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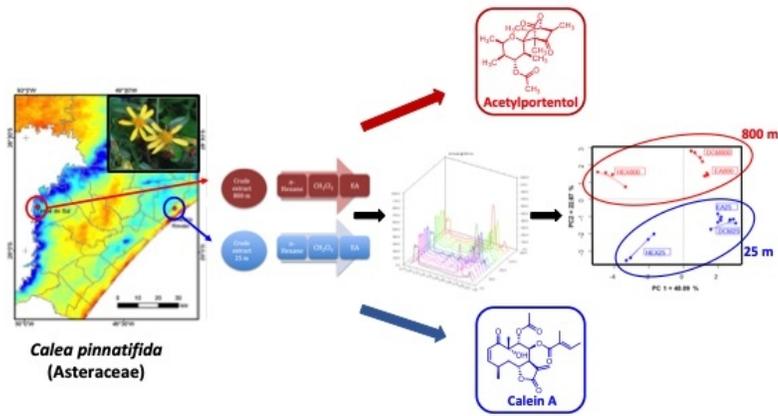
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**Chemical fingerprinting of the Brazilian medicinal plant
Calea pinnatifida (R. Br.) Less. (Asteraceae) collected at
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Keywords:	HPTLC, Environmental factors, Calein A, Acetylportentol, Chemometric analysis

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4 ***pinnatifida* (R. Br.) Less. (Asteraceae) collected at different altitudes**
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Chemical fingerprinting of the Brazilian medicinal plant *Calea pinnatifida* (R. Br.) Less. (Asteraceae) collected at different altitudes

Calea pinnatifida (R. Br.) Less. is a plant of Brazilian folk medicine. We evaluated the influence of environmental factors on the chemical profile of *C. pinnatifida* collected during the winter season. *C. pinnatifida* leaves, alongside soil samples, were collected from two sites of different altitude. Plant samples were sequentially extracted, while soil samples were subject to compositional analysis. Plant extracts were compared using HPTLC-UV, using chemometric analyses to compare samplings harvested at both altitudes. Two marker metabolites, calein A (1) and acetylportentol (2), were isolated from samples collected at the respective altitudes. The differing metabolic profiles observed may be a result of the influence of environmental factors.

Keywords: Chemometric analysis; HPTLC; environmental factors; calein A; acetylportentol

1. Introduction

The accumulation of secondary metabolites in plants is influenced by environmental factors, and fluctuations in these can have pronounced effects on the metabolome (Yang et al. 2018). We were interested in how the chemical fingerprint of the medicinally important species *Calea pinnatifida* L (Asteraceae) varied with environmental factors. *C. pinnatifida* is used in Brazilian folk medicine to treat stomach pain, giardiasis and amoebiasis, with the plant having reported leishmanicidal, trypanocidal and anticancer effects (Lima et al. 2015; Marchetti et al. 2012; Lima et al. 2017; de Aguiar Amaral et al. 2017). Bioactive compounds isolated from the genus *Calea* include chromenes, sesquiterpene lactones, phenolic acids, polyacetylenes, fatty esters, monoterpenes and sterols (Ferreira et al. 1980; Kato et al. 1994). The aims of the current study were to ascertain whether altitude and soil composition affected the metabolome of *C. pinnatifida* collected from two different sites within the state of Santa Catarina, Brazil.

2. Results and Discussion

Soil pH at both collection sites was low (Table S1), consistent with expectations (SBCS 2016). Clay content classification of soil collected at an altitude of 25 m was Class 3 (21-40% clay), with 'very low' (<10 mg / dm³) P content, while soil collected at 800 m was Class 4 (20% clay) with 'very low' (<17 mg / dm³) P. Among other soil nutrients in both samples, K, Ca, Mg, Zn and Mn levels were all determined to be 'high' or 'very high'. Significant differences were observed between the two samples with respect to % argile, % organic matter, pH-water and humidity, and also the soil nutrients K, Fe and Cu, but not among other minerals assessed. Successive extraction (Figure S1) of crude extracts led to various solvent fractions. Photodensitometric analyses (Table S2) allowed detection of peaks characterising each fraction. Due to the variability in the presence/absence and concentration of 16 defined peaks among the various fractions, multivariate analysis was used as a discriminatory tool (Figure S2). Chemical variability contributed significantly in allowing discrimination of plant extracts collected at 25 m from those collected at 800 m (Figure S4). HPTLC plates were then derivatised (Figure S3), showing variable metabolic profiles. In particular, one compound (**1** on Figure S3) was notable in plants collected at 25 m ($R_f = 0.35$), and a second (**2**) in plants collected at 800 m ($R_f = 0.25$). Samples of both compounds were isolated using preparative TLC. Compound **1** was deduced to be C₂₂H₂₈O₈, due to its HRMS signal at $m/z = 443.1666$ (calcd. for C₂₂H₂₈O₈Na: 443.16764; 9 degrees of unsaturation). Comparison of NMR data acquired (Figures S5-S13) and the literature identified this compound as either Calein A or B (Herz et al. 1980; Quijano et al. 1979). As the signals for protons 8 and 9 overlapped, ¹H, HSQC and HMBC spectra were recorded in benzene-*d*₆ to definitively determine the relative position of acetate and angelate groups, allowing identification as calein A (Figure S14A). Relative configuration was confirmed by NOESY (Figure

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3 S14B). Although **1** has been isolated from several *Calea* species, it has not previously
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5 been isolated from *C. pinnatifida*, in which two other caleins have been
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7 described, namely calein D (Ferreira et al. 1980) and calein C (Caldas et al.
8
9 2018). Compound **2** was identified as the polyketide metabolite acetylportentol (Figure
10
11 1), heretofore known as a lichen constituent (Huneck et al. 1996). Its molecular formula
12
13 was found to be $C_{19}H_{28}O_6$ from the HRMS signal at $m/z = 375.1779 [M + Na]^+$ (calcd.
14
15 for $C_{19}H_{28}O_6Na$: 375.17836; 6 units of unsaturation). NMR data (Figures S15-S19) was
16
17 similar to previously described data for **2** (Parrot et al. 2015). Interestingly, the related
18
19 compound portentol, also first isolated from various lichens including *Roccella*
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21 *portentosa*, was subsequently found in extracts from the Brazilian nut tree *Gustavia*
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23 *hexapetala* (Cheng et al. 2015).

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30 Fig. 1

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33 Several studies have shown the impact of topography on the content of plant
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35 metabolites (Cirak et al. 2017; Seyis et al. 2020). These studies support the notion that
36
37 altitudinal variation reflects the influence of abiotic factors on plant metabolite
38
39 responses. *C. pinnatifida* collection took place at very different climatic points; one at
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41 25 m in the coastal region and another at 800 m, where temperatures may be very low
42
43 during winter. Cold acclimation can lead to biochemical changes, including
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45 accumulation of carbohydrates and amino acids (Jorge et al. 2016). Temperature
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47 variation can either elevate or decrease metabolite content (Yang et al. 2018). Calein A
48
49 was notably present in specimens collected at 25 m, where the temperature was higher.
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51 Analogous variation in sesquiterpene profile with temperature has been noted by others
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53 (Wallaart et al. 2000). Solar radiation can also influence metabolite profile. Factors
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55 include photoperiod, intensity and wavelength. Variation in these parameters has been
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3 shown to impact secondary metabolite profiles (Pan et al. 2014; Yang et al. 2018;
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5 Huaranca et al. 2020). In our work, at 25 m, where UV radiation, and therefore
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7 oxidative stress is less intense, this may have impacted upon the metabolite profile as
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9 compared to 800 m. Regarding the impact of water availability, as the annual rainfall
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11 between the studied altitudes is almost identical, one may consider that this also holds
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13 true for water availability. Alongside abiotic factors, chemical and edaphic factors like
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15 soil fertility can also vary with elevation gradient, although soil composition was largely
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17 comparable between our two study sites. Other studies have reported a positive
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19 correlation between N, PO₄³⁻ and K availability and concentration of metabolites
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21 (Martinez et al. 2013; Kumar et al. 2014; Kováčik et al 2018; Yang et al. 2018). Thus,
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23 nutrient levels can affect plant growth. Intuitively, varied concentrations of
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25 micronutrients and macronutrients could play a positive role in plant growth and
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27 metabolite synthesis. In our case, the synthesis of compounds like acetylportentol found
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29 in *C. pinnatifida* growing at 800 m may be more favoured than those of the
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31 sesquiterpene lactone calein A. The abiotic factors described above can influence
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33 enzyme activities involved in carbon metabolism (Kumar et al. 2008) and in secondary
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35 metabolite synthesis (Earnshaw et al. 1987; Misra et al. 2011). Mapping of metabolic
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37 and enzymatic networks have shown that metabolic pathways differ with altitude (Zhao
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39 et al. 2019). Adaptability of secondary plant product synthesis to environmental factors
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41 is quite difficult to describe because they are modulated simultaneously, acting as
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43 synergistic or antagonistic factors. We report herein the influence of some abiotic
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45 factors on metabolites of *C. pinnatifida*, although biotic parameters such as ecological
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47 interactions between plants and other organisms can also affect the metabolism of
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49 secondary compounds.
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3. Conclusion

