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Complete ^{1}H NMR assignments of pyrrolizidine alkaloids and a new eudesmanoid from *Senecio polypodioides*

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Chemical investigation of the aerial parts of *Senecio polypodioides* lead to the isolation of the new eudesmanoid 1β-angeloyloxyeudesm-7-ene-4β,9α-diol (1) and the known dirhamnosyl flavonoid lespidin (3), while from roots, the known 7β-angeloyloxy-1-methylene-8α-pyrrolizidine (5) and sarracine *N*-oxide (6), as well as the new neosarracine *N*-oxide (8), were obtained. The structure of 1 and 8 was elucidated by spectral means. Complete assignments of the ^{1}H NMR data for 5, 6, sarracine (7), and 8 were made using one-dimensional and two-dimensional NMR experiments and by application of the iterative full spin analysis of the PERCH NMR software. Copyright © 2014 John Wiley & Sons, Ltd.

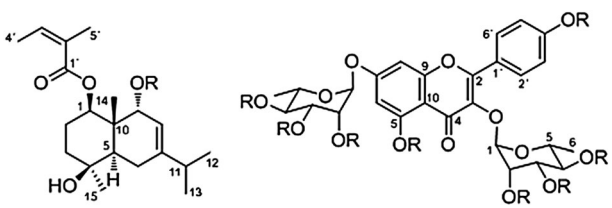
Keywords: *Senecio polypodioides*; 1β-angeloyloxyeudesm-7-ene-4β,9α-diol; 7β-angeloyloxy-1-methylene-8α-pyrrolizidine; sarracine *N*-oxide; sarracine; neosarracine *N*-oxide; iterative ^{1}H NMR analysis

Introduction

The genus *Senecio* (Asteraceae) has been extensively investigated for their natural compounds and biological activity. Chemical studies of several species have shown eremophilanoids and pyrrolizidine alkaloids (PAs) as the main secondary metabolites.[1] Some eremophilanoids have been described as herbivorous insect antifeedant,[2,3] active against some phytopathogenic fungi,[2] and as cytotoxic compounds.[4,5] In addition to the well-known toxicity to cattle and humans,[6] PAs have also been studied for a vast range of biological activities in ecological interactions,[7] including insect antifeedant properties.[8]

From the CH_2Cl_2 soluble fraction of the MeOH extract of *Senecio polypodioides*, treated with Zn°/H_2SO_4, the PAs platyphylline and platyphylline *N*-oxide were reported.[9]

In continuation of our studies related to natural compounds from the *Senecio* species,[3,10] the constituents of *S. polypodioides* were reinvestigated. From the aerial parts, we were able to isolate the new eudesmanoid 1β-angeloyloxyeudesm-7-ene-4β,9α-diol (**1**), along with the known dirhamnosyl flavonoid lespidin (**3**),[11] while from the roots, 7β-angeloyloxy-1-methylene-8α-pyrrolizidine (**5**),[12] sarracine *N*-oxide (**6**),[13] which after Zn° dust treatment gave sarracine (**7**),[14,15] and the new neosarracine *N*-oxide (**8**) were obtained. Structural assignment of **1–8** was achieved by spectral means. In addition, complete assignments of the ^{1}H NMR data for **5–8** were made by application of the iterative full spin analysis using the PERCH NMR software.[16]



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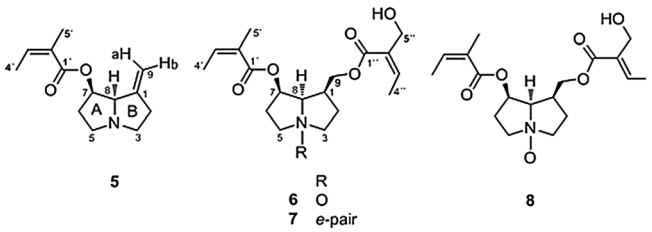

