Chemistry of New Zealand Apiaceae: A Rare Phenylpropanoid and Three New Germacrane Derivatives from *Anisotome lyallii*

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A phytochemical investigation of the New Zealand endemic Apiaceae species *Anisotome lyallii* Hook.f. yielded (+)-α-angeloyloxylatifolone (**1**), 6-O-angeloyl-8-O-tigloyl-6β,8α,11-trihydroxygermacra-1(10)*E*,4*E*-diene (**2**), 6-O-tigloyl-8-O-tigloyl-6β,8α,11-trihydroxygermacra-1(10)*E*,4*E*-diene (**3**) and 6-O-tigloyl-8-O-tigloyl-1α,6β,8α,11-tetrahydroxygermacra-4*E*,10-(14)diene (**4**). The structures were elucidated by HR mass spectrometry and 1D- and 2D-NMR spectroscopy. A chemosystematic survey for compounds **1–3** in other New Zealand Apiaceae by HPLC-MS revealed that **1–3** were confined to *A. haastii* Cockayne & Laing and *A. lyallii*, and that some minor compounds in other species of *Anisotome* were isomers of **2** and **3**.

Introduction

A. lyallii, a herb of up to 45 cm height, is an endemic species of coastal areas of the southern third of the New Zealand South Island and Steward Island (Dawson, 1961). A chemotaxonomic survey by HPLC-MS revealed that *A. lyallii* contains anisotomenes (Zidorn *et al.*, submitted), irregular diterpenes, which have recently been discovered in sub-alpine members of the New Zealand and Tasmanian endemic genus *Anisotome* (van Klink *et al.*, 1999).

HPLC-MS investigations also revealed the presence of three major unidentified compounds in *A. lyallii*. We now report the isolation and structure elucidation of these compounds and their distribution within the genus *Anisotome*.

Results

Air-dried whole plants of *A. lyallii* (298 g) were exhaustively extracted with acetone. The crude extract obtained after evaporating the solvent *in vacuo* (36.2 g) was further separated by repeated silica gel column chromatography (CC) using gradients of cyclohexane and acetone and subsequent Sephadex LH-20 CC with acetone as eluant. Enriched fractions of **1–4** (Fig. 1) were finally purified by semi-preparative RP-18 HPLC using an isocratic mixture of acetonitrile and water containing 0.01% trifluoroacetic acid to yield **1** (10.0 mg), **2** (32.3 mg), **3** (63.1 mg) and **4** (4.0 mg).

Compound **1** was identified as (+)-α-angeloyloxylatifolone, a rare phenylpropanoid, on the basis of its on-line LC-mass spectrum {*m/z* = 307 $[M + H]^+$ (100), 339 $[M + MeOH + H]^+$ (12), 371 $[M + 2MeOH + H]^+$ (18), congruent with a molecular formula of $C_{16}H_{18}O_6$}, its optical rotation and its ^{1}H NMR and ^{13}C NMR data. This compound so far has only been found in two other Apiaceae, *Anthriscus sylvestris* Hoffm. and *Laserpitium siler* L. (Stefanovic *et al.*, 1977; Kozawa *et al.*, 1978; Micovic *et al.*, 1985). As ^{13}C NMR data have not been published yet, these are given in the experimental section.

Compound **2** showed on-line mass signals at *m/z* = 419 $[M + H]^+$ and 401 $[M - H_2O + H]^+$, appropriate for a molecular mass of $C_{25}H_{38}O_5$. This molecular formula was verified by HR mass spectrometry. ^{1}H and ^{13}C NMR data (Tables I-II) revealed the presence of a sesquiterpene moiety and two hemiterpenic acid moieties. The sesquiterpene part of the molecule was identified on the basis of 1D- and 2D-NMR experiments as a derivative of 6β,8α,11-trihydroxygermacra-1(10)*E*,4*E*-