The profile of a plant's secondary metabolites is called a fingerprint, and is a true marker of biological complexity. The fingerprints for the fractions of *Calea pinnatifida* collected at 25 m and 800 m were similar between fractions, however, there were also subtle differences in chromatographic profile at both altitudes, emphasizing that environmental factors may be influential. Two metabolites, the sesquiterpene lactone calein A (**1**) and the polyketide metabolite acetylportentol (**2**) were isolated from fractions of plants collected at altitudes of 25 m and 800 m respectively. Variation in abiotic factors at both altitudes, such as temperature, water availability, light irradiation and soil fertility with respect to macro- and micronutrients are suggested to influence the observed metabolic changes. As *C. pinnatifida* is a medicinal plant, further studies are needed to ascertain how its pharmacological activity is affected by variation in its metabolite profile.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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Fig. 1. Structure of (+)- acetylportentol (2)

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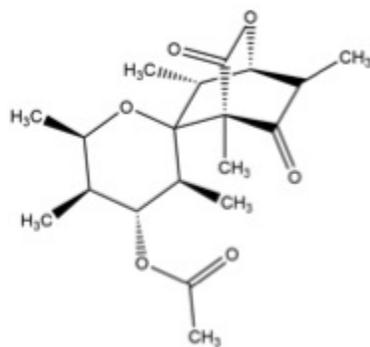


Figure 1

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SUPPLEMENTARY MATERIAL

Chemical fingerprinting of the Brazilian medicinal plant *Calea pinnatifida* (R. Br.) Less. (Asteraceae) collected at different altitudes

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Calea pinnatifida (R. Br.) Less. is a plant of Brazilian folk medicine. We evaluated the influence of environmental factors on the chemical profile of *C. pinnatifida* collected during the winter season. *C. pinnatifida* leaves, alongside soil samples, were collected from two sites of different altitude. Plant samples

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3 were sequentially extracted, while soil samples were subject to compositional
4 analysis. Plant extracts were compared using HPTLC-UV, using chemometric
5 analyses to compare samplings harvested at both altitudes. Two marker
6 metabolites, calein A (1) and acetylportentol (2), were isolated from samples
7 collected at the respective altitudes. The differing metabolic profiles observed
8 may be a result of the influence of environmental factors.
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14 Keywords: Chemometric analysis; HPTLC; environmental factors; calein A; acetylportentol
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16 17 **Experimental section:**

18 19 *Reagents and chemicals*

20
21 All solvents were of analytical grade and were purchased from Sigma-Aldrich (São Paulo,
22 Brazil). HPTLC was carried out on silica gel plates (Merck silica gel 60GF₂₅₄). The standard
23 butein was purchased from Sigma-Aldrich (CAS 487-52-5).
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29 30 *Plant material*

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32 Whole fresh plant material of *Calea pinnatifida* (R. Br.) Less. (Asteraceae) was collected from
33 two areas in the state of Santa Catarina, Brazil, during the non-flowering winter season. The
34 plant has no fruit and experiments were only conducted with the leaves. The first area, (latitude
35 28°47'32.8"S, longitude 49°20'13.6"W), at an altitude of 25 meters, is located in the city of
36 Balneário Rincão. The other area, (latitude 28°48'34.0"S, longitude 49°56'10.9"W), is at an
37 altitude of 800 meters in the "Serra da Rocinha", connecting the municipalities of Timbé do Sul
38 - SC and São José dos Ausentes- RS. The collected specimens were botanically identified and
39 authenticated by botanical researchers of the Herbarium Pe. Dr. Raulino Reitz at the
40 Universidade do Extremo Sul Catarinense (UNESC), Criciúma, Santa Catarina, Brazil. Voucher
41 specimens (CRI 11964) were also deposited at the Herbarium.
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Environmental factor analysis

Soil collection followed the procedures recommended by the Agricultural Research and Rural Extension Company of Santa Catarina (EPAGRI). Soil collection was performed in the winter, on the same day as the collection of *C. pinnatifida*. Soil was collected at fifteen points near the site where the plant naturally develops at both altitudes. After sampling at the fifteen points, a proportion of the soil was homogenised and dried in the shade, totaling 500 g in weight. This procedure was performed three times, totaling three samples of 500 g each for each altitude. Subsequently, the samples were sent to the Soil Chemical Analysis Laboratory of EPAGRI for analysis of basic soil aspects (% argile, pH, phosphorus, potassium, aluminium, calcium, magnesium and organic matter) and evaluation of micronutrients (copper, iron, zinc and manganese). Moisture content of the soils was also calculated. For this, freshly collected soil samples from each altitude were weighed and, subsequently, the percentage humidity was calculated (obtained by drying in a drying oven until constant weight was achieved). Eight soil samples from each altitude were analysed in order to determine the moisture content of the soil. The Student's t-test was performed to verify the hypothesis of differences in the average concentration of nutrients between different altitudes, adopting a significance level of $P < 0.005$ in the concentration of macronutrients, micronutrients and moisture in the soil of each collection site.

Extraction of plant leaves

The leaves of *Calea pinnatifida* from both sites (26 g collected at 25 meters and 24 g collected at 800 meters) were dried, and manually reduced to the smallest possible size. They were then macerated with ethanol 96% for 15 days at room temperature with occasional shaking, and then filtered. The filtrates were evaporated in a rotary evaporator under pressure. The resulting ethanol extracts were re-dissolved in water and sequentially fractionated with solvents of increasing polarity; *n*-hexane, dichloromethane (DCM) and ethyl acetate (EA). Solvents were evaporated from all fractions in a rotary evaporator under pressure. In all, six samples were

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3 prepared, three from 25 meters (*n*-hexane fraction, DCM fraction, EA fraction), and three
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5 samples from 800 meters (*n*-hexane fraction, DCM fraction, EA fraction).
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8 ***High Performance Thin Layer Chromatography (HPTLC) analysis***

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11 From each of the six fractions, four aliquots were weighed and dissolved in absolute ethanol to
12 reach a concentration of 0.5 mg/mL, as was the standard butein. Samples were spotted on pre-
13 coated silica gel 60GF₂₅₄ aluminium sheets (20 x 10 cm) using the automated sample applicator
14 CAMAG Automatic TLC Sampler III (ATS3), which was programmed through WINCATS
15 software. Before use, plates were prewashed with isopropanol and dried at room temperature.
16 The operating conditions were as follows: syringe delivery speed: 10 mm/s; injection volume:
17 30 µL; bandwidth: 6 mm; start position: 15 mm and distance from bottom of plate: 15 mm. The
18 plates were then developed in chloroform / ethyl acetate (75:25), and after drying, plates were
19 scanned at six wavelengths: 220, 260, 280, 310, 340 and 370 nm. They were then derivatised
20 using the anisaldehyde sulfuric reagent, followed by heating to 105°C in order to visualise the
21 non-aromatic compounds.
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34 ***Multivariate analyses***

