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The recurvatianes: A suite of oxygenated guaiane sesquiterpenes from *Perezia recurvata*

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ABSTRACT

Six guaiane sesquiterpenes recurvatiane A–F, including the previously known recurvatiane E and xanthomicrol, were isolated from the Andean *Perezia recurvata* and their structures determined by spectroscopic evidence. The absolute stereochemistry of recurvatiane A was established by derivatization with (*R*)- and (*S*)-α-methoxy-α-phenylacetic acids (MPA). The suite of guaiane-based metabolites here reported, which we have named recurvatianes A–F, provide an example of a pathway by which molecular diversity is generated by the occurrence of specific oxidation reactions in the late biosynthetic steps of the frame structure.

1. Introduction

The Nassauvieae tribe (Asteraceae) contains approximately 24 genera and 370 species and is an important component in the flora of the Central and Southern Andean region (Panero and Funk, 2008). Indigenous communities still maintain traditional uses linked to biodiversity, and medicinal plants are sometimes the only available treatment for certain illnesses. Some examples are: *Perezia multiflora* (local name: escorzonera, chanqoroma, used as diuretic, antipyretic, expectorant and particularly valued as a means of controlling bleeding in childbirth and uterine hemorrhages); *Perezia coerulescens*, *Perezia pinnatifida* (valeriana: sedative, diuretic, and diaphoretic) and *Perezia purpurata* (marancel: antiinflammatory, antiviral, carminative, soft sedative) (De-la-Cruz et al., 2007).

The results from molecular phylogenetic studies of the tribe Nassauvieae based on chloroplast DNA coding regions show that the genus *Perezia* is not monophyletic since *Perezia nutans* and *Perezia prenanthoides* are not related to other species of *Perezia*. The removal of *P. nutans* and *P. prenanthoides* from *Perezia* to form the new genus *Calorezia* (Panero, 2007) was proposed, so the genus *Perezia* includes 30 species instead of the 32 so far recognized, 18 of which are found in Chile (www.florachilena.cl).

The genus *Perezia* has been separated (Reveal and King, 1973; Crisci, 1980; Kim et al., 2002) from genus *Acourtia* (previously a section of Perezia). In earlier literature, *Acourtia* genus from North and Central America has usually been treated as *Perezia*, section *Acourtia*, which can be misleading. Chemically, these two genera differ by the occurrence of isocedrenes in the genus *Perezia* (Bohlmann and Zdero, 1979; Zdero et al., 1986, 1988; Bittner et al., 1989), and perezone (Archer and Thompson, 1965; Joseph-Nathan, 1974) and related compounds in the species classified in the genus *Acourtia* (Zdero et al., 1991).

Thirty years ago the literature available on the chemistry of the Nassauvieae tribe was almost nonexistent (Heywood et al., 1977). Since then, contributions to the knowledge of *Perezia* genus have been made as a result of the number of chemistry-related studies of the species *Perezia carthamoides*, *Perezia lactucoides*, *Perezia linearis*, *Perezia lyrata*, *Perezia megalantha*, *Perezia pedicularifolia*, *Perezia pilifera*, *Perezia recurvata* (Bittner et al., 1989), *Perezia multiflora* (Bittner et al., 1989; Joseph-Nathan et al., 1978), *P. coerulescens* (Angeles et al., 1984), and *Perezia ocorzonera* (De-Israilev and González, 1994). These studies indicate that isocedrene derivatives are widely distributed within this genus.

In this paper we describe the structures of six guaiane-derived sesquiterpenes **1**–**6** (Fig. 1) which, along with xanthomicrol **7** (Stout and Stout, 1961) were isolated from *P. recurvata* (Vahl) Less collected in Sierra Baguales (Magellan Region, Chile). A previous study (Bittner et al., 1989) of *P. recurvata* led to the isolation of compound **5** as the unique guaiane derivative. However, in this work we have isolated a suite of guaiane-based metabolites that

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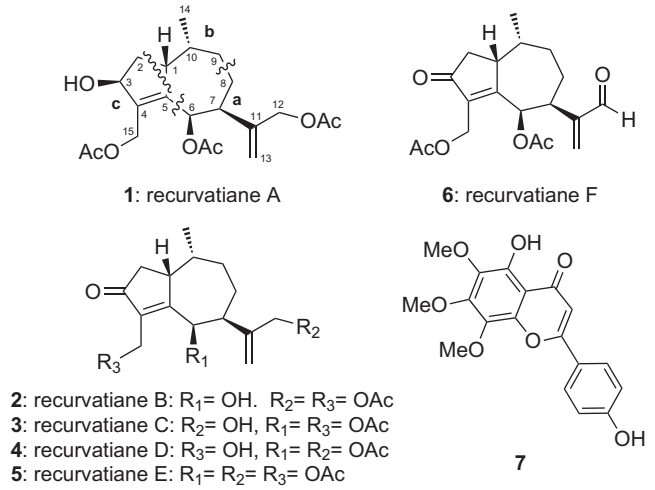


Fig. 1. Structure of metabolites isolated from *Perezia recurvata*.

