-12*β*-acetoxy-9,13,14-ortho-1α-decenoate-resiniferonol-10′-oic acid [(9′*R*)-7 and (9′*S*)-8]: oily mixture in a 40:60 ratio (**7**:**8**); ^{1}H ($CDCl_3$, 750 MHz) and ^{13}C NMR ($CDCl_3$, 188 MHz), Table 4; ESIMS 613 $[M + Na]^+$, 629 $[M + K]^+$; HRESIMS *m*/*z* 613.2983 (calcd for $C_{32}H_{46}O_{10}Na$, 613.2989).

Pimelotides C (10) and D (11): oily mixture in a 1:2 ratio (**10**:**11**). **10**: ^{1}H ($CDCl_3$, 750 MHz) and ^{13}C NMR ($CDCl_3$, 188 MHz), Table 4; ESIMS 569 $[M + Na]^+$, 585 $[M + K]^+$; HRESIMS *m*/*z* 569.2739 (calcd for $C_{30}H_{42}O_9Na$, 569.2726). **11**: ^{1}H ($CDCl_3$, 750 MHz) and ^{13}C NMR ($CDCl_3$, 188 MHz), Table 4; ESIMS 627 $[M + Na]^+$, 643 $[M + K]^+$; HRESIMS *m*/*z* 627.2793 (calcd for $C_{32}H_{44}O_{11}Na$, 627.2781).

Gnidiglaucin (14): oil; $[\alpha]_D$ +41.2 (*c* 0.03 $CHCl_3$); ^{1}H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), Table 3; ESIMS 613 $[M + Na]^+$, 629 $[M + K]^+$; HRESIMS *m*/*z* 613.2983 (calcd for $C_{32}H_{46}O_{10}Na$, 613.2988).

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Supporting Information Available: 1D and 2D NMR spectra for compounds **1**−**3**, **5**, **7**, **8**, **10**, and **11**, Chem 3D c3d files and HMBC and 2D ROE correlation figures for compounds **7**, **8**, **10**, and **11**, and figure of the possible biosynthetic pathway for diterpenoid orthoesters and esters **1**−**20**. This material is available free of charge via the Internet at http://pubs.acs.org.

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