Results and Discussion

The structure of **1** was determined after MS and NMR analysis. The molecular formulae were established as $C_{20}H_{32}O_4$ by HREIMS that showed *m/z* 336.2308 (calcd 336.2301). Also, the exact mass of an ion at *m/z* 318.2192 (calcd for $C_{20}H_{30}O_3$ 318.2195) corresponding to $M^+–H_2O$ was observed. The ^{1}H NMR spectrum of **1** showed an olefinic signal at δ 6.13 (qq, J = 7.3, 1.5 Hz, H-3′), which, together with two methyl group signals at δ 2.01 (dq, J = 7.3, 1.5 Hz, Me-4′) and 1.90 (quint, J = 1.5 Hz, Me-5′), were indicative of an angeloyloxy group.[17] The ^{1}H NMR spectrum and the gHSQC experiment further showed five methine groups at δ 5.53 (dddd, J = 6.0, 2.0, 1.0, and 1.0 Hz, H-8), 5.17 (dd, J = 12.0, 4.2 Hz, H-1), 2.24 (br hept, J = 6.0 Hz, H-11), 3.41 (br d, J = 6.0 Hz, H-9), and 1.80 (dd, J = 11.5, 5.8 Hz, H-5); three methylene groups at δ 2.16 (m, H-2*R*) and 1.67 (m, H-2*S*), 1.76 (ddd, J = 17.6, 3.8, and 3.8 Hz, H-3*S*) and 1.64 (ddd, J = 17.6, 13.8, and 3.8 Hz, H-3*R*), and 2.12 (m, H-6*S*) and 2.09 (m, H-6*R*). In addition, there are a tertiary methyl group at δ 0.94 (s, Me-14) and two secondary methyl groups that correspond to an isopropyl moiety at δ 1.06 (d, J = 6.0 Hz) and 1.04 (d, J = 6.0 Hz) attached to C-7. These data, and the observed *g*HMBC correlations (Table 1), were consistent with the eudesmanoid **1**. The stereochemistry shown in the molecular structure was assigned taking into account biogenetic considerations[18] and the NOESY two-dimensional (2D) correlations observed between the signal at δ 3.41 (H-9) and that at δ 0.94 (Me-14), the signal at δ 5.17 (H-1) and those at 1.80 (H-5) and 1.67 (H-2*S*), and the signal at δ 1.23 (Me-15) and that at 2.09 (H-6*R*), in concordance with other eudesmane derivatives isolated from the *Senecio* species.[19,20] Complete ^{1}H and ^{13}C NMR assignments (Table 1) were made with the aid of one-NMR and two-NMR experiments including gCOSY, gHSQC, gHMBC, and NOESY 2D. Additional structural evidence followed from the *O*-acetyl derivative **2**, whose ^{1}H NMR spectrum showed the acetyl methyl group singlet at δ 2.02, while the nine signals shifted to δ 4.84 (d, 5.7 Hz). ^{1}H and ^{13}C NMR signal assignments (Table 1) followed after one-NMR and two-NMR experiments.

From the aerial parts' methanol extract, which was negative to the Dragendorff test, lespedin (**3**) was isolated as a yellow powder. Its identification was possible after one-dimensional (1D) and 2D NMR experiments and by comparison of its spectral data with those described.[11] Further evidence was obtained after assignment of the peracetylated derivative **4**, whose ^{1}H NMR spectrum showed eight acetyl group signals at δ 2.33 (s, 3H), 2.20 (s, 3H), 2.12 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), and 1.99 (s, 6H). Individual NMR assignment for the rhamnose residues followed after 1D and 2D NMR experiments starting with the COSY spectrum using the anomeric proton signals as a starting point. The ^{1}H and ^{13}C NMR assignments are given in the Experimental section.

Table 1. NMR data for **1–2** in $CDCl_3$ at 500 MHz, δ (J in Hz)

	1			**2**		
Atom	δ ^{1}H	δ ^{13}C	HMBC	δ ^{1}H	δ ^{13}C	HMBC
1	5.17 (dd, 12.0, 4.2)	74.8	C-2, C-9, C-10, C-1′	5.04 (dd, 11.9, 3.9)	73.4	C-14
2*S*	1.67 (m)	23.5		1.73 (m)	22.9	C-1, C-4
2*R*	2.16 (m)			1.75 (m)		C-1, C-4
3*R*	1.64 (ddd, 17.6, 13.8, 3.8)	39.1	C-2	1.71 (m)	39.1	
3*S*	1.76 (ddd, 17.6, 3.8, 3.8)		C-2	1.74 (m)		
4		71.0			70.9	
5	1.80 (dd, 11.5, 5.8)	39.9	C-6, C-10, C-14	1.71 (dd, 11.8, 5.2)	41.5	C-6, C-10, C-14
6*R*	2.09 (m)	23.9	C-5, C-7, C-8, C-10, C-11	2.09 (ddd, 17.1, 5.2, 0.9)	23.6	C-5, C-7, C-8, C-10
6*S*	2.12 (m)		C-5, C-7, C-8, C-10, C-11	2.19 (ddd, 17.1, 11.8, 2.1)		C-5, C-7, C-8
7		147.2			149.4	
8	5.53 (dddd, 6.0, 2.0, 1.0, 1.0)	117.7	C-6, C-9, C-10, C-11	5.50 (dddd, 5.7, 2.1, 1.1, 1.1)	115.0	C-6, C-10, C-11, C-9
9	3.41 (d, 6.0)	69.7	C-5, C-7, C-8	4.84 (d, 5.7)	71.9	C-5, C-7, C-8, C-10, C-14
10		41.8			39.9	
11	2.24 (br hept, 6.0)	34.8	C-5, C-8, *i*Pr(Me)	2.24 (br hept, 6.9)	34.9	C-7, C-8, C-12
12	1.06 (d, 6.0)	21.6	C-7, C-11	1.05 (d, 6.9)	21.3	C-7
13	1.04 (d, 6.0)	21.2	C-7, C-11	1.03 (d, 6.9)	21.1	C-7
14	0.94 (s)	12.4	C-1, C-5, C-9, C-10	1.11 (s)	12.7	C-1, C-5, C-9, C-10
15	1.23 (s)	29.7	C-4	1.26 (s)	29.9	C-4, C-5
1′		169.1			167.4	
2′		127.6			128.3	
3′	6.13 (qq, 7.3, 1.5)	139.1	C-4′, C-5′	6.01 (qq, 7.2, 1.5)	137.4	C-5′
4′	2.01 (dq, 7.3,1.5)	16.0	C-2′, C-3′	1.95 (dq, 7.2, 1.5)	15.7	C-2′,C-3′
5′	1.90 (quint, 1.5)	20.7	C-1′, C-2′, C-3′	1.87 (q, 1.5)	20.6	C-1′, C-2′, C-3′

2: Ac; δ ^{1}H 2.02 (s, CH_3C O); δ ^{13}C 170.6 (CH_3C O), 21.6 (CH_3C O).

