Diterpenoids from *Baccharis pingraea*

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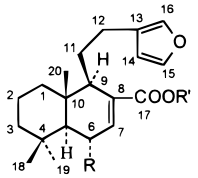
From the aerial parts of *Baccharis pingraea* the known furolabdane, angeloyl-gutierrezianolic acid (**1**); two novel diterpenoids, furolabda-6,8-dien-17-oic acid (**2**) and furolabd-7-en-17-oic acid (**3**); and the known linear diterpenoid, (10*E*)-centipedic acid (**4**), were isolated. LC/MS suggested the presence of gutierrezianolic acid (**5**). The structures of the new compounds were elucidated by 1D and 2D NMR methods.

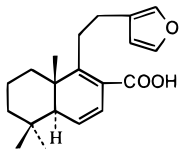
During our investigation of Latin American plants as sources of biologically active agents, we investigated the chemistry of *Baccharis pingraea* DC. (family Asteraceae, tribe Astereae) collected in Chile. This species has been studied extensively by Zdero *et al.*,[1,2] and variations of the chemical composition depending on the origin of the samples have been observed. Regardless of the origin, the accumulation of *ent*-labdane glycosides has been reported as an important chemotaxonomic feature of *B. pingraea*.[1,2] In this paper we report the isolation of two known furoditerpenoid acids, angeloyl-gutierrezianolic acid [3] (**1**) and (10*E*)-centipedic acid [4] (**4**), as well as two novel compounds, furolabda-6,8-dien-17-oic acid (**2**) and furolabd-7-en-17-oic acid (**3**), from a hexane-soluble fraction. The absolute stereochemistries of compounds **1**−**3** were not determined. LC/MS and TLC also suggested the presence of the known gutierrezianolic acid (**5**).[3,5]

Compound **1** was isolated as the major component of the hexane-soluble fraction. Its HRFABMS was in agreement with a molecular formula of $C_{25}H_{34}O_5$ (requires *m/z* 415.2484; found 415.2486). The appearance of an intense signal for a fragment $C_{20}H_{27}O_3$ (requires *m/z* 315.1946; found 315.1953) together with ^{1}H, ^{13}C, HMQC, and HMBC spectra suggested the presence of an angelate ester in **1**. Except for the absence of a methyl ester signal, the ^{1}H NMR spectrum of the free acid **1** is identical with the spectrum of angeloyl-gutierrezianolic acid methyl ester obtained from *Guterrezia espinosae* by Zdero *et al.*[3]

Because no optical rotation was reported for angeloyl-gutierrezianolic acid or its methyl ester, a comparison with the optical rotation of **1**, $[\alpha]^{25}_D$ +72° (*c* 1.0, $CHCl_3$), was not possible. However, Bohlmann *et al.* reported the optical rotation of a methyl ester acetate of gutierrezianolic acid isolated from *G. mandonii*.[5] We prepared this methyl ester acetate (**6**) from **1** by saponification of **1** and methylation of the resulting carboxylic acid **5** with diazomethane, followed by acetylation with acetic anhydride. Compound **6** had a positive optical rotation as reported for the corresponding compound prepared from gutierrezianolic acid, and the ^{1}H NMR spectral data of both compounds were identical.[5] Therefore, **5** is identical with gutierrezianolic acid from *G. mandonii*, which was assigned normal labdane stereochemistry by Bohlmann *et al.*[5] based only on the comparison of its optical rotation with the optical rotation of agathenoic acid and because of the frequent occurrence of normal labdanes in the tribe Astereae. Because *ent*-labdanes have been reported from *B. pingraea*,[1,2] which is also placed in the tribe Astereae, and because normal labdanes are generally rare in this genus,[6] no unambiguous assignment of the absolute stereochemistry of compound **1** is possible with the available data.