35 *Data processing*

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38 Each densitogram of a track on the HPTLC plate set at 254 nm defines a fingerprint associated
39 with a retention time for each peak correlating with an integrated area. All data points were
40 exported into an Excel sheet to build a matrix table. All peaks were integrated automatically
41 (minimum slope: 5; minimum height: 10 AU; minimum area: 40 AU; maximum height: 990
42 AU) and then normalised based on their retention time and area, using butein as internal
43 standard.
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54 *Statistical analysis*

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58 The matrix generated from the HPTLC data was used for multivariate analyses. A fourth-root
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3 transformation of the values was applied, centering and autoscaling as null values and great
4 differences in concentrations according to chemical profiles were observed (van den Berg et al.
5 2006). Principal Component Analysis (PCA) was performed on the normalised values to
6 discriminate the extracts obtained from plants harvested at the two altitudes on the basis of their
7 metabolic profiles (function `dudi.pca`, package “ade4”). All statistical analyses were made using
8 R software V. 3.3.1 (R Core Team, 2016).

16 ***Isolation and identification of differential compounds present in both extracts***

19 Preparative TLC plates 30 x 30 cm, with a thickness of 1 mm, were prepared using the
20 stationary phase silica gel 60GF₂₅₄ and developed in the mobile phase chloroform / ethyl acetate
21 (75:25). Relevant areas were scraped from the plates and compounds eluted from the silica with
22 methanol. NMR spectra were recorded with a Bruker Avance I 300 MHz spectrometer, and the
23 residual solvent signals were used as internal references: MeOD δ H = 3.3 ppm, δ C = 49 ppm.
24 Additional spectra were recorded with a Bruker AVANCE III HD 500 MHz spectrometer in
25 C₆D₆ (δ H = 7.16 ppm, δ C = 128.06 ppm). The ESI HRMS data were recorded in positive mode
26 using two spectrometers, an AGILENT 6510 and a THERMO FISHER SCIENTIFIC Q-
27 Exactive.
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43 **List of Supplementary Figures and Tables**

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36 **Figure S14.** 2D-NMR correlations of compound **1** (Calein A). Most significant HMBC
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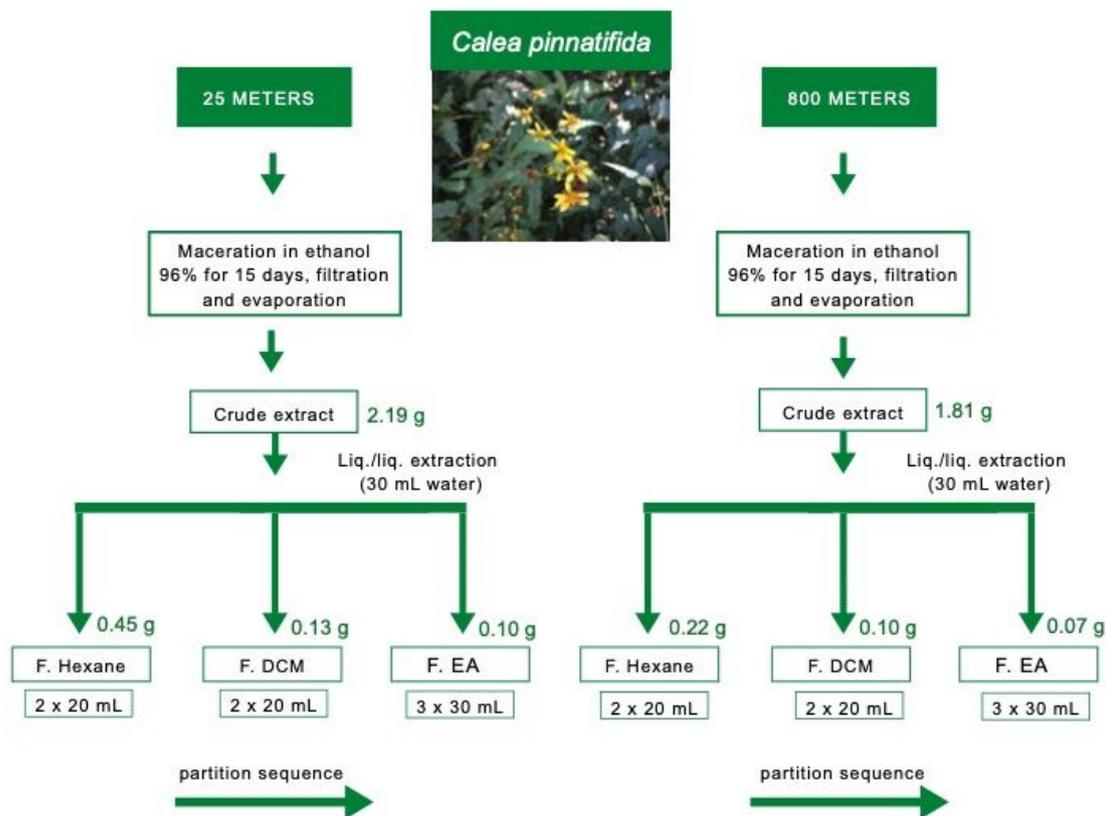
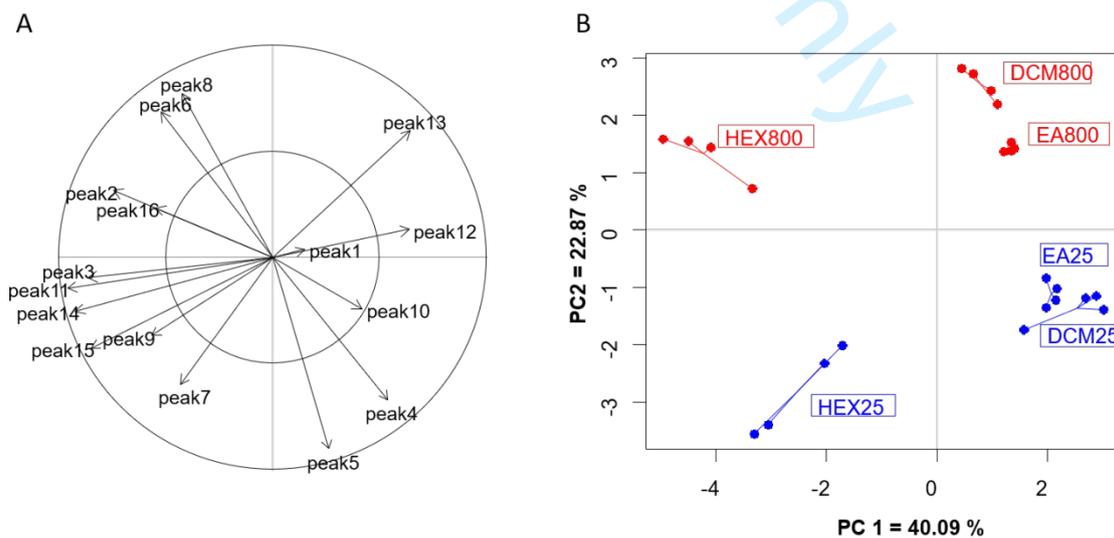
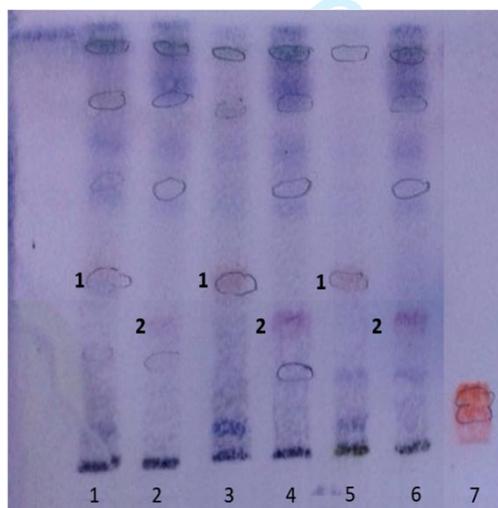


Figure S1. Extraction scheme of *Calea pinnatifida* collected at two altitudes (25 meters and 800 meters). *C. pinnatifida* leaves were first macerated in ethanol 96%, after which the extracts were evaporated and extracted with three solvents of increasing polarities: *n*-hexane, dichloromethane (DCM) and ethyl acetate (EA).