Table 2. ^{1}H NMR parameters for ring A of **5–8** in $CDCl_3$ at 500 MHz

Atom	5[a]	6	7	8
H-1	—	3.497	2.779	3.478
H-2*R*	2.528	1.995	1.834	1.993
H-2*S*	2.555	2.518	1.883	2.513
H-3*R*	2.760	3.881	2.805	3.876
H-3*S*	3.139	3.747	3.176	3.752
H-9*R*	4.862[b]	4.269	4.258	4.259
H-9*S*	5.040[c]	4.404	4.416	4.384
H-3″	—	6.394	6.372	6.953
H-4″	—	2.041	2.055	1.923
H-5″*R*	—	4.225	4.245	4.343
H-5″*S*	—	4.225	4.224	4.342
$J_{1,2R}$	—	10.21	11.24	10.26
$J_{1,2S}$	—	8.25	7.65	8.18
$J_{1,9R}$	—	9.19	8.26	9.24
$J_{1,9S}$	—	6.97	7.68	7.00
$J_{2R,2S}$	−15.36	−12.63	−11.74	−12.72
$J_{2R,3R}$	7.45	8.75	8.83	8.67
$J_{2R,3S}$	7.34	8.11	9.13	8.16
$J_{2S,3R}$	7.44	4.82	2.90	4.85
$J_{2S,3S}$	5.48	7.76	6.99	7.74
$J_{3R,3S}$	−9.72	−11.65	−10.75	−11.66
$J_{9R,9S}$	0.58	−11.03	−10.90	−10.98
$J_{3'',4''}$	—	7.26	7.26	7.25
$J_{3'',5''R}$	—	−0.99	−0.93	−0.45
$J_{3'',5''S}$	—	−0.98	−1.06	−0.52
$J_{4'',5''R}$	—	0.87	0.98	0.47
$J_{4'',5''S}$	—	0.87	0.88	0.43
$J_{5''R,5''S}$	—	−12.00	−12.62	−12.00

[a]Long-range coupling constants in **5** are $J_{2R,9Z} = -2.32$; $J_{2R,9E} = -2.20$; $J_{2S,9Z} = -2.02$; $J_{2S,9E} = -2.10$; $J_{8,2R} = -0.82$; $J_{8,2S} = -1.50$; $J_{8,9Z} = -1.86$; $J_{8,9E} = -1.97$.

[b]For (*Z*) atom.

[c]For (*E*) atom.

Table 3. ^{1}H NMR parameters for ring B of **5–8** in $CDCl_3$ at 500 MHz

Atom	5	6	7	8
H-5*R*	3.262	3.794	3.298	3.806
H-5*S*	2.817	3.831	2.733	3.828
H-6*R*	2.159	2.081	2.021	2.083
H-6*S*	2.085	2.911	2.086	2.912
H-7	5.508	5.722	5.316	5.699
H-8	4.088	4.086	3.571	4.084
H-3′	6.026	6.213	6.105	6.215
H-4′	1.967	2.025	2.009	2.026
H-5′	1.830	1.922	1.909	1.920
$J_{5R,5S}$	−10.05	−11.70	−10.10	−11.75
$J_{5R,6R}$	2.32	4.00	1.51	3.94
$J_{5R,6S}$	8.32	8.84	8.26	8.77
$J_{5S,6R}$	6.49	7.10	6.33	7.07
$J_{5S,6S}$	10.77	9.59	11.25	9.68
$J_{6R,6S}$	−13.77	−14.75	−14.04	−14.73
$J_{6R,7}$	1.44	1.48	1.01	1.48
$J_{6S,7}$	4.76	5.35	3.91	5.34
$J_{7,8}$	4.55	5.68	3.65	5.70
$J_{8,1}$	—	8.59	8.12	8.61
$J_{3',4'}$	7.25	7.27	7.25	7.26
$J_{3',5'}$	−1.47	−1.48	−1.51	−1.53
$J_{4',5'}$	1.58	1.55	1.58	1.56

Pyrrolizidine alkaloids **5** and **6** were isolated throughout open silica gel chromatography from the $CHCl_3$ soluble fractions of the root MeOH extract. The main fraction obtained using CH_2Cl_2–MeOH (97 : 3) as an eluent gave a positive Dragendorff test and was subjected to TLC separation to afford **5**, whose ^{1}H and ^{13}C NMR spectra showed an angeloyloxy group,[17] an exocyclic double bond, and additional four methylene groups and two methine groups. Total assignment was made with the aid of a one-NMR and two-NMR experiment, and its identification was confirmed by comparison with described NMR data.[12,21]

By default, PERCH calculations provide chemical shift and coupling constant values with six and five decimal places, respectively. The experimental 500-MHz spectra were acquired with a digital resolution better than 0.3 Hz, and therefore, chemical shifts and coupling constant values with three and two digits after a decimal point, respectively, constitute a proper description as has been done previously.[22–26]