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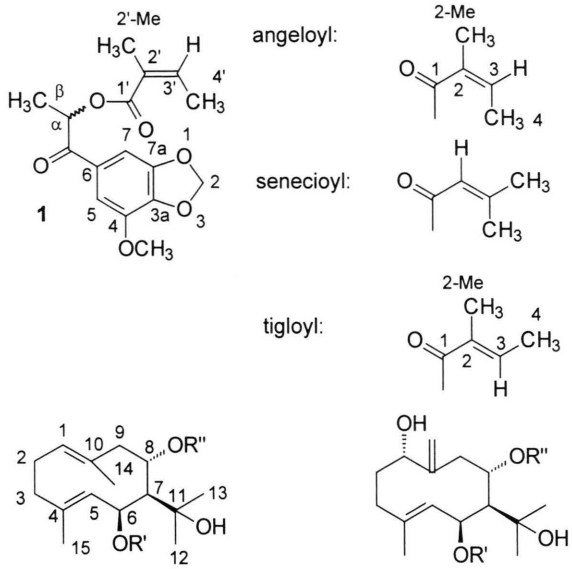

2: R' = angeloyl, R'' = tigloyl
3: R' = R'' = tigloyl

4: R' = R'' = tigloyl

5: R' = H, R'' = senecioyl

Fig. 1. Structures of a phenylpropanoid and four germacranes from *Anisotome lyallii* (**1**–**4**) and *A. pilifera* (**5**). (+)-α-angeloyloxylatifolone (**1**), 6-O-angeloyl-8-O-tigloyl-6β,8α,11-trihydroxygermacra-1(10)*E*,4*E*-diene (**2**), 6-O-tigloyl-8-O-tigloyl-6β,8α,11-trihydroxygermacra-1(10)*E*,4*E*-diene (**3**), 6-O-tigloyl-8-O-tigloyl-1α,6β,8α,-11-tetrahydroxygermacra-4*E*,10(14)-diene (**4**), and 8-O-senecioyl-6β,8α,11-trihydroxygermacra-1(10)*E*,4*E*-diene (**5**).

diene. NMR data of the sesquiterpene moiety were very similar to the data reported for 8-O-senecioyl-6β,8α,11-trihydroxygermacra-1(10)*E*, 4*E*-diene **5** (Fig. 1), which we recently isolated from *A. pilifera* (Hook. f.) Cockayne & Laing (Zidorn and Perry, submitted). The main difference between the spectra of **2** and **5** was the pronounced downfield-shift of the signal assignable to H-6 in **2** (δ_H = 5.80 ppm instead of 4.91 ppm) indicating esterification in that position. The hemiterpenic acid moieties in **2** were identified by ^{1}H NMR, ^{13}C NMR, HSQC and HMBC spectroscopy as angeloyl and tigloyl (Table II). The signal assignable to H-6 showed a HMBC crosspeak to the carbonyl signal at 167.0 ppm (C-1′) and the signal of H-8 showed a crosspeak to the carbonyl signal at 166.5 ppm (C-1″). A signal indicative of an angeloyl moiety (H-3′ at 6.04 ppm) showed a crosspeak to C-1′ at 167.0 ppm, and the signal of a proton indicative of a tigloyl moiety (H-3″ at 6.81 ppm) showed a crosspeak to C-1″ at 166.5 ppm. Therefore, **2** was identified as 6-O-angeloyl-8-O-tigloyl-6β,8α,11-trihydroxygermacra-1(10)*E*,4*E*-diene, a previously unreported compound.

On-line and HR mass spectra of **3** were identical to those obtained for compound **2**, suggesting that **3** was an isomer of **2**. This was verified by ^{1}H and ^{13}C NMR spectroscopy (Table II), showing almost perfectly superimposable signals for the sesquiterpene moiety of the molecule, plus signals assignable to two tigloyl moieties. The signals of protons H-6 and H-8 showed HMBC crosspeaks to the carbonyls of the tigloyl moieties. Thus, compound **3** is 6-O-tigloyl-8-O-tigloyl-6β,8α,11-trihydroxygermacra-1(10)*E*,4*E*-diene, another new compound.