Table 1
^{13}C NMR data of compounds **1–6** [125 MHz, δ_C ppm, $CDCl_3$].

#	1	2	3	4	5	6
1	47.7	44.4	44.5	44.3	44.4	44.3
2	34.3	37.2	37.3	37.4	37.2	37.2
3	75.8	206.7	205.9	207.8	205.5	205.7
4	135.0	133.4	135.0	138.5	135.0	135.0
5	148.5	181.6	177.5	174.5	177.0	176.5
6	71.7	71.6	72.7	73.0	73.0	72.6
7	47.9	50.1	45.9	47.1	47.4	41.1
8	30.3	29.2	30.2[h]	30.2[c]	30.4	30.1
9	29.9	30.2	30.2[h]	30.3[c]	30.2	29.6
10	34.1	33.4	33.8	33.8	33.7	33.8
11	145.1	145.8	149.1	144.6	144.4	150.7
12	66.9	66.2	66.1	66.8	66.5	193.4
13	114.6	114.8	112.8	115.6	115.4	134.9
14	20.1	20.9	20.9	20.9	20.6[a]	20.9
15	59.1	54.7	54.5	55.0	54.7	54.6
6–C=O	170.0	–	170.0	170.1	169.8	170.8[g]
6–$COCH_3$	20.8	–	20.8[b]	20.8[d]	20.7[e]	20.7[f]
12–C=O	170.6	170.7	–	170.8	170.5	–
12–$COCH_3$	20.8	20.8[a]	–	20.9[d]	20.7[e]	–
15–C=O	171.2	170.7	171.2	–	170.4	170.9[g]
15–$COCH_3$	20.8	20.9[a]	20.9[b]	–	20.8[e]	20.8[f]

[a–h] Overlapped signals. Chemical shifts confirmed by 2D HSQC and HMBC experiments.

provide an interesting example of the pathway by which molecular diversity is generated by specific oxidation reactions on a guaiane intermediate in the late biosynthetic steps. Although the known compound **5** has been referred to by its IUPAC name (Bittner et al., 1989), it might be convenient to group it together with **1**–**4**, and **6** as recurvatianes A–F.

2. Results and discussion

From the methanolic extract of *P. recurvata*, collected in the Sierra Baguales (Magellan Region, Chile), recurvatianes A–F (**1**–**6**), were isolated after gel filtration on Sephadex LH-20 chromatography followed by normal phase HPLC.

Recurvatiane A (**1**) was obtained as a colourless oil $[\alpha]_D^{20} - 182$ (*c*, 0.11, CH_2Cl_2). Its EIMS spectrum showed a peak at 394, which corresponds to the empirical formula $C_{21}H_{30}O_7$ $[M]^+$ (HREIMS *m/z* 394.2001 (calcd. for $C_{21}H_{30}O_7$ 394.1992)). The IR spectrum showed absorption for hydroxyl and carbonyl groups at 3460 and 1740 cm^{-1}, respectively. The ^{13}C NMR and DEPT spectra of **1** (Table 1) showed the presence of 21 carbon signals assigned to 4 × CH_3 (three from acetate methyl groups), 6 × CH_2 (one olefinic), 5 × CH (two bearing oxygen) and six quaternary carbons (three olefinic). The lack of 2D NMR spectroscopic information of most of the guaiane-derived metabolites reported from the Nassauvieae tribe renders a detailed 2D NMR spectral analysis of **1** expedient.