The ^{1}H NMR spectrum of the compound isolated from the fractions eluted with CH_2Cl_2–MeOH (4 : 1) showed signals for an angeloyloxy group and an additional sarracinoyloxy group[14] that, along with a methylene group as part of an ABX system at δ 4.404 (H-9*S*), 4.269 (H-9*R*), and 3.497 (H-1), suggest a diester PA. The downfield ^{1}H (Tables 2 and 3) and ^{13}C NMR (Experimental section) chemical shifts for the CH_2-3, CH_2-5, and CH-8 signals in comparison with NMR data for analogous free bases[27,28] advised the *N*-oxide **6**.[13] Although this compound is referred to in several papers[9,13,29], no detailed NMR data are available. The relative stereochemistry followed from NOESY 1D experiments, and further evidence was obtained after Zn° dust reduction to afford the amine **7**, which was identified by comparison of ^{1}H and ^{13}C NMR data with those described.[14,15]

The ^{1}H NMR spectrum of a fraction mainly containing **6** showed a small signal at δ 6.953 as a broad quartet, suggesting the presence of a neosarracinoyl group. After several failed purification attempts by column chromatography (CC), a sample of this mixture was subjected to HPLC separation using a reverse phase semipreparative C18 column, and CH_3CN–H_2O (53 : 47) mixtures adjusted to 7.5 pH with an 15-mM NH_4OH solution as the eluent, to afford, in addition of **6**, 0.6 mg of a compound whose ^{1}H NMR spectrum showed signals for an angeloyl group. The additional vinyl signal at δ 6.953 (H-3″), along with methylene group signals at δ 4.343 (H-5″*R*) and 4.342 (H-5″*S*), and a methyl group at δ 1.923 (Me-4″) were consistent with a neosarracinoyl group.[15] Other signals for the pyrrolizidine bicycle were almost identical to those of sarracine *N*-oxide (**6**). Furthermore, the ^{13}C NMR spectrum showed a signal at δ 86.5 (C-8), which is considered as a diagnostic signal for PAs *N*-oxide.[14,30] These data suggest the presence of neosarracine *N*-oxide (**8**).

The ^{1}H NMR substituent chemical shifts (SCSs) from **7** to **6** are 1.076, 0.571, 0.496, 1.098, and 0.515 ppm for the H-3*R*, H-3*S*, H-5*R*, H-5*S*, and H-8 signals, respectively, while ^{13}C NMR SCSs are 15.0, 14.6, and 17.6 for the C-3, C-5, and C-8 signals, respectively. Thus, complete ^{1}H and ^{13}C NMR assignments of neosarracine *N*-oxide (**8**) were possible by applying these SCS values and by comparing NMR data of **6**, **7**, and those described for neosarracine.[15]

Since the early ^{1}H NMR study of 7-hydroxy-1-hydroxymethyl PA stereoisomerics,[31] the $J_{1,8}$ and $J_{7,8}$ values and the sum of the J_7 values estimated from the signal width at half height have been used to establish the configuration in saturated pyrrolizidines,[14,32,33]

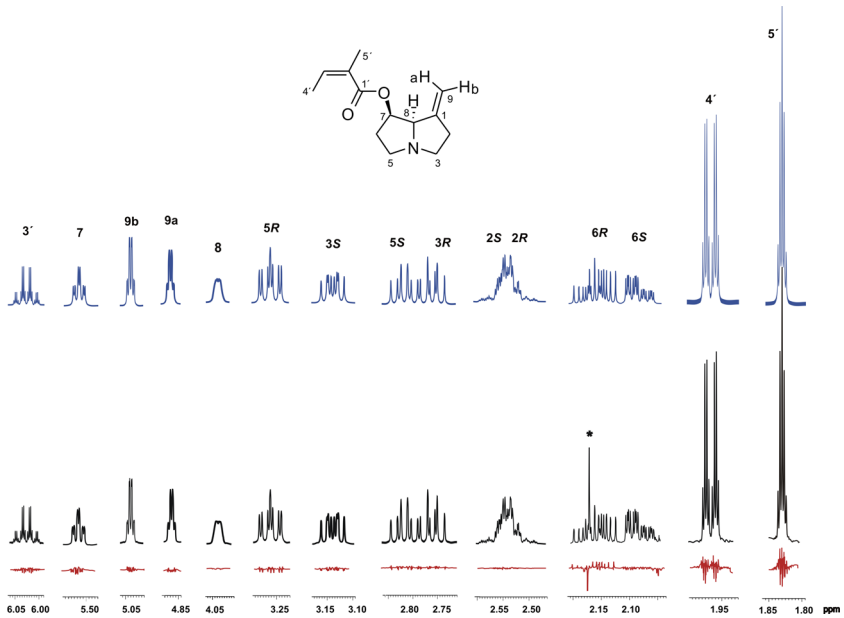

Figure 1. Comparison of the PERCH calculated (top) and the experimental (center) ^{1}H NMR of **5** (in $CDCl_3$ at 500 MHz). Residuals are shown in the bottom plot.