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3 Figure S2. Multivariate analysis on the 24 extracts of *Calea pinnatifida* collected at two
4 altitudes. Graphs of Principal Component Analysis (PCA) score plot were performed on
5 the HPTLC-UV data obtained from all extracts of increasing polarities: *n*-hexane
6 (HEX), dichloromethane (DCM) and ethyl acetate (EA). (A) Score plot obtained on the
7 two first axes (PC1, PC2) which explains 62.96 % of the observed variance; (B)
8 Corresponding loading plots of UV detected metabolites.
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39 Figure S3. Comparative HPTLC fingerprints of six extracts after derivatisation with
40 anisaldehyde-sulfuric reagent (ANS). Extracts 1-6 were obtained with solvents of
41 increasing polarity: *n*-hexane (lanes 1,2), dichloromethane (lanes 3,4) and ethyl acetate
42 (lanes 5,6). Plant extracts 1, 3 and 5 were prepared from plants collected at 25 m while
43 extracts 2, 4 and 6 were prepared from plants collected at 800 m. The standard butein
44 was spotted on the plates to avoid any R_f deviation. Two differential compounds were
45 detected in the extracts: compound 1 was identified in fractions of plants collected at 25
46 m and compound 2 in those of plants collected at 800 m. The mobile phase was
47 chloroform / ethyl acetate 75:25.
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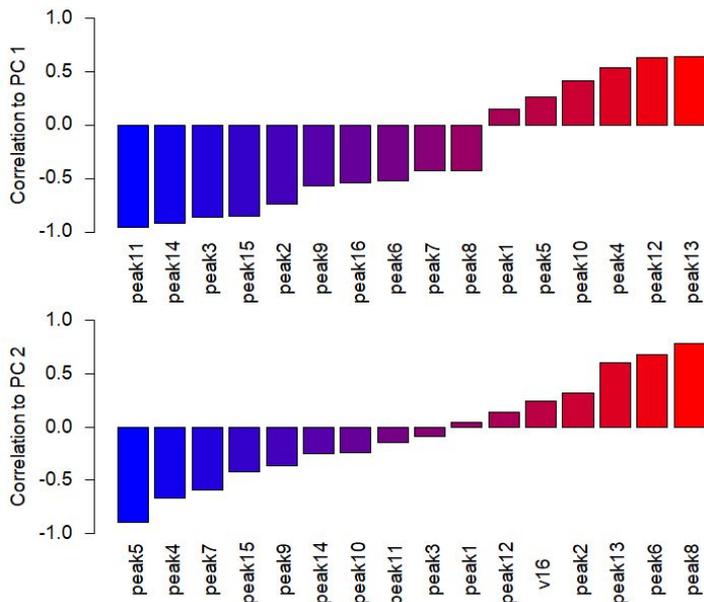


Figure S4. Projections to the axes PC1 and PC2 of the metabolites of *Calea pinnatifida* analysed by Principal Component Analysis.