1 R = angeloyloxy, R' = H
3 R, R' = H
5 R = OH, R' = H
6 R = OAc, R' = Me



2

Three characteristic signals at δ_H 6.32, 7.24, and 7.31 in the ^{1}H NMR spectrum of **2**, together with the base peak in the LREIMS at *m/z* 81, revealed the presence of a furan ring. A molecular formula $C_{20}H_{26}O_3$ was determined by HREIMS (requires *m/z* 314.1882; found 314.1882). The signals of two vinylic protons at δ_H 6.45 (H-7) and 5.85 (H-6), which appeared as double doublets with coupling constants of 3.0 and 10.2 Hz, suggested the presence of a *cis*-disubstituted double bond next to an electron-withdrawing group. Two signals in the ^{13}C NMR spectrum at δ_C 163.7 and 123.2 indicated an additional tetrasubstituted double bond. A signal at δ_C 172.2 in the ^{13}C NMR spectrum suggested the presence of a carboxylic acid. A HMBC spectrum showed correlations for the methyl groups Me-18 and Me-19, as well as for H-6 to C-5. The methyl group Me-20 showed cross peaks to C-1, C-5, C-9, and C-10. Proton H-7 had three-bond correlations with carbons C-5, C-17, and C-9. Based on these results and by comparison of its ^{13}C NMR and DEPT spectra with those of **1**, the structure of **2** could be assigned as furolabda-6,8-dien-17-oic acid.

A molecular composition $C_{20}H_{28}O_3$ was determined for **3** by HREIMS (requires *m/z* 316.2038; found 316.2040). The ^{1}H NMR spectrum of **3** also showed the presence of signals characteristic for a furan ring at δ_H 6.24, 7.17, and 7.29. The base peak at *m/z* 82 in the LREIMS was in agreement with the presence of a furylmethylene group. A signal for one vinyl proton appeared at δ_H 6.92 suggesting the presence of a carboxylic acid at the same double bond. Based on a comparison of its ^{1}H and ^{13}C NMR spectra with those of **1**, we have assigned **3** as furolabd-7-en-17-oic acid. No assignment of the absolute stereochemistry of the related diterpenes **2** and **3** as either normal or *ent*-labdanes was made.

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For compound **4** a molecular composition $C_{20}H_{28}O_3$ was determined by HREIMS (requires *m/z* 316.2038; found 316.2034). From this formula, seven degrees of unsaturation were calculated, which were accounted for by the presence of a furan ring, a carboxylic acid, and three additional double bonds. Therefore, **4** is a linear diterpenoid acid. *E*-configurations for the double bonds in positions C-6 and C-10 followed from the chemical shifts of H-7 (δ_H 6.86) and the Me-20 (δ_C 15.9). Based on this evidence and HMQC and HMBC spectra, we assigned **4** the structure of (10*E*)-centipedic acid previously reported from *Gutierrezia resinosa*.[4]

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO P1020 polarimeter and IR spectra on a Buck Scientific 500 IR spectrometer. NMR spectra were recorded in $CDCl_3$ on a Varian Unity 300 NMR spectrometer at 300 (^{1}H) and 75.4 MHz (^{13}C) with residual $CHCl_3$ ($\delta_H = 7.24$) and $CDCl_3$ ($\delta_C = 77.0$) as references. HMBC spectra were acquired with $1/2J = 0.06$ s. LREIMS were obtained with a Hewlett−Packard 5988A (70 eV). HRFABMS and HREIMS were recorded on a JEOL HX 110 with a resolution of 10 000 with *m*-NBA as matrix for FAB spectra. Visualization of compounds **1**−**5** on Si gel TLC was carried out by spraying with a mixture of 0.5% anisaldehyde, 10% HOAc, and 5% H_2SO_4 in MeOH followed by heating at 120 °C for 10 min. Initially purple spots changed to a characteristic blue after several hours.

Plant Material. Aerial parts of *B. pingraea* DC. were collected and identified by Luis Gonzalez in April 1995, at Laguna Dolores, Departamento Colina, Region Metropolitana, Santiago, Chile. A voucher specimen (no. 0666) has been deposited at the herbarium of the Pontificia Universidad Católica de Chile, Santiago, Chile. Intellectual Property Rights Agreements for plant collections and collaborative research have been fully executed between The University of Arizona and the collaborating institution in this study.

Extraction and Isolation. The air-dried and ground plant material (150 g) was extracted repeatedly with CH_2Cl_2−MeOH (1:1). From the dried extract (13 g), 2.4 g of hexane-soluble material was obtained, which was then chromatographed on Si gel with 20% EtOAc and 0.1% HOAc in hexane. Two major fractions were collected, of which the one eluting earlier weighed 300 mg on drying and contained **2**−**4** and the one eluting later weighed 180 mg when dried and consisted mainly of **1**. The final isolation of 65 mg **1**, 7 mg **2**, 7 mg **3**, and 15 mg **4** was carried out on a HPLC system equipped with a Varian Star 9040 refractive index detector and a reversed-phase column (Alltech Econosil C_{18} − 10 μm, 10 × 250 mm) using a mixture of MeCN, MeOH, and H_2O (75:10:15) as isocratic eluent.