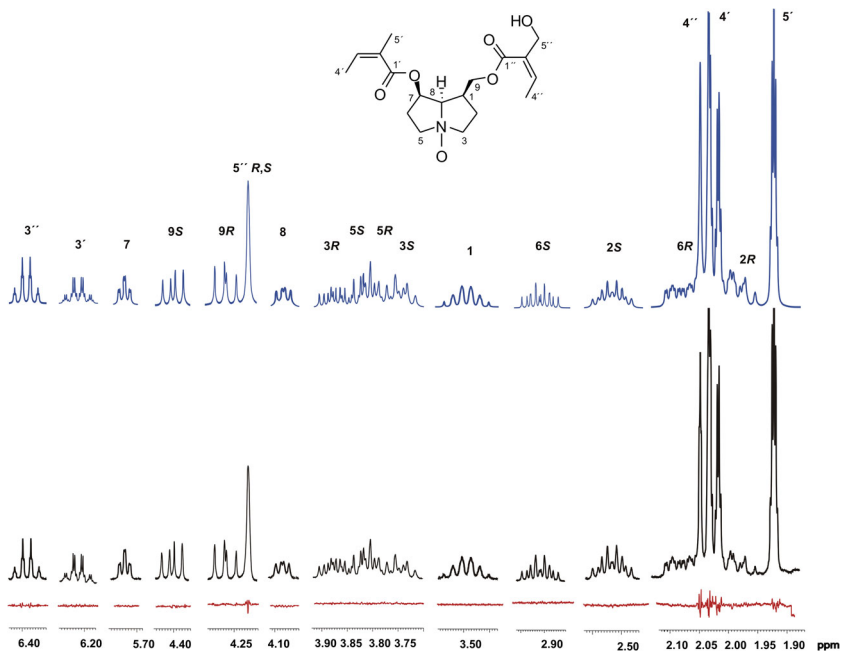

Figure 2. Comparison of the PERCH calculated (top) and the experimental (center) ^{1}H NMR of **6** (in $CDCl_3$ at 500 MHz). Residuals are shown in the bottom plot.

and no detailed measurement of coupling constant values for this type of compounds is available. The presence of small allylic coupling constants and the overlapping of several signals in **5–8** difficult the total coupling constant description. Complete ^{1}H NMR analysis was therefore carried out using the PERCH NMR software, as recently described for some natural products.[22–26] The 500-MHz free induction decay data for **5–8** were used for the preparation of the experimental frequency domain spectra, which were subjected to phase and baseline correction in the preparation module of the PERCH shell. The molecular structures of **5–8** were constructed using the PERCH molecular modeling software and subjected to Monte Carlo analysis using molecular mechanics geometry optimization. After conformational analysis, the respective minimum energy structures for **5–8** were used to predict the initial δ values, as well as the sign and magnitude of coupling constants. Some known chemical shifts and coupling constant values were manually adjusted before the predicted δ_H and $J_{H,H}$ values were optimized in the PERCHit shell using the total-line-shape fitting (T) mode. The iteration process for **5–8** was repeated until convergence was reached, and the total root-mean-square deviation (rms) values were 0.10, 0.06, 0.05, and 0.10%, respectively. Total rms represents the overall 'root mean square' between theoretical and experimental spectra. The results of the ^{1}H NMR full analysis of **5–8** are summarized in Tables 2 and 3 and Figs 1–4.

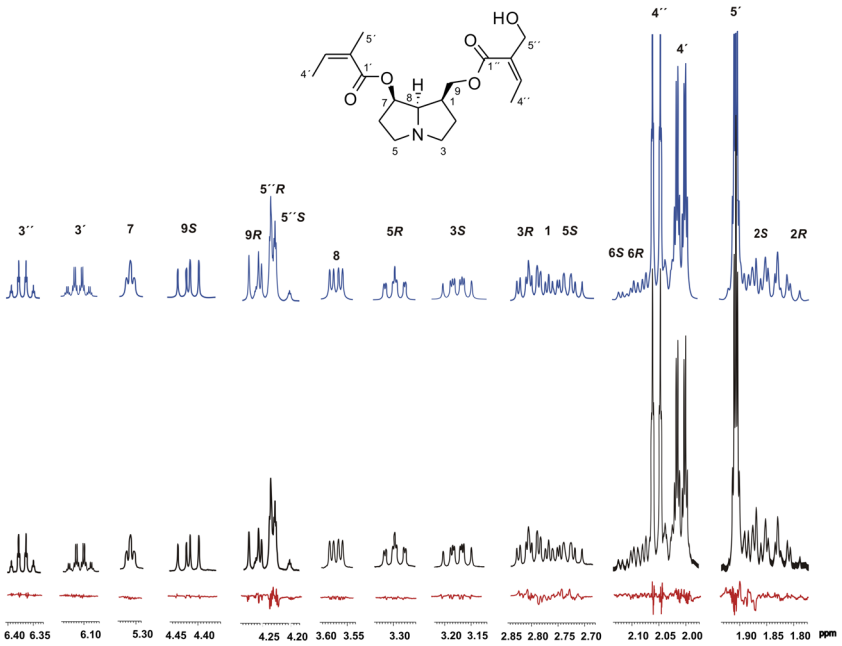

Figure 3. Comparison of the PERCH calculated (top) and the experimental (center) ^{1}H NMR of **7** (in $CDCl_3$ at 500 MHz). Residuals are shown in the bottom plot.