HRMS data of **4** indicated a molecular formula of $C_{25}H_{38}O_6$. ^{1}H and ^{13}C NMR spectra (Tables I, II) showed signals assignable to a sesquiterpene moiety and two tigloyl moieties. Results from HSQC and HMBC experiments identified the sesquiterpene part of the molecule as a 1,6,8,11-tetraoxygermacra-4,10(14)-diene derivative. The ^{1}H NMR shifts of H-6 and H-8, and HMBC crosspeaks from the signals of these protons to the two carbonyl signals, revealed that the tigloyl moieties were attached to C-6 and C-8. Therefore C-1 and C-11 both bore hydroxyl groups.

NOE spectra showed two sets of interactions: between H-1, H-5 and H-8; and between H-6, H-7, one H-14 and Me-15. This suggested that these two sets of protons were on opposite faces of the molecule. Assuming the usual absolute stereochemistry at C-7, this led to a proposed structure **4** with 1α-OH, 6β-tigloyl and 8α-tigloyl groupings. Conformational searching and molecular modeling of this proposed structure led to two predicted ring conformations (Fig. 2 and Table III) with various rotations around C-11–OH and C-7–C-11. The major conformation (about 75% populated) explained the NOE interactions given above. However, this major conformation did not account for the observed NOE interaction between H-5 and one or other H-9, since these protons are over 0.44 nm apart in this conformation. In the minor conformation (about 25% populated), H-5 and H-9α are 0.21 nm apart (Fig. 2), which would lead to a strong NOE interaction. Other evidence for the

Table I. ^{1}H NMR data of compounds **2–4**[a].

Position	**2**	**3**	**4**
Sesquiterpene moiety			
1	5.16 1H, dq (12.0, 1.5)	5.15 1H, br d (12.0)	4.25 1H, br d (8.5)
2	2.41 1H, m	2.39 1H, m	2.11 2H, m
	2.15 1H, m	2.13 1H, m	
3	2.18 2H, m	2.15 2H, m	2.14 2H, m
5	5.13 1H, d (7.0)	5.13 1H, br d (6.5)	5.24 1H, br d (5.5)
6	5.80 1H, br d (6.0)	5.77 1H, br d (6.0)	5.82 1H, br d (5.5)
7	1.87 1H, m	1.86 1H, m	2.22 1H, dd (4.5, 2.5)
8	5.65 1H, dd (12.0, 6.0)	5.69 1H, dd (12.0, 6.0)	5.88 1H, dd (9.0, 4.5, 4.5)
9	2.76 1H, dd (13.0, 6.0)	2.73 1H, dd (13.0, 6.0)	2.67 2H, m
	1.98 1H, m	1.98 1H, dd (13.0, 12.0)	
12	1.44 3H, s	1.41 3H, s	1.37 3H, s
13	1.39 3H, s	1.37 3H, s	1.29 3H, s
14	1.69 3H, [b]	1.69 3H, s	5.36 1H, br s
			5.31 1H, br s
15	1.55 3H, s	1.53 3H, s	1.55 3H, s
Hemiterpenic acid moiety I			
3′	6.04 1H, qq (7.5, 1.5)	6.66 1H, qq (7.0, 1.5)	6.76 1H, qq (7.5, 1.0)
4′	1.95 3H, dq (7.5, 1.5)	1.69 3H, m[b]	1.75 3H, q (1.5)
2′-Me	1.69 3H, [b]	1.67 3H, dq (7.0, 1.5)	1.74 3H, dq (7.5, 1.0)
Hemiterpenic acid moiety II			
3″	6.81, qq (7.0, 1.0)	6.85 1H, qq (7.0, 1.5)	6.87 1H, qq (7.5, 1.0)
4″	1.86 3H, br s	1.87 3H, q (1.5)	1.88 3H, q (1.5)
2″-Me	1.81 3H, dq (7.0, 1.0)	1.83 3H, dq (7.0, 1.5)	1.83 3H, dq (7.5, 1.0)

[a] Measured in $CDCl_3$ at 500 MHz, referenced to solvent residual signals of $CHCl_3$ at 7.25 ppm.
[b] Overlapping signals.