The ^1H NMR spectrum of **1** displayed singlets at δ 5.03 and δ 5.09 due to olefinic protons. Signals at δ 4.55 (*d*, *J* = 6.6) and at 5.82 (*s*) were assigned to protons geminal to oxygen, as well as the pair of methylene protons at δ 4.50 (*d*, *J* = 13.2), 4.60 (*d*, *J* = 13.2) and 4.72 (*d*, *J* = 12.9), 4.75 (*dd*, *J* = 12.9, 2.2), respectively. Nine well-resolved signals, each attributed to one of the remaining aliphatic protons, appeared between 1.14 and 3.11 ppm. Three acetate methyl groups (δ 2.01, 2.04 and 2.05) and a secondary methyl group at δ 0.86 (*d*, *J* = 7.2) accounted for all the protons of the molecule (Table 2). According to the spectral data and the molecular formula, compound **1** has to be bicyclic. Since no IR absorption for a free acid was observed and only one isoprenic methyl group (Me-14) was evident, the remaining three methyl groups expected for a sesquiterpene skeleton must be modified, two of them as methyl acetate (C-12 and C-15) and the other as a terminal methylene (C-13). Thus, the remaining three oxygen atoms must be involved in a secondary acetate at C-6, and a hydroxyl group at C-3, respectively. Oxygenated functionalities are consistent with the observed IR absorptions.

All C–H correlations for **1** were inferred from the HSQC spectrum. An ^1H–^1H COSY experiment showed coupling between a methylene (H_2-2) with two well-resolved methine protons (H-1 and H-3). This spin system is indeed extended from H-1 through H-6. The mutual HMBC correlations of H_2-13/C-12, H_2-12/C-13 and their long-range correlations of both H_2-13 and H_2-12 with C-7 and with an olefinic quaternary carbon C-11 secured an acetoxy isopropenyl group at C-7. The long-range correlations of H_2-8 and H-6 with C-11 defined the fragment **a**. Fragment **b** was established by the long range correlation of the secondary methyl group H_3-14 with C-1, C-9 and C-10. Fragments **a** and **b** were connected through C-8 and C-9 by the correlations H-10/C-8, and H-9/C-7. The remaining carbons of the molecule must form part of a cyclopentene ring with a methyl acetate appendage. As the spin system indicated three contiguous carbon (C-1 to C-3), the correlation H-15/C-4, C-3, C-5; H-3/C-4, C-5, C-15 established a cyclopentane ring fused to fragments **a**/**b** by the correlation H-6/C-5, C-4, C-1. Therefore the connectivity information obtained from COSY, HSQC and HMBC experiments unambiguously determined the planar structure of compound **1** as a highly oxidized guaiane network with a hydroxyl at C-3 and acetate groups at C-6, C-12 and C-15.

The relative stereochemistry of compound **1** was deduced by NOESY experiments, analysis of coupling constants and molecular mechanic calculations (PCModel Software). NOE correlation of H-6 with H-7 indicated that the secondary acetate and acetoxy isopropenyl groups are located on the same face of the molecule. The NOE effects observed between H-1 with H-10 and the methylenic proton H-2β (1.77, *m*) together with the NOE correlation of H-2α (1.61, *m*) and H-3 established a syn relationship for H-1, H-10 and the hydroxyl group at C-3. On the other hand, the correlation of the methylenic proton H-9β (1.25, *m*) with H-10 and the NOE effect observed between H-9α (1.14, *m*) with H-7 placed H-7 and H-6 on the opposite side of the molecule relative to H-10, establishing the whole relative stereochemistry of **1** as depicted in Fig. 2. Molecular mechanic calculations indicated theoretical dihedral angles of 80° for H-6/H-7 and 86° for H-3/H-2β, respectively. These theoretical values are in good agreement with the absence of coupling for H-6 (δ 5.82, *s*, Table 2) and the presence of a doublet

Table 2
^{1}H NMR data of compounds **1**–**6** 500 MHz, δ_H ppm, (*J*) Hz, $CDCl_3$.