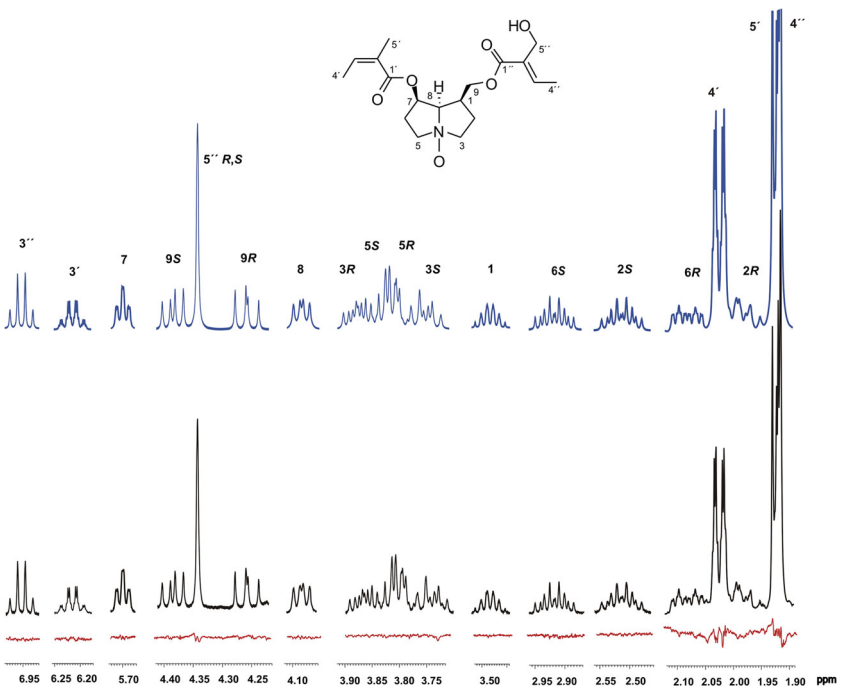

Figure 4. Comparison of the PERCH calculated (top) and the experimental (center) ^{1}H NMR of **8** (in $CDCl_3$ at 500 MHz). Residuals are shown in the bottom plot.

In an attempt to carry out a conformational analysis, molecular models of **5–8** were built and subjected to the Monte Carlo protocol using MMFF94. The resulting dihedral angles for the main conformers of **5–8** were subjected to Altona evaluation[34] to obtain coupling constant values that were weighed according to the conformational distribution. The results were not in agreement with the experimental values, presumably because of the large conformational freedom, as observed in our laboratory for other compounds containing five-member rings.

Experimental

General

Melting points were determined on an Electrothermal capillary melting point apparatus and are uncorrected. Optical rotations were measured on a PerkinElmer 341 polarimeter. IR spectra were acquired on a PerkinElmer 2000 FT-IR spectrophotometer. High-resolution mass spectra were recorded, using the electron

impact mode (70 eV), on a Jeol GCmateII spectrometer. For column chromatography, Natland silica gel (100–200 mesh ASTM) was used. TLC was performed on precoated silica gel aluminum sheets (silica gel 60 F_{254}, 0.20 mm, Merck). Fractions and pure compounds were monitored by a Dragendorff reagent, UV (254 nm), and by a ceric sulfate reagent followed by heating. HPLC purification was achieved on a Varian Prostar 215 chromatograph using a semipreparative Prevail C18 5 μ column with a length of 250 mm and internal diameter of 10 mm and a Varian Prostar 320 UV–vis detector.

NMR data

All NMR experiments were recorded on a Varian System 500 spectrometer at 298 K operating at 500 and 125 MHz for ^{1}H and ^{13}C, respectively. Detections were carried using $CDCl_3$ for **1–2**, **4–8**, and MeOH-d_4 for **3** containing 0.03% TMS. The g-COSY, NOESY 2D, g-HSQC, and g-HMBC experiments were carried out with standard pulse sequences provided by the spectrometer manufacturer. NOESY experiments were obtained, after sample degassing with simultaneous slow bubbling of N_2 and ultrasound during 20 min, using a preacquisition delay of 1 s, 256 transients, acquisition time of 2 s, 32-k data, and a mixing time of 1.5 s. ^{1}H NMR spectra for PERCH simulation of **5–8** were acquired using a 90° pulse. Four transients with spectral widths of 9.5 ppm and 32-k data points were collected for **5**, **6**, and **8**, while for **7**, a spectral width of 16 ppm and 128-k data points were used, providing digital resolutions of 0.29 and 0.12 Hz/point, respectively.

^{1}H NMR full spin-spin analysis

Full spin-spin simulation of **5–8** was achieved using the PERCH software (PERCH Solutions Ltd., Kuopio, Finland). The ^{1}H NMR experimental spectra were imported and subjected to phase and baseline correction, peak picking, and integration in the preparation module (PAC) into the PERCH shell. Molecular models for **5–8** were built using the molecular modeling software (MMS), and after geometry optimization, they were submitted to Monte Carlo analysis. The most stable conformer was used to obtain the initial calculated spectra; next, some known coupling constant and chemical shift values were incorporated in the parameters table of the graphical spectral parameters editor (PMS). The optimization of the spectral parameters was carried out using the total-line-shape fitting (T) mode in the PERCH iterator until an excellent agreement between the experimental and calculated spectra was obtained. The total root-mean-square errors were 0.10, 0.06, 0.05, and 0.1% for **5**, **6**, **7**, and **8**, respectively.