Table II. ^{13}C NMR data of compounds **2–4**[a].

Position	**2**	**3**	**4**	Position	**2**	**3**	**4**
Sesquiterpene moiety				*Hemiterpenic acid moiety I*			
1	132.1 d	132.1 d	87.0 d	1′	167.0 s	167.3 s	167.0 s
2	24.8 t	24.8 t	28.6 t	2′	127.4 s	128.7 s	128.6 s
3	38.7 t	38.7 t	37.6 t	3′	139.6 d	137.3 d	138.0 d
4	135.7 s	135.6 s	134.9 s	4′	15.7 q	11.7 q	11.9 q
5	130.6 d	130.5 d	128.5 d	2′-Me	20.2 q	14.3 q	14.6 q
6	70.7 d	71.1 d	71.2 d				
7	52.9 d	53.0 d	50.0 d	*Hemiterpenic acid moiety II*			
8	75.2 d	75.2 d	71.0 d				
9	40.9 t	40.9 t	43.1 t	1″	166.5 s	166.6 s	166.5 s
10	129.6 s	129.7 s	142.9 s	2″	128.7 s	128.6 s	128.6 s
11	73.5 s	73.5 s	73.2 s	3″	137.5 d	137.6 d	138.3 d
12	30.4 q	30.2 q	29.7 q	4″	12.3 q	12.3 q	12.3 q
13	29.5 q	29.4 q	29.0 q	2″-Me	14.5 q	14.5 q	14.6 q
14	20.7 q	20.7 q	118.6 t				
15	16.5 q	16.8 q	17.4 q				

[a] Measured in $CDCl_3$ at 125 MHz, referenced to solvent signals of $CDCl_3$ at 77.00 ppm. Multiplicities were derived from DEPT experiments.

presence of both ring conformations of **4** in $CDCl_3$ solution was given by the couplings of the H-8 signal. The major ring conformation (Fig. 2) has H-8 *transoid* to H-9α, with a predicted coupling constant of 11.5 Hz (Table III). The actual largest H-8 to H-9 coupling constant for **4** was 9.0 Hz (Table II). This is in good agreement with the weighted mean of the H-8 to H-9α coupling con-

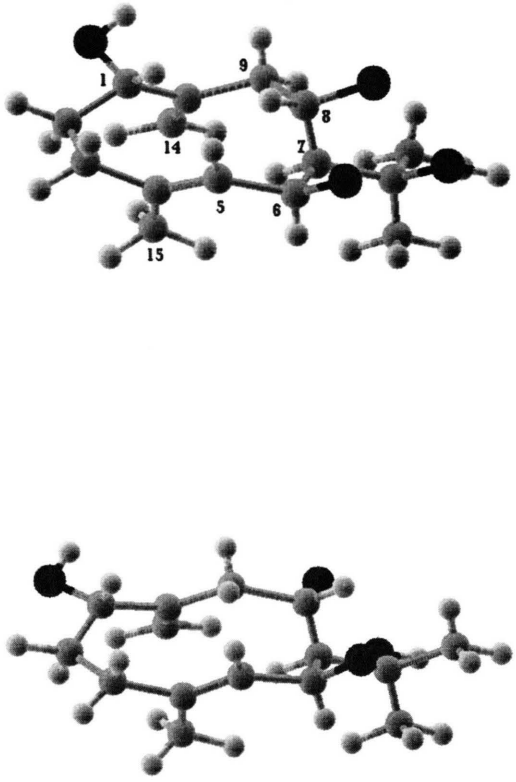

Fig. 2. Major (above) and minor (below) ring conformations predicted for germacrane **4** (tigloyl moieties omitted).

stants predicted for the main conformations (> 1% populated) found by conformational searching and molecular modeling (Table III). These ring conformations seem to be exchanging rapidly at room temperature, since there was no sign of broadening in the ^{13}C NMR spectrum.