#	1	2	3	4	5[c]	6
1	3.11 *m*	3.32 *m*	3.03 *m*	3.00 *m*	3.00 *m*	3.04 *m*
2	α: 1.61 *m*	α: 2.15 *m*	α: 2.15 *dd* (18.3, 4.1)	α: 2.14 *dd* (18.3, 3.8)	α: 2.14 *dd* (18.3, 4.2)	α: 2.17 *dd* (18.3, 4.1)
	β: 1.77 *m*	β: 2.46 *dd* (18.3, 6.3)	β: 2.44 *dd* (18.3, 6.6)	β: 2.41 *dd* (18.3, 6.3)	β: 2.42 *ddd* (18.3, 6.6, 1.0)	β: 2.45 *dd* (18.3, 6.6)
3	4.55 *d* (6.6)	–	–	–	–	–
4	–	–	–	–	–	–
5	–	–	–	–	–	–
6	5.82 *s*	5.19 *s*	6.12 *s*	6.07 *s*	6.05 *s*	5.97 *s*
7	2.10 *dd* (12.9, 4.7)	2.17 *m*	2.37 *m*	2.33 *m*	2.27 *m*	2.85 *dd* (12.9, 4.1)
8	1.63 *m*	α: 1.66 *m*	α: 1.77 *m*	α: 1.77 *m*	α: 1.78 *m*	α: 1.70 *m*
	1.75 *m*	β: 1.91 *m*	β: 1.90 *m*	β: 1.90 *m*	β: 1.89 *m*	β: 1.88 *m*
9	α: 1.14 *m*	α: 1.08 *m*	α: 1.13 *m*	α: 1.15 *m*	α: 1.12 *m*	α: 1.21 *m*
	β: 1.25 *m*	β: 1.37 *dd* (14.8, 6.6)	β: 1.41 *dd* (14.5,5.7)	β: 1.40 *dd* (14.2, 5.7)	β: 1.40 *dd* (5.0, 13.8)	β: 1.43 *dd* (14.8, 5.33)
10	2.18 *m*	2.53 *m*	2.35 *m*	2.33 *m*	2.32 *m*	2.36 *m*
11	–	–	–	–	–	–
12	4.50 *d* (13.2)	4.65 *d* (13.6)	4.12 *d* (13.2)	4.54 *d* (13.2)	4.54 *d* (13.3)	9.50 *s*
	4.60 *d* (13.2)	4.57 *d* (13.6)	4.20 *d* (13.2)	4.68 *d* (13.2)	4.60 *d* (13.3)	–
13	5.03 *s*	5.19 *s*	5.01 *s*	5.14 *s*	5.18 *m*	6.11 *s*
	5.09 *s*	5.24 *s*	5.13 *d* (1.0)	5.19 *s*	5.11 *s*	6.37 *s*
14	0.86 *d* (7.3)	0.98 *d* (7.3)	0.99 *d* (7.3)	0.99 *d* (7.3)	0.97 *d* (7.2)	1.01 *d* (7.3)
15	4.75 *dd* (12.9, 2.2)	4.63 *d* (12.3)	4.88 *dd* (12.6,1.6)	4.37 *dd* (13.2, 1.3)	4.73 *dd* (12.7, 1.1)	4.94 *dd* (12.6, 1.3)
	4.72 *d* (12.9)	4.81 *d* (12.3)	4.79 *d* (12.6)	4.47 *dd* (13.2, 1.6)	4.83 *dd* (12.7, 1.3)	4.82 *d* (12.6)
6-C=O	–	–	–	–	–	
6-$COCH_3$	2.01 *s*	–	2.09 *s*	2.11 *s*	2.09 *s*	2.10 *s*
12-C=O	–	–	–	–	–	–
12-$COCH_3$	2.05 *s*	2.10 *s*[a]	–	2.09 *s*	2.05 *s*[b]	–
15-C=O	–	–	–	–	–	–
15-$COCH_3$	2.04 *s*	2.04 *s*[a]	2.06 *s*	–	2.04 *s*[b]	2.05 *s*

[a,b] Interchangeable signals.
[c] Recorded at 400 MHz.

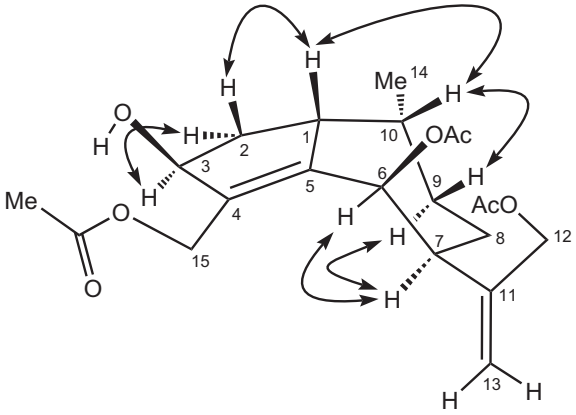
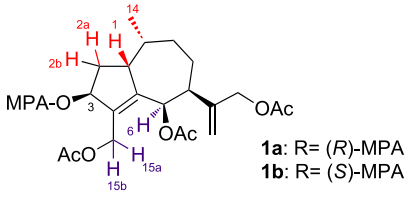


Fig. 2. Selected NOEs of recurvatiane A (**1**).

Table 3
^{1}H NMR $\Delta\delta$ ($\delta_R-\delta_S$) values ($CDCl_3$, ppm, recorded at 500 MHz) of the diastereomeric MPA esters **1a** and **1b**.