Plant material

Roots and aerial parts of *S. polypodioides* (Greene) were collected from San Miguel Suchixtepec, Miahuatlan, Oaxaca, Mexico, in March 2009. A voucher specimen (65049) is deposited in the Herbarium of Forest Sciences, Universidad Autónoma de Chapingo, Texcoco, Mexico.

Extraction and isolation

Air-dried and powdered aerial parts of *S. polypodioides* (1.08 kg) were successively extracted with 3.5 l of hexane (3 × 6 h), EtOAc (3 × 6 h), and MeOH (3 × 6 h) under reflux. Filtrates were evaporated to dryness under reduced pressure to afford 9.6 (0.89%), 15.3 (0.89%), and 80.0 g (7.42%), of hexane, EtOAc, and MeOH extracts, respectively.

The hexane extract was defatted by precipitation with MeOH. After solvent evaporation, 6.5 g was obtained, a portion (3.5 g) of which was chromatographed over silica gel eluting with hexane–EtOAc mixtures. The fraction eluted with 4 : 1 mixtures (193.1 mg) was purified by TLC (20 × 20 cm) eluting with hexane–EtOAc (7 : 3) to afford 67.6 mg of **1**.

A portion of the aerial parts' methanol extract (9 g) was chromatographed over silica gel using hexane–EtOAc gradients followed by EtOAc–MeOH. Fractions eluted with EtOAc–MeOH (3 : 2) afford 47.9 mg of flavonoid **3**.[11]

Air-dried powdered roots of *S. polypodioides* (1.34 kg) were successively extracted with 3.5 l of hexane, EtOAc, and MeOH under reflux (3 × 6 h). Solvents were evaporated under reduced pressure to yield 2.75 (0.20%), 11.88 (0.88%), and 118.0 g (8.8%) of the hexane, EtOAc, and MeOH extracts, respectively.

A sample of 20 g of the dried methanol extract was suspended in water (200 ml) and extracted with $CHCl_3$ (200 ml × 4) to give, after solvent evaporation, 4.6 g of a dark residue. This residue was chromatographed over silica gel (200 g) using a CH_2Cl_2–MeOH gradient collecting fractions of 50 ml. The composition of the 384 obtained fractions was monitored by TLC, and those chromatographically similar fractions were combined to yield four main fractions (A–D).

Fraction B obtained from CH_2Cl_2–MeOH (97 : 3) (54 mg) was rechromatographed over silica gel to give a main fraction that revealed a positive Dragendorff test and was further purified by TLC using CH_2Cl_2–MeOH (7 : 3) to afford 18.5 mg of **5**.[12]

Fraction C (341 mg) obtained from the CH_2Cl_2–MeOH (4 : 1) eluates gave a white solid, which was slowly precipitated from a hexane–CH_2Cl_2 solution to afford 300 mg of **6** (mp 119.6–120.4 °C, recrystallized from acetone 125–126 °C[13]).

Fraction D (100 mg) obtained from CH_2Cl_2–MeOH (3 : 1) eluates gave a white solid. After several failed purification attempts by CC, 20 mg of this fraction was subjected to HPLC separation using a semipreparative reverse phase C18 5 μ column with a length of 250 mm and internal diameter of 10 mm and CH_3CN–H_2O (53 : 47) mixtures adjusted to 7.5 pH with a 1.5-mM NH_4OH solution as an eluent to afford 0.6 mg of **8** (t_R = 22.3 min) and additional 10.8 mg of **6** (t_R = 25.6 min).

Compounds

*1β-Angeloyloxyeudesm-7-ene-4β,9α-diol (**1**)*

Colorless oil; UV (EtOH) λmax (log ε) 201 (4.2) and 214 (4.1); IR ($CHCl_3$) νmax 3467, 3254, 2996, 2935, 1722, 1458, 1384, 1267, 1244, 1158, and 1080/cm; and $[\alpha]_{589}$ −12.9, (c 1.6, $CHCl_3$). ^{1}H and ^{13}C NMR data are given in Table 1. EIMS: *m/z* (rel int) 336 $[M]^+$ (2), 318 (8), 279 (20), 235 (23), 217 (28), 200 (19), 175 (50), 149 (77), 107 (37), and 83 (100); EIHRMS: *m/z* 336.2308 $[M]^+$, calcd. for $C_{20}H_{32}O_4$, 336.2301; 318.2192 $[M^+–H_2O]$, calcd. for $C_{20}H_{30}O_3$, 318.2192.