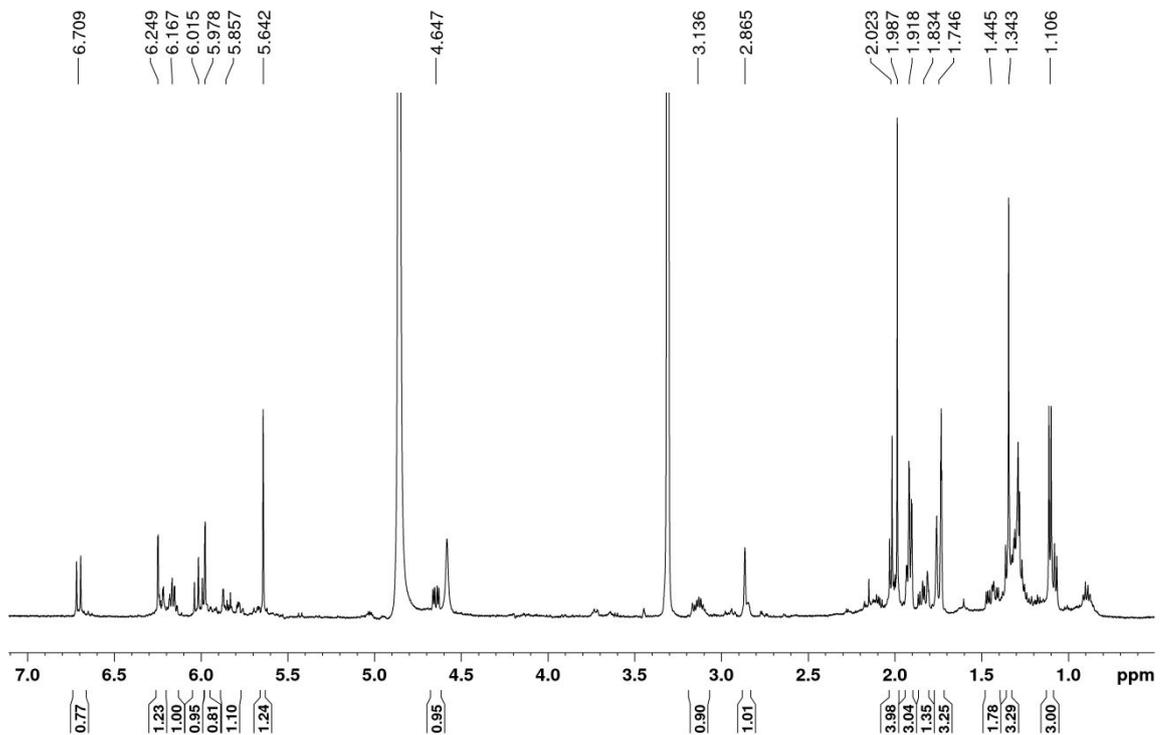


Figure S5. ¹H NMR (MeOD, 500 MHz) spectrum of compound 1

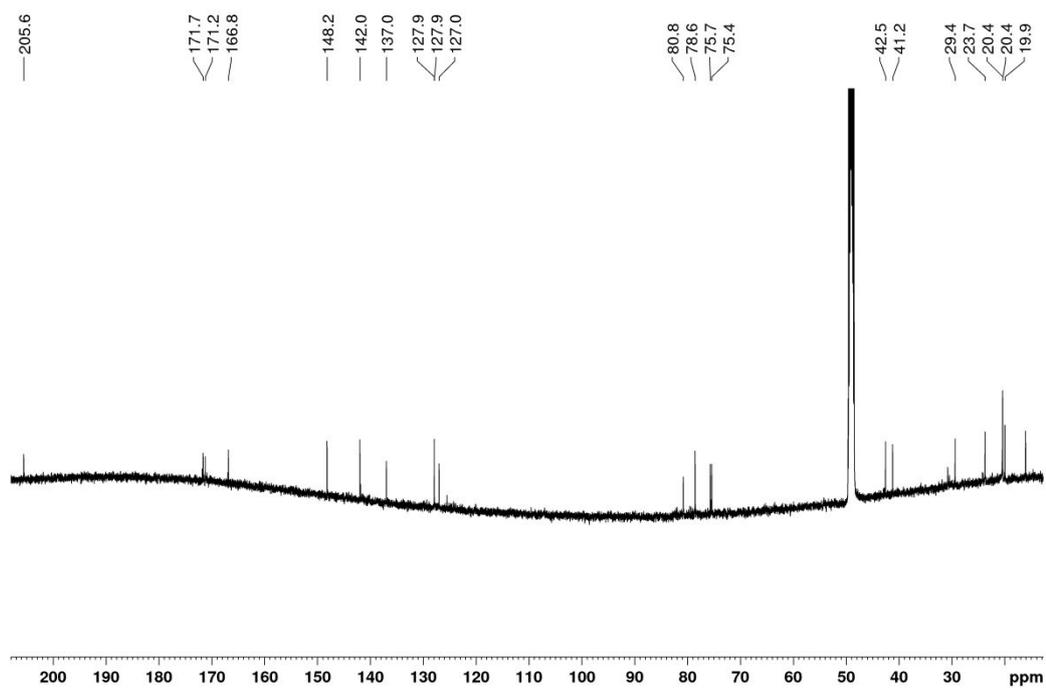


Figure S6. ^{13}C NMR (MeOD, 125 MHz) spectrum of compound **1**

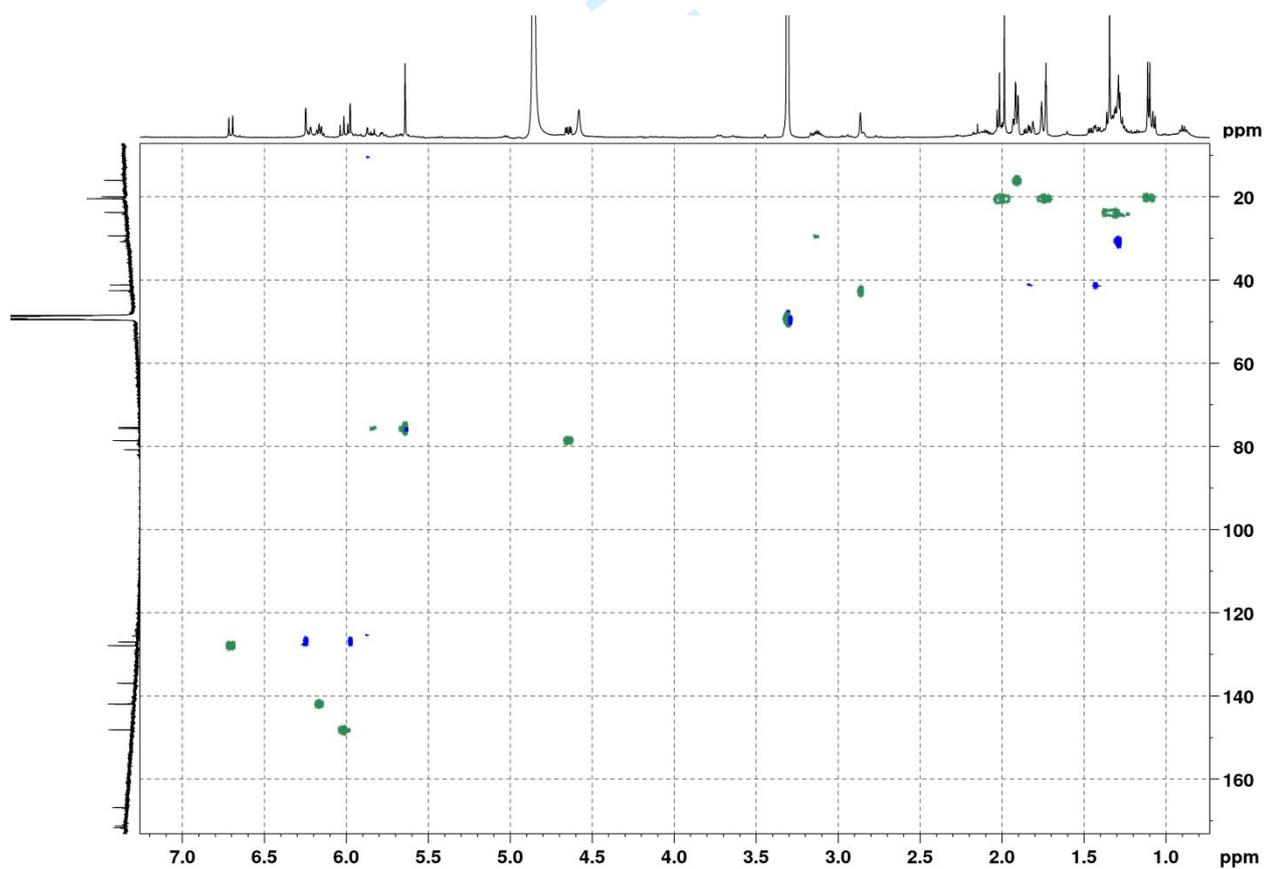