The α-position of the hydroxy group at C-1 was corroborated by a comparison of the ^{1}H NMR coupling pattern of H-1 with literature data for 1α,6β-dihydroxygermacra-4*E*,10(14)-diene and 1ß,6β-dihydroxygermacra-4*E*,10(14)-diene (Barrero *et al.*, 1999). The minor ring conformation of **4** (Fig. 2) is similar to the predominant ring conformation of 1α,6β-dihydroxygermacra-4*E*,10(14)-diene predicted by molecular modeling (Barrero *et al.*, 1999). Consequently **4** is the new compound 6-O-tigloyl-8-O-tigloyl-1α,6β,8α,11-tetrahydroxygermacra-4*E*,10(14)-diene.

A survey for **1–3** (**4** was hardly detectable in crude extracts of *A. lyallii*) by HPLC-MS in all seventeen known species of *Anisotome* (Dawson, 1961; Webb, 1986; Parsons *et al.*, 1998) revealed that these compounds were confined to samples of *A. haastii* and *A. lyallii*. These analyses also revealed that compounds exhibiting the same on-line mass spectra as **2** and **3** but with slightly differing retention times occurred in *A. aromatica* Hook. f., in both varieties of *A. imbricata* (Hook.f.) Cockayne, and in *A. lanuginosa* (Kirk) J. W. Dawson. These isomers, which may be germacra-1(10)*E*,4*E*-diene-6,8,11-triol derivatives with different combinations of two hemiterpenic acids, were only present as minor compounds and were not isolated. We recently reported 8-O-senecioyl-6β,8α,11-trihydroxygermacra-1(10)*E*,4*E*-diene **5** from *A. pilifera* (Zidorn and Perry, submitted).

A vast number of different germacra-1(10)*E*,4*E*-diene derivatives has been isolated from various plants. Interestingly, germacra-1(10)*E*,4*E*-diene-6,8,11-triol derivatives have so far only been found in members of the genus *Anisotome* (this work; and Zidorn and Perry, submitted). Therefore germacranes with that particular substitution pattern are chemosystematic markers

Table III. Molecular modeling and selected NMR data for compound **4**.

Ring Conformation	Energy (kcal/mol)	Population (% at 300K)	Proton-proton couplings (Hz)		
			H-8 – H-7	H-8 – H-9α	H-8 – H-9ß
Major	42.858	36.40	1.1	11.5	3.4
Major	43.045	26.60	1.1	11.5	3.4
Minor	43.452	13.44	11.5	3.3	3.6
Minor	43.550	11.41	11.5	3.3	3.6
Major	44.056	4.88	1.1	11.5	3.4
Major	44.649	1.81	1.1	11.5	3.4
Major	44.907	1.17	1.1	11.5	3.4
Other	44.927	1.13	11.5	3.2	3.7
Weighted mean	–	–	3.8	9.0	3.3
Actual (in $CDCl_3$)	–	–	4.5	9.0	4.5

for this umbelliferous genus, but are not detectable in all *Anisotome* species. A similar, but not correlated, pattern of patchy occurrence only in *Anisotome* has been found for the irregular anisotomene diterpenes (Zidorn *et al.*, submitted). These observations reinforce taxonomic questions about the boundaries of this genus (Dawson, 1961).