Preparation of Gutierrezianolic Acid Methyl Ester Acetate (6).[5] The angelate ester **1** (20 mg) was heated to reflux in 10% methanolic KOH for 8 h. After neutralization with HOAc and evaporation of the solvent, the residue was extracted with EtOAc to give the alcohol **5**. This product was methylated with excess CH_2N_2 in Et_2O and, after evaporation of the solvent, heated for 2 h at 100 °C in 1 mL of Ac_2O. The resulting gutierrezianolic acid methyl ester acetate (**6**) was purified by HPLC on an econosil Si gel column with 8% EtOAc in hexane.

Detection of Compound 5 by LC/MS. For the LC/MS detection of compound **5** a Finnegan TSQ 7000 mass spectrometer in APCI positive mode with a Si gel column (Alltech Econosil 5 μm, 4.6 × 250 mm), 20% EtOAc and 0.1% HCO_2H in hexane as mobile phase, a flow rate of 1 mL/min, and ion traces at *m/z* 297 $[M - 2H_2O + 1]^+$ and 271 were used. Under these conditions compound **5** appeared in the chromatogram at 20.5 min. Injection of the EtOAc-soluble portion of the CH_2Cl_2−MeOH extract of *B. pingraea* gave a peak with the same retention time, and a mass spectrum recorded at this time was identical to the mass spectrum of **5**.

Table 1. ^{13}C and ^{1}H NMR Data of Compounds **2** and **3**[a]

	2		**3**	
position	^{13}C	^{1}H	^{13}C	^{1}H
1a	34.2 t	1.48 m	39.4 t	1.01 m
1b		1.94 m		1.85 m
2a	18.8 t	1.61 m	18.5 t	1.49 m
2b		1.61 m		1.49 m
3a	40.7 t	1.18 m	41.9 t	1.15 m
3b		1.47 m		1.42 m
4	33.3 s		32.8 s	
5	50.7 d	2.00 t (3.0)	49.3 d	1.20 m
6a	127.9 d	5.85 dd (3.0, 10.2)	24.3 t	2.01 m
6b				2.20 m
7	123.6 d	6.45 dd (3.0, 10.2)	140.7 d	6.92 m
8	123.2 s		134.3 s	
9	163.7 s		50.6 d	2.10 m
10	41.0 s		36.9 s	
11a	30.1 t	2.50 m	29.2 t	1.44 m
11b		2.86 m		1.82 m
12a	25.8 t	2.57 m	26.5 t	2.37 m
12b		2.73 m		2.76 m
13	125.1 s		125.7 s	
14	110.9 d	6.32 m	111.1 d	6.24 m
15	142.7 d	7.31 t (1.8)	142.5 d	7.29 t (1.8)
16	138.7 d	7.24 s	138.6 d	7.17 s
17	172.2 s		173.7 s	
18	22.9 q	0.97 s	21.9 q	0.89 s
19	32.4 q	0.94 s	33.1 q	0.85 s
20	14.7 q	0.90 s	14.3 q	0.81 s

[a] ^{1}H chemical shifts were obtained from HMQC experiment, multiplicities and coupling constants from 1D ^{1}H NMR, and ^{13}C multiplicities from DEPT experiment.

Furolabda-6,8-dien-17-oic acid (2): colorless oil; $[\alpha]^{25}_D$ −35° (*c* 0.9, $CHCl_3$); IR ν_{max} (dry film) 1680, 1560, 1460, 1382, 1024, 871 cm^{-1}; ^{13}C and ^{1}H NMR, see Table 1; EIMS *m/z* 314 (3), 269 (4), 232 (4), 217 (18), 163 (20), 149 (52), 105 (52), 81 (100); HREIMS *m/z* 314.1882 ($[M]^+$ requires 314.1882).

Furolabd-7-en-17-oic acid (3): colorless oil; $[\alpha]^{25}_D$ −27° (*c* 0.9, $CHCl_3$); IR ν_{max} (dry film) 1686, 1457, 1384, 1020, 870 cm^{-1}; ^{13}C and ^{1}H NMR, see Table 1; EIMS *m/z* 316 (2), 234 (4), 191 (2), 149 (3), 124 (7), 109 (44), 82 (100); HREIMS *m/z* 316.2040 ($[M]^+$ requires 316.2038).

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