Figure S7. HSQC (MeOD 500/125 MHz) spectrum of compound **1**

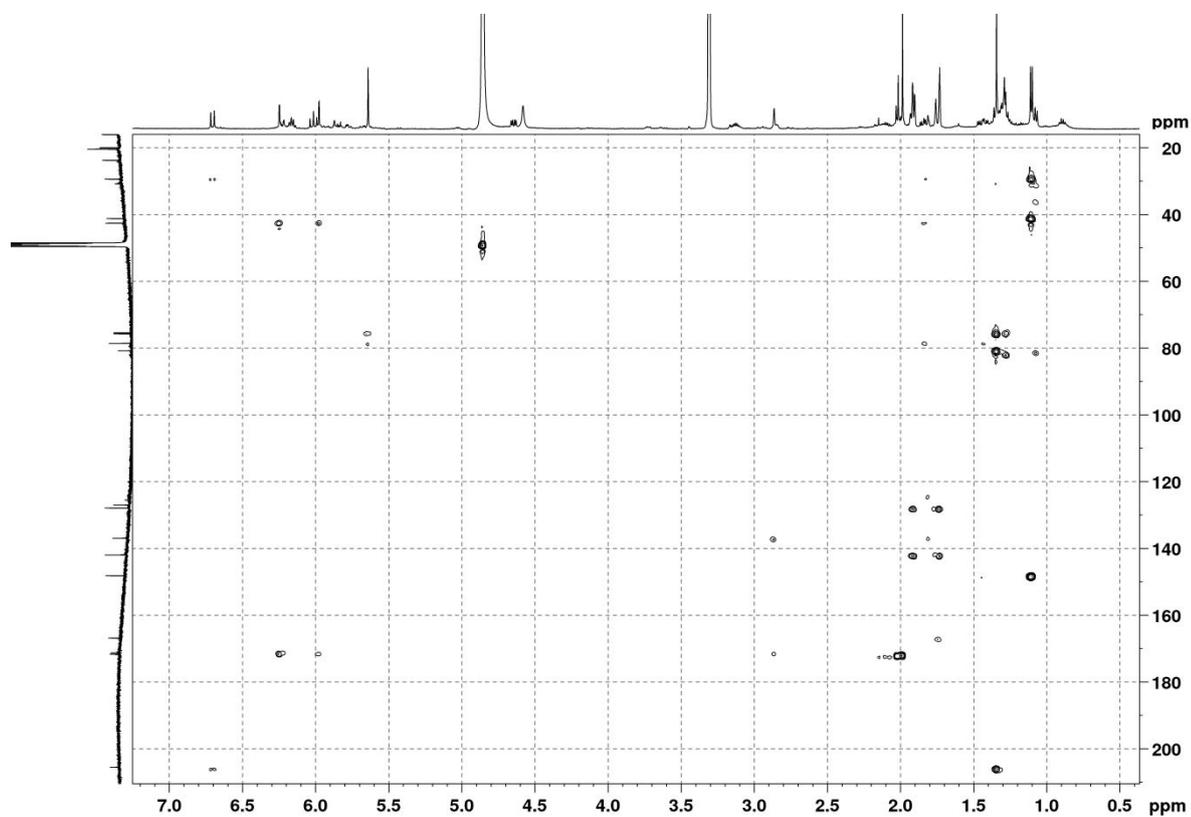


Figure S8. HMBC (MeOD, 500/125 MHz) spectrum of compound **1**

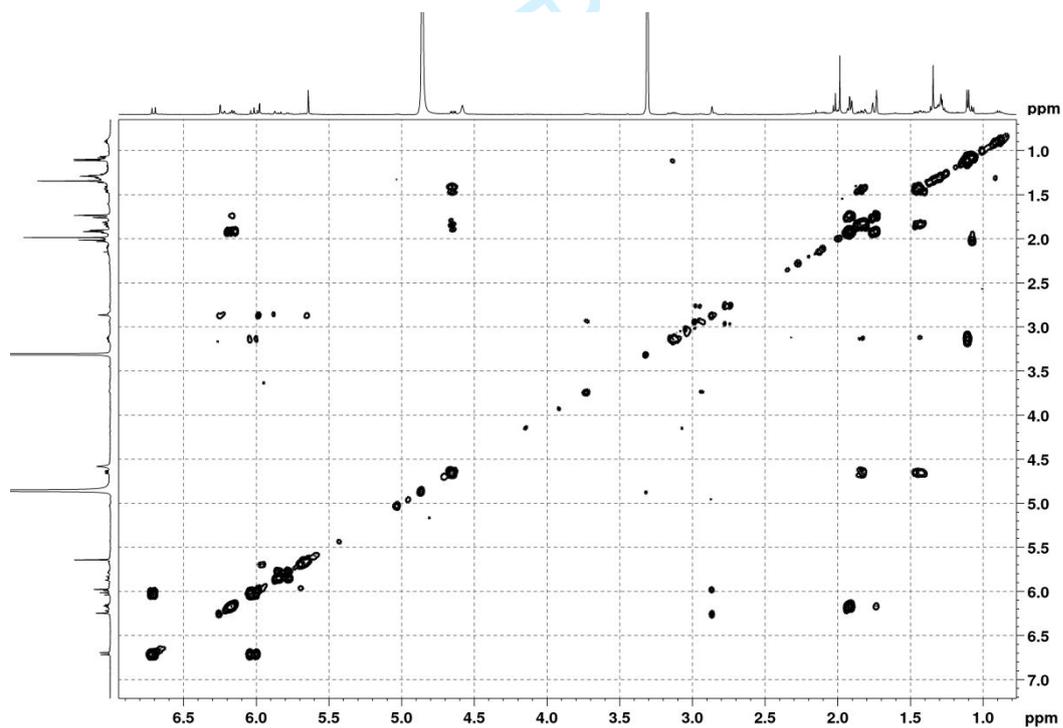
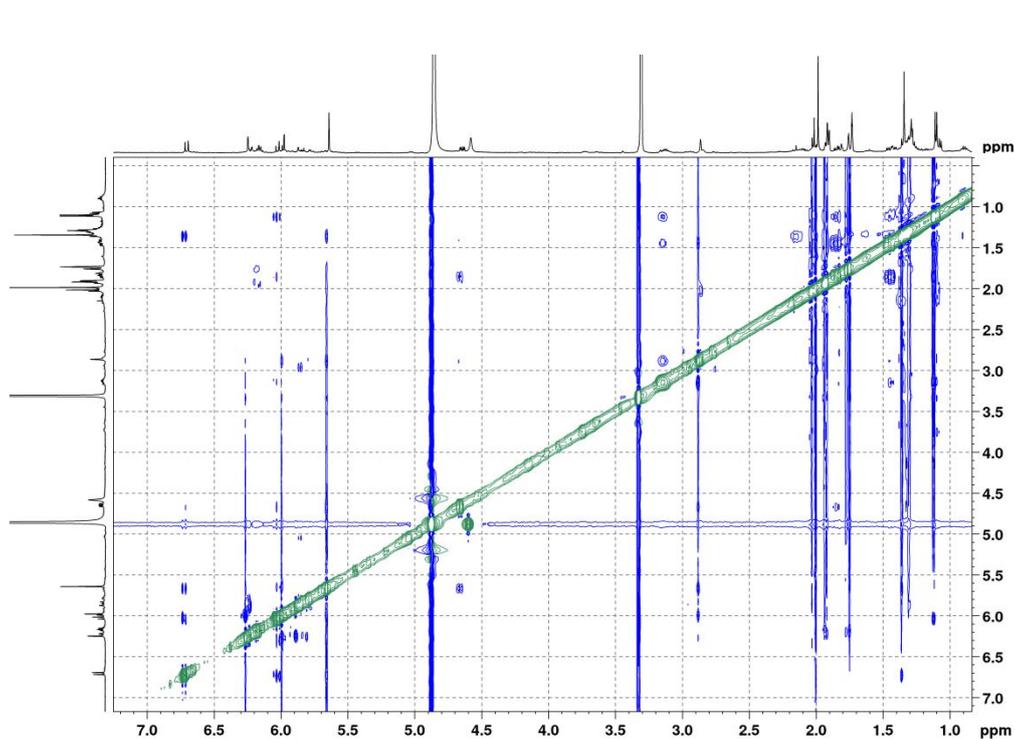
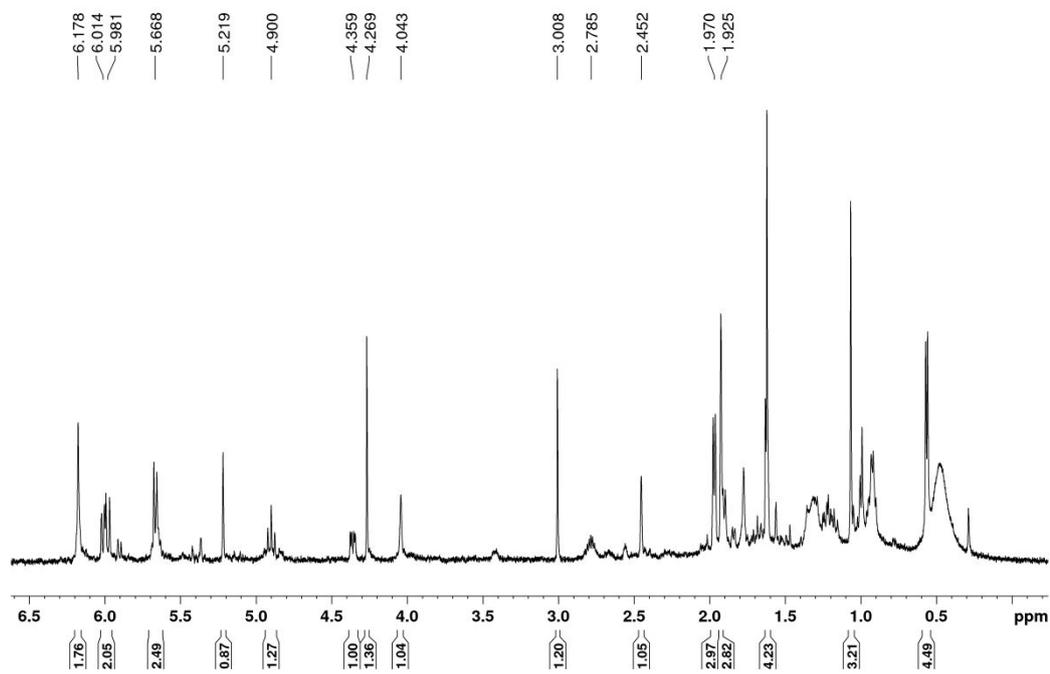