Experimental

Plant material. – *A. lyallii* was collected at Cannibal Bay/Otago/New Zealand (S 46°28′, E 169°46′, alt.: 10 m a.m.s.l.) in January 2000. A voucher specimen was deposited in the herbarium of the Plant Extracts Research Unit (voucher code: CZ-000118–3). Collection data of other *Anisotome* species investigated by HPLC-MS and extraction procedures and HPLC-MS parameters are described in full in Zidorn *et al.* (submitted). Air dried subaerial parts of the plants were ground, 500 mg plant material was mixed with 10.0 ml of a methanolic stock solution containing 0.200 mg/ml of *N*-phenyl-undecanamide (Perry *et al.*, 1996) as internal standard and extracted three times with 25 ml of methanol for 7.5 min. with an IKA-25 Ultraturrax apparatus at 24000 cycles/min. Extracts were combined and brought to dryness *in vacuo*. The residue was dissolved in 4 ml of methanol, filtered and used for HPLC analysis; HPLC system 1 (for quantification), oven temperature: 40 °C; column: Zorbax Rx-C18, 4.6 mm × 150 mm, particle size 3.5 μm ; guard column: Phenomenex C18, 4 mm × 3.00 mm; detection wavelength: 205 nm; injection volume: 10 μl; mobile phase A: 0.01% trifluoroacetic acid in water; mobile phase B: CH_3CN; flow rate: 1.00 ml/min; linear gradient: 0 min 80% A, 20% B; 5 min 80% A, 20% B; 10 min 50% A, 50% B; 31 min 46.5% A, 53.5% B; 55 min 5% A, 95% B; stop time: 60 min; post time: 15 min; HPLC system 2 (for HPLC-MS analyses) oven temperature: 40 °C; column: Zorbax Rx-C18, 4.6 mm × 150 mm, particle size 3.5 μm; guard column: LiChroCart 4 × 4 mm packed with LiChrospher RP-18 material (5 μm particle size); detection wave length: 205 nm; injection volume: 10 μl; mobile phase A: 0.15% acetic acid in water; mobile phase B: 0.15% acetic acid in MeOH; flow rate: 1.00 ml/min; linear gradient: 0 min 70% A, 30% B; 5 min 70% A, 30% B; 10 min 40% A, 60% B; 31 min 37.5% A, 63.5% B; 55 min 2% A, 98% B; stop time: 60 min; post time: 15 min. LC-MS analysis were performed with a Finnigan MAT SSQ 7000 mass spectrometer by APCI in the positive mode, employing a CID value of – 5 V, a corona amperage of 5 μA, a sheath gas pressure of 50 PSI and a capillary temperature of 150 °C.

The following retention times in HPLC-systems 1 and 2 were observed, respectively: **1** (23.9, 19.6), **2** (42.9, 39.8), **3** (39.9, 35.4), **4** (23.0, n.o.). The HPLC analyses for quantification were run in triplicate. The following amounts of compounds **1–4** were estimated in subaerial parts of *A. lyallii* by comparing the peak area of the internal standard and the peak areas of compounds **1–4**: **1** (1.09 ± 0.01 mg/g), **2** (2.59 ± 0.07), **3** (1.17 ± 0.02 mg/g), **4** (0.04 ± 0.00 mg/g).

Semi-preparative HPLC – Column: Phenomenex Luna 250 × 10 mm (5 μm particles, product-nr.: 006–4252-N0), guard column: Phenomenex C18, 4 mm × 3.00 mm, flowrate: 5.00 ml, detection wavelength: 205 nm, isocratic 50% MeCN, 50% H_2O + 0.01% TFA (**1**, retention time: 20 min; **4**, retention time: 17 min); isocratic 68% MeCN, 32% H_2O + 0.01% TFA (**2**, retention time: 20 min; **3**, retention time: 18 min).