	δ_R	δ_S	$\Delta\delta^{RS}$
Me-14	0.78	0.89	−0.11
H-1	2.84	3.10	−0.26
H-2a	1.59	1.72	−0.13
H-2b	1.64	1.78	−0.14
H-15a	4.79	4.64	+0.15
H-15b	4.59	4.20	+0.39
H-6	5.88	5.83	+0.05

for H-3 (δ 4.55, *d*, *J* = 6.6), and therefore with the proposed relative stereochemistry.

The absolute stereochemistry of **1** was established by derivatization with (*R*)- and (*S*)-α-methoxy-α-phenylacetic (MPA) acids (Seco et al., 2001). Subsequent NMR analysis of the Δδ values for the two MPA esters **1a** and **1b** provided clear evidence to assign the absolute stereochemistry at C-3 as *S*, Table 3. This information together with NOESY data established the absolute stereochemistry for recurvatiane A (**1**) as 1*R*, 3*S*, 6*R*, 7*S*, 10*R*.

The fragmentation pattern of the EIMS spectra of recurvatianes B-D, **2**–**4**, showed a molecular ion at *m/z* 290 $[M-AcOH]^+$ for each compound, as well as an ion at *m/z* 230 $[M-2AcOH]^+$. Furthermore, comparison of their NMR spectroscopic data with those of the known compound **5** (Bittner et al., 1989), also isolated in this work, suggested they are positional isomers, the main differences being a hydroxyl substituent at C-6 ($\delta_{H\text{-}6}$ 5.19, *s*, δ_C 71.6), C-12 (δ_H 4.12, *d*, *J* = 13.2; δ_H 4.20, *d*, *J* = 13.2, δ_C 66.1 ppm) and C-15 (δ_H 4.37, *dd*, *J* = 13.2, 1.3; δ_H 4.47, *dd*, *J* = 13.2, 1.6; δ_C 55.0), for **2**, **3**, **4**, respectively, instead of the corresponding acetate groups found in **5**. We have named compound **5** recurvatiane E.

The relative stereochemistry of compounds **2**–**5** were deduced by NOESY experiments and analysis of coupling constants. They all showed the same relative stereochemistry as compound **1**, also confirming the previously proposed (Bittner et al., 1989) for **5**. Since compounds **1**–**5** were isolated from a unique extract of *Perezia*, it should be expected that they all belong to the same enantiomeric series as recurvatiane A, **1**. Thus, the absolute stereochemistry of these compounds has been assigned as follows: recurvatiane B, **2**, 1*R*, 6*R*, 7*S*, 10*R*; recurvatiane C, **3**, 1*R*, 6*R*, 7*S*, 10*R*; recurvatiane D, **4**, 1*R*, 6*R*, 7*S*, 10*R*; and recurvatiane E, **5**, 1*R*, 6*R*, 7*S*, 10*R*.

Recurvatiane F, **6** was isolated as a colourless oil $[\alpha]_D^{20}$ − 50 (*c*, 0.03, CH_2Cl_2). Its EIMS spectrum showed a peak at *m/z* 305

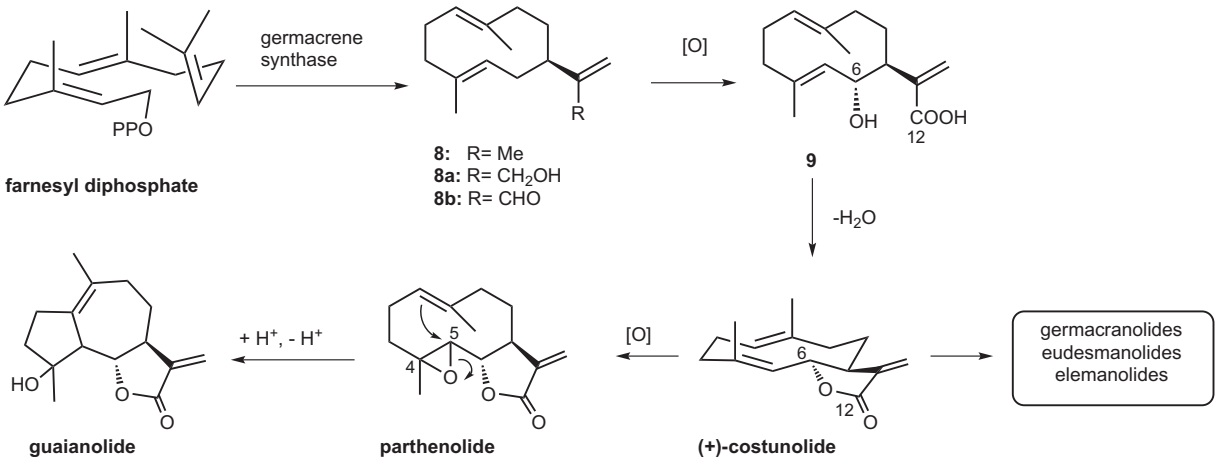


Fig. 3. Biosynthetic pathway for guaianolides in Asteraceae (De Kraker et al., 2002, 2003).