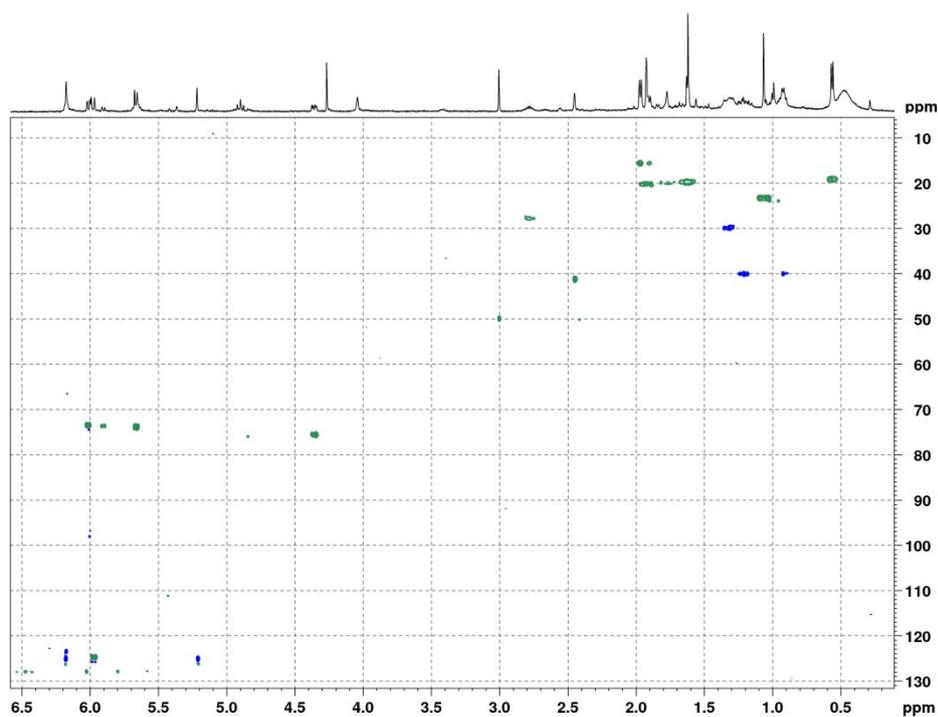
Figure S9. COSY (MeOD, 500 MHz) spectrum of compound **1**



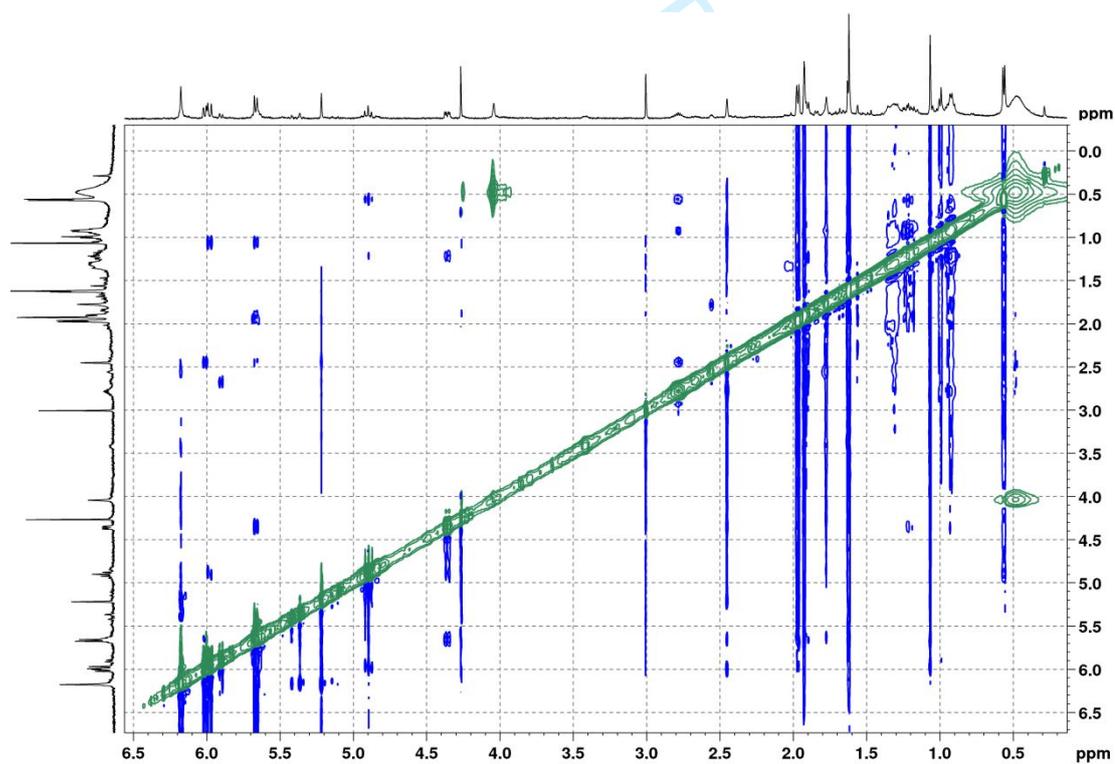
25
26 Figure S10. NOESY (MeOD, 500 MHz) spectrum of compound **1**