NMR data of **1** (measured in $CDCl_3$ at 500 and 125 MHz, respectively). ^{1}H NMR: phenylpropane moiety, δ_H = 7.27 (d, J = 1.5 Hz, 1H, H-7), 7.14 (d, J = 1.5 Hz, 1H, H-5), 6.06 (s, 2H, H-2), 5.91 (q, J = 7.5 Hz, 1H, H-α), 3.92 (s, 3H, OMe), 1.55 (d, J = 7.5 Hz, 3H, H-β); angeloyl moiety, δ_H = 6.12 (dd, J = 7.5, 1.5 Hz, 1H, H-3′), 1.99 (dq, J = 7.5, 1.5 Hz, 3H, H-4′), 1.92 (dq, J = 1.5, 1.5 Hz, 3H, H-2′Me). ^{13}C NMR: phenylpropane moiety, δ_C = 195.0 (C=O), 149.0 (C-7a), 143.7 (C-4), 140.1 (C-3a), 129.0 (C-6), 109.3 (C-7), 102.9 (C-5), 102.4 (C-2), 70.9 (C-α), 56.6 (OMe), 17.3 (C-β); angeloyl moiety, δ_C = 167.3 (C-1′), 139.1 (C-3′), 127.1 (C-2′), 20.4 (C-2′Me), 15.8 (C-4′); signal assignments were verified by HSQC and HMBC experiments. $[\alpha]_D^{27}$ + 2.3° (*c* 0.200, MeOH).

2 was obtained as a pale yellow oil; $[\alpha]_D^{15}$ –28° (*c* 0.200, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 214 (4.23) nm; IR (film) ν_{max} 3430 (br), 2966, 2931, 2849, 1696, 1649, 1449, 1437, 1261, 1232, 1155, 1132 cm^{-1}; ^{1}H and ^{13}C NMR in Tables I-II; on-line AP-CIMS *m/z* 419 $[M + H]^+$ (5), 401 $[M - H_2O + H]^+$ (100), 301 [M – hemiterpenoyl – $2H_2O$ + H]$^+$ (16), 261 [M – hemiterpenoyl – isopropanoxy –

H_2O + H]$^+$ (90), 161 [M – 2hemiterpenoyl – isopropanoxy – $2H_2O$ + H]$^+$ (96); HREIMS *m/z* 441.2620 [M + Na]$^+$ (calcd. for $C_{25}H_{38}O_5Na$, 441.2617).

3 was obtained as a pale yellow oil; $[\alpha]_D^{27}$ –57° (*c* 0.200, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 214 (4.45) nm; IR (film) ν_{max} 3510 (br), 2962, 2925, 2856, 1709, 1650, 1440, 1381, 1268, 1135, 1073, 1015, 982 cm^{-1}; ^{1}H and ^{13}C NMR in Tables I-II; on-line APCIMS *m/z* 419 [M + H]$^+$ (6), 401 [M – H_2O + H]$^+$ (64), 319 [M – hemiterpenoyl – H_2O + H]$^+$ (44), 301 [M – hemiterpenoyl – $2H_2O$ + H]$^+$ (16), 261 [M – hemiterpenoyl – isopropanoxy – H_2O + H]$^+$ (100), 161 [M – 2hemiterpenoyl – isopropanoxy – $2H_2O$ + H]$^+$ (90); HREIMS *m/z* 441.2633 [M + Na]$^+$ (calcd. for $C_{25}H_{38}O_5Na$, 441.2617).

4 was obtained as a pale yellow oil; $[\alpha]_D^{27}$ strongly positive [between +40 and +60° (*c* 0.029, $CHCl_3$)], no exact result is given because of the low concentration; UV (MeOH) λ_{max} (log ε) 208 (4.24) nm; IR (film) ν_{max} 3420 (br), 2968, 2921, 2848, 1704, 1690, 1645, 1443, 1383, 1261, 1139 cm^{-1}; ^{1}H and ^{13}C NMR in Tables I-II; HREIMS *m/z* 457.2588 [M + Na]$^+$ (calcd. for $C_{25}H_{38}O_6Na$, 457.2566).

Conformational searching and molecular modeling used PCMODEL V 7.0 (Serena Software, Bloomington Ind., USA). The 'Bonds' method was used to randomly rotate bonds C-1–O, C-6–O, C-7–C-11, C-8–O, C-11–O, C-5–C-6, C-6–C-7, C-7–C-8, C-8–C-9, C-9–C-10 and C-10–C-1 to generate starting conformations. These were minimized with the MM3 force-field (Allinger *et al.*, 1989). Results are given in Table III.

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