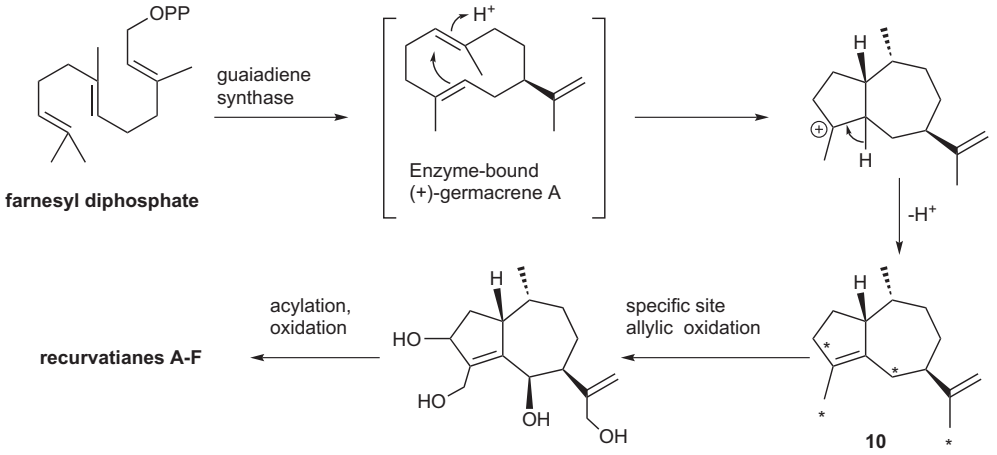


Fig. 4. Proposed biogenetic pathway for recurvatianes A–F.

$[M–C_2H_3O]^+$, which corresponds to the empirical formula $C_{19}H_{24}O_6$ (HREIMS *m/z* 305.1401 (calcd. for $C_{17}H_{21}O_5$ 305.1389)). Absorption for carbonyl groups at 1740 and 1720 cm^{-1} were observed in the IR spectrum. ^{13}C NMR and DEPT spectra of **6** (Table 1) showed the presence of 19 carbon signals assigned to 3 × CH_3 (two from acetyl groups), 5 × CH_2 (one olefinic, one bearing oxygen), 5 × CH (one bearing oxygen, one aldehyde) and six quaternary carbons (three carbonylic, three olefinic). The ^1H and ^{13}C NMR data were very similar to those of recurvatiane C, **3** particularly the chemical shifts of the carbon skeleton. Connectivity information obtained from COSY, HSQC and HMBC experiments unambiguously determined the planar structure of **6** as an aldehyde derived by oxidation of the hydroxymethyl side chain of **3**. NOESY experiments and coupling constants indicate the compound possesses the same relative stereochemistry as compounds **1**–**5**, thus we also propose its absolute configuration as 1*R*, 6*R*, 7*S*, 10*R*.

The complete biosynthesis of guaine sesquiterpenes has not yet been established (Drew et al., 2009). It is assumed that the backbone of guaiane sesquiterpene originates from a common germacrene precursor that is formed via the acetate-mevalonate-FPP pathway by a germacrene synthase, which cyclizes farnesyl diphosphate (FPP) to (+)-germacrene A, **8** (De Kraker et al., 1998), Fig. 3.

It has been hypothesized that a number of oxidative steps of the methyl of the isopropenyl moiety (**8** → **8a** → **8b** →, **9**, Fig. 3) followed by 6,12-lactonization to (+)-costunolide (De Kraker et al., 2002, 2003), precedes cyclization to the guaiane nucleus and to other sesquiterpene scaffolds, (Fig. 3) and also that guaianolides from Asteraceae are generated from parthenolide with a unique ring closure forming 5 and 7 carbon rings via a 4,5 epoxidation (De Kraker et al., 2002, 2003). Thus, (+)-costunolide, a 6α,7β-germacranolide, appears to be the presumed branching point in the biosynthesis of guaianolides from Asteraceae, as well as to the majority of sesquiterpene lactones found in plants, Fig. 3. The enzymes that catalyze the production of (+)-costunolide, have recently been identified (De Kraker et al., 1998, 2002; De-la-Cruz et al., 2007).