*9-O-Acetyl-1β-angeloyloxyeudesm-7-ene-4β,9α-diol (**2**)*

A solution of **1** (17.5 mg) in pyridine (0.1 ml) was treated with Ac_2O (0.3 ml), allowed to stand overnight at room temperature, poured over ice H_2O, and extracted with EtOAc. The organic layer was washed with HCl 10%, H_2O, aqueous $NaHCO_3$, and H_2O, dried over anh. Na_2SO_4, filtered, and evaporated under vacuum. The residue was subjected to TLC purification using hexane–EtOAc (9 : 1) mixtures as the eluent to afford 17.3 mg (87.9%) of **2** as colorless oil. ^{1}H and ^{13}C NMR data are given in Table 1. EIMS: *m/z* (rel int) 378 $[M]^+$ (2), 335 (57), 218 (54), 200 (23), 175 (38), 160 (65), 133

(37), and 83 (100); EIHRMS: *m/z* 378.2406 $[M]^+$, calcd. for $C_{22}H_{34}O_5$, 378.2348; 335.2227 $[M^+–Ac]$, calcd. for $C_{20}H_{31}O_4$, 335.2222.

*Lespidin octaacetate (**4**)*

A solution of 12.5 mg of **3** in pyridine (0.3 ml) was treated with acetic anhidride (0.6 ml) at 90 °C for 43 h. After reaction workup as for **2**, the crude reaction mixture was purified by TLC using hexane–EtOAc (1 : 1) as an eluent to give 10.6 mg (53.6%) of **4**. Amorphous yellow powder; mp 72.3–73.0 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.91 (*d*, *J* = 8.9 Hz, 2H, H-2′ and H-6′), 7.29 (*d*, *J* = 8.9 Hz, 2H, H-3′ and H5′), 7.09 (*d*, *J* = 2.4 Hz, 1H, H-8), 6.79 (*d*, *J* = 2.4 Hz, 1H, H-6), 7.91 (*d*, *J* = 8.9 Hz, 2H, H-2′ and H-6′), 5.64 (*dd*, *J* = 3.4 and 1.8 Hz, H-2 Rha7), 5.56 (*d*, *J* = 1.8 Hz, H-1 Rha7), 5.54 (*d*, *J* = 1.8 Hz, H-1 Rha3), 5.26 (*dd*, *J* = 10.0 and 3.4 Hz, H-3 Rha3), 5.18 (*dd*, *J* = 9.8 and 9.8 Hz, H-4 Rha3), 4.93 (*dd*, *J* = 10.0 and 10.0 Hz, H-4 Rha7), 3.92 (*dq*, *J* = 9.8 and 6.2 Hz, H-5 Rha3), 3.32 (*dq*, *J* = 10.0 and 6.2 Hz, H-5 Rha7), 1.24 (*d*, *J* = 6.2 Hz, Me-6 Rha3), and 1.24 (*d*, *J* = 6.2 Hz, Me-6 Rha3). Acetyl groups δ 2.33 (s, 3H), 2.20 (s, 3H), 2.12 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), and 1.99 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.2 (C-4), 159.2 (C-7), 157.5 (C-9), 155.0 (C-2), 152.7 (C-4′), 150.9 (C-5), 136.9 (C-3), 130.1 (C-2′ and C-6′), 127.6 (C-1′), 122.1 (C-3′ and C5′), 112.9 (C-10), 101.8 (C-8), 98.2 (C-1 Rha3), 95.8 (C-1 Rha7), 70.5 (C-4 Rha3), 70.4 (C-4 Rha7), 69.2 (C-2 Rha3), 69.1 (C-2 Rha7), 68.9 (C-3 Rha4), 68.5 (C-3 Rha3), 68.4 (C-5 Rha7), 68.0 (C-5 Rha3), 17.7 (C-6 Rha3), and 17.0 (C-6 Rha7). Acetyl groups: CH_3CO δ 170.0, 169.94, 169.91, 169.88, 169.59, 169.53, 168.67; CH_3CO δ 21.11, 21.09, 20.87, 20.80, 20.75, 20.71, 20.69, and 20.65.

*Sarracine N-oxide (**6**)*

mp 119.6–120.4 °C (lit.,[13] recrystallized from acetone 125–126 °C); UV (EtOH) λmax (log ε) 217 (4.0). $[\alpha]_{589}$ −89, (c 0.26, EtOH). Complete ^{1}H NMR assignments are given in Tables 2 and 3; ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.7 (C-1″), 165.8 (C-1′), 141.8 (C-3′), 140.3 (C-3″), 132.2 (C-2″), 125.8 (C-2′), 86.3 (C-8), 73.7 (C-7), 70.1 (C-3), 68.1 (C-5), 63.7 (C-5″), 61.9 (C-9), 37.5 (C-1), 32.3 (C-6), 28.5 (C-2), 20.6 (C-5′), 15.8 (C-4″), and 15.6 (C-4′).

*Sarracine (**7**)*

A solution of **6** (33 mg) in MeOH (2 ml) was acidified to pH 2 by addition of H_2SO_4 2.5%, Zn° dust[35] (120 mg) was added, and the mixture was stirred for 9 h at room temperature. The solution was filtered, alkalinized with NH_4OH (pH 11), and extracted with $CHCl_3$ (10 ml × 3). The organic layer was dried over anh. Na_2SO_4 and filtered, and the solvent was evaporated under reduced pressure to afford 26.4 mg of sarracine (**7**). UV (EtOH) λmax (log ε) 215 (4.7); $[\alpha]_{589}$ −110, (c 0.56, EtOH). Complete ^{1}H NMR assignments are given in Tables 2 and 3.

*Neosarracine N-oxide (**8**)*

Complete ^{1}H NMR assignments are given in Tables 2 and 3.

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