In the Asteraceae family, with the exception of the species *Warionia saharae* (Hilmi et al., 2002, 2003a,b; Katinas et al., 2008), the lactone ring of guaianolides is always 6α,7β, while guaianolides with a 6β,7β have only been found in Apiaceae (De Kraker et al., 2002). Therefore, as the recurvatianes A–F, for example **6**, *en route* to a guaianolide, possess a 6β,7β stereochemistry, which is unexpected for metabolites belonging to the Asteraceae family, an alternative biosynthetic precursor to (+)-costunolide could be considered. Accordingly, the set of guaiane-derived metabolites from *P. recurvata* suggests a possible strategy for the assembling of a guaiane backbone **10** with the same oxidation state as the original five-carbon building block precursor, Fig. 4. A guaiadiene synthase might be able to facilitate the transformation of FPP to **10**

through an enzyme-bound (+)-germacrane A (FPP→ (+)-germacrene A→ (**10**) (Rising et al., 2000). The guaiane skeleton **10** can be further modified by enzymes that catalyze precise and controlled allylic hydroxylation, oxidation, and acylation at the periphery of the bicyclic scaffold leading to the structural diversity of recurvatianes. Although **10** has not been isolated as a natural product it is worth noting that its ent-7-epimer, aciphyllene, has been isolated from the liverworth *Dumortiera hirsuta* (Saritas et al., 1998; Blay et al., 2007).

3. Conclusions

In conclusion, five novel highly oxygenated guaiane metabolites have been isolated from the Andean *P. recurvata*; their structures were determined by spectroscopic analysis and their absolute stereochemistry by derivatization with (*R*)- and (*S*)-α-methoxy-α-phenylacetic acids. This set of guaiane-based metabolites, five of which are new compounds, which we have named recurvatianes A–F, **1**–**6**, led us to propose an alternative biosynthetic pathway that suggests that Nature uses the strategy of assembling the guaiane core at the same oxidation state as the original building blocks. This suite of metabolites provides an example of a pathway by which molecular diversity is generated by the occurrence of specific oxidation reactions in the late biosynthetic steps of the frame structure.

4. Experimental

4.1. General procedures

Optical rotations were measured on a Perkin–Elmer model 343 Plus polarimeter using a Na lamp at 25 °C. IR spectra were recorded in a Perkin–Elmer 1650/FTIR spectrometer. ^{1}H NMR and ^{13}C NMR, HSQC, HMBC and COSY spectra were measured employing a Bruker AMX 500 instrument operating at 500 MHz for ^{1}H NMR and at 125 MHz for ^{13}C NMR. Two-dimensional NMR spectra were obtained using the standard Bruker software. EIMS and HRMS data were taken on a Micromass Autospec spectrometer. HPLC separations were performed on an Agilent 1200 Series Quaternary LC system (Jaigel-Sil semipreparative column, 10 μm, 20 × 250 mm) with hexane–EtOAc mixtures. The gel filtration column (Sephadex LH-20) used hexane-MeOH-CH_2Cl_2 (3:1:1) as eluent. The spray reagent for TLC was H_2SO_4–H_2O–AcOH (1:4:20). PCModel ((v. 7.0) Serena Software, Bloomington, IN) was used to calculate the energy-minimized model of recurvatianes A–F (**1**–**6**).

4.2. Biological material

Perezia recurvata was collected in the Sierra Baguales (Magellan Region, Chile) in November 2008. A voucher specimen has been deposited in the Chemistry Department of the Universidad de Magallanes (Punta Arenas, Chile) with code PZ-rec-2008/11.

4.3. Extraction and isolation

Dry samples (925 g) were extracted with MeOH at room temperature, and concentrated to give a dark residue (18.4 g). The extract was partitioned between EtOAc (3 × 100 ml) and water (75 ml). The EtOAc extracts were combined and concentrated to obtain a brown oil (8.9 g) that was separated on a Sephadex LH-20 column, followed by silica-gel chromatography. Fractions containing guaiane-derivatives, as indicated by their ^{1}H NMR spectra, were further separated by HPLC. Normal phase HPLC chromatography of fraction A using hexane:EtOAc (7:3) as eluent afforded the known compound **5** (16.1 mg) (t_R 35 min). Whereas from fraction C, compounds **1** (10.9 mg) (t_R 29 min), **2** (0.7 mg) (t_R 41 min) (t_R 41 min), **3** (4.3 mg) (t_R 35 min), **4** (3.5 mg) (t_R 37 min), and the aldehyde **6** (6.1 mg) (t_R 32 min), were separated after normal phase HPLC using hexane: EtOAc (6:4) as eluent.

4.3.1. Recurvatiane A [(−)-6β,12,15-triacetoxy -1β,7α,10β-H-guaia-4,11(13)-dien-3-ol] (**1**)

Colourless oil, $[\alpha]_D^{20} = -182$ (*c* 0.11, CH_2Cl_2); ^{1}H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 2 and 1; EIMS *m/z* 394 (1) $[M]^+$, 334 (2) $[M–AcOH]^+$, 274 (31) $[M–2AcOH]^+$, 214 (100) $[M–3AcOH]^+$; HREIMS *m/z* 394.2001 (calcd. for $C_{21}H_{30}O_7$ 394.1992); IR (film) ν_{max} 3460, 1740, 1373, 1234, 1030 cm^{-1}.

4.3.2. Recurvatiane B [(−)-6β,12-diacetoxy-15-hidroxy-1β,7α,10β-H-guaia-4,11(13)-dien-3-one] (**2**)

Colourless oil, $[\alpha]_D^{20} = -225$ (*c* 0.08, CH_2Cl_2); ^{1}H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 2 and 1; EIMS *m/z* 290 (29) $[M–AcOH]^+$, 230 (53) $[M–2AcOH]^+$, 201 (49), 91 (74); HREIMS *m/z* 290.1510 (calcd. for $C_{17}H_{22}O_4$ 290.1518); IR (film) ν_{max} 3431, 1736, 1375, 1234, 1032 cm^{-1}.

4.3.3. Recurvatiane C [(−)-12,15-diacetoxy-6β-hydroxy-1β,7α,10β-H-guaia-4,11(13)-dien-3-one] (**3**)

Colourless oil, $[\alpha]_D^{20} = -194$ (*c* 0.16, CH_2Cl_2); ^{1}H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 2 and 1; EIMS *m/z* 290 (8) $[M–AcOH]^+$, 230 (100) $[M–2AcOH]^+$, 91 (90); HREIMS *m/z* 290.1528 (calcd. for $C_{17}H_{22}O_4$ 290.1518); IR (film) ν_{max} 3420, 1740, 1371, 1232, 1028 cm^{-1}.

4.3.4. Recurvatiane D [(−)-6β,15-diacetoxy-12-hydroxy-1β,7α,10β-H-guaia-4,11(13)-dien-3-one] (**4**)

Colourless oil, $[\alpha]_D^{20} = -247$ (*c* 0.07, CH_2Cl_2); ^{1}H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 2 and 1; EIMS *m/z* 290 (50) $[M–AcOH]^+$, 230 (100) $[M–2AcOH]^+$, 215 (45), 91 (90); HREIMS *m/z* 290.1531 (calcd. for $C_{17}H_{22}O_4$ 290.1518); IR (film) ν_{max} 3423, 1736, 1375, 1234, 1030 cm^{-1}.

4.3.5. Recurvatiane E [(−)-6β,12,15-triacetoxy-1β,7α,10β-H-guaia-4,11(13)-dien-3-one] (**5**)

Colourless oil, $[\alpha]_D^{20} = -67$ (*c* 0.9, $CHCl_3$); lit (Bittner et al., 1989) $[\alpha]_D^{20} = -22$ (*c* 1.43, $CHCl_3$); ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Tables 2 and 1; EIMS *m/z* 392 (26) $[M]^+$, 349 (18) $[M–C_2H_3O]^+$, 332 (25) $[M–AcOH]^+$, 230 (100); HREIMS *m/z* 392.1844 (calcd. for $C_{21}H_{28}O_7$ 392.1835); IR (film) ν_{max} 1740, 1373, 1232, 1030 cm^{-1}. The physical and spectral data agree with the literature values (Bittner et al., 1989).

4.3.6. Recurvatiane F [(−)-6β,15-diacetoxy-3-oxo-1β,7α,10β-H-guaia-4,11(13)-dien-12-al] (**6**)

Colourless oil, $[\alpha]_D^{20} = -50$ (*c* 0.03, CH_2Cl_2); ^{1}H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Tables 2 and 1; EIMS *m/z* 305 (4) $[M–C_2H_3O]^+$, 277 (29), 246 (100); HREIMS *m/z* 305.1401 (calcd. for $C_{17}H_{21}O_5$ 305.1389); IR (film) ν_{max} 1740, 1720, 1230, 1029 cm^{-1}.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2010.11.021.

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