55 Figure S11. ^1H NMR (C_6D_6 , 500 MHz) spectrum of compound **1**



26
27 Figure S12. HSQC (C_6D_6 , 500/125 MHz) spectrum of compound **1**



56
57 Figure S13. NOESY (C_6D_6 , 500 MHz) spectrum of compound **1**

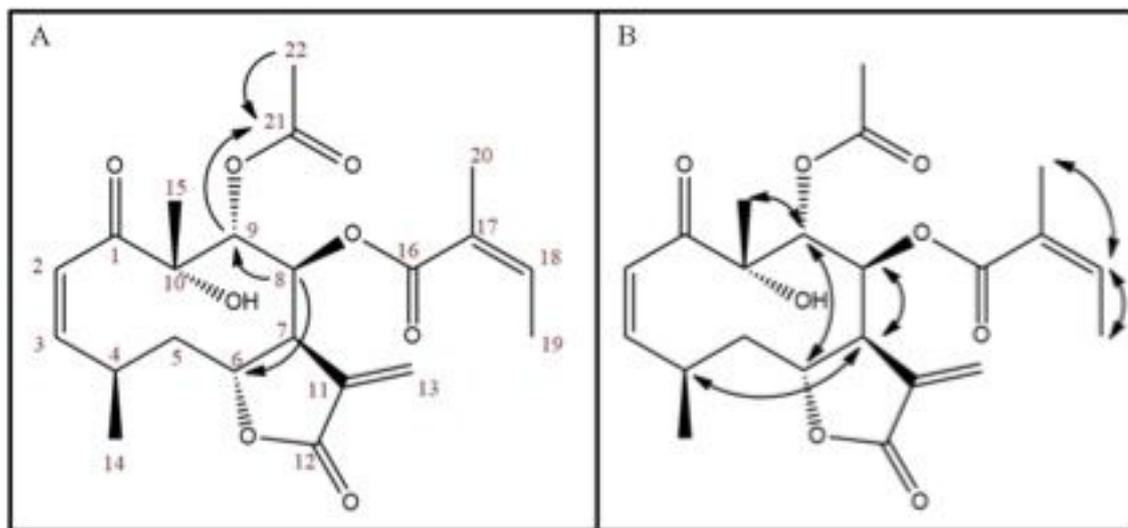
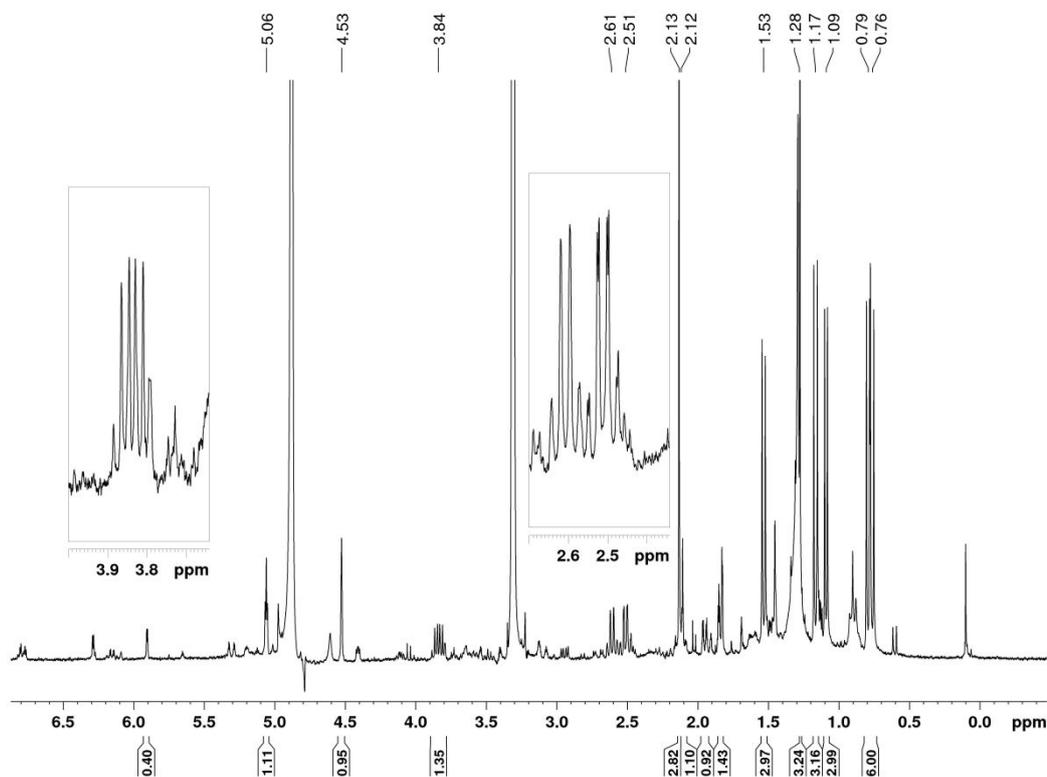
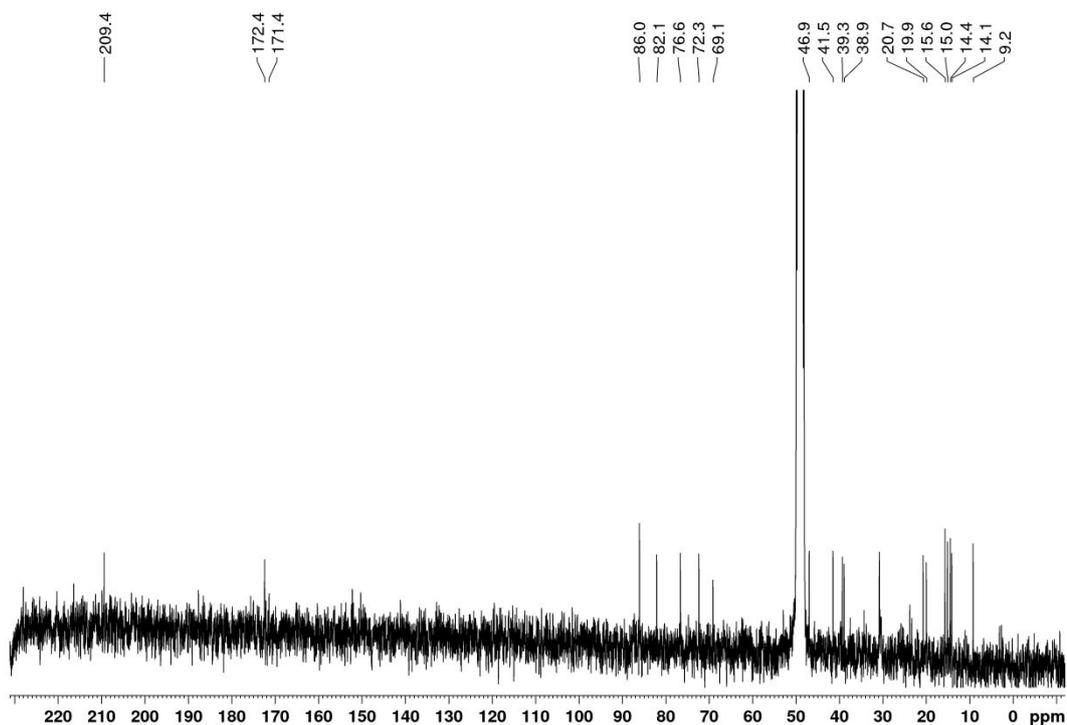


Figure S14. 2D-NMR correlations of compound **1** (Calein A). Most significant HMBC (H → C) correlations (A) confirmed positions of acetate and angelate groups while important NOE correlations (B) established relative configuration.

Figure S15. ^1H NMR (MeOD, 300 MHz) spectrum of compound **2**Figure S16. ^{13}C NMR (MeOD, 75 MHz) spectrum of compound **2**

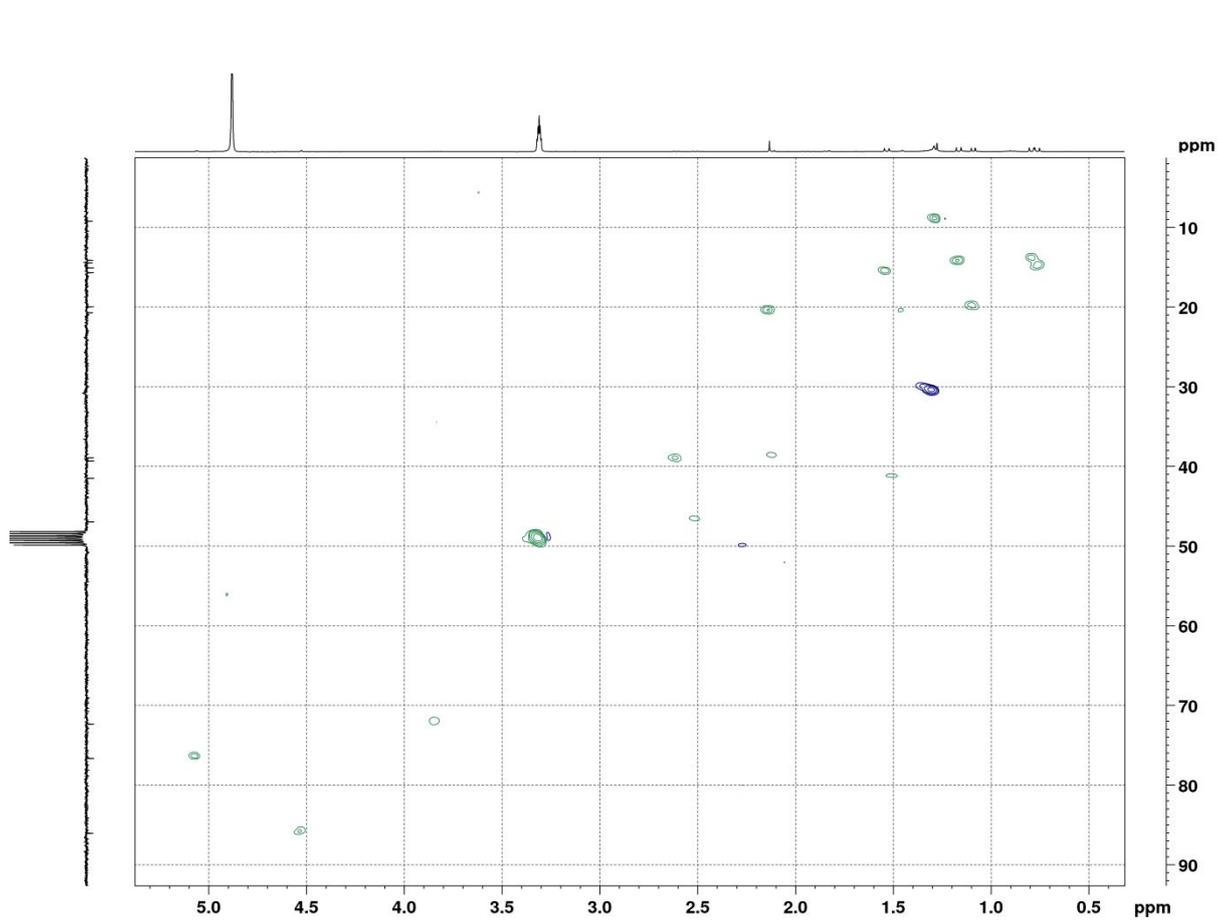


Figure S17. HSQC (MeOD 300/75 MHz) spectrum of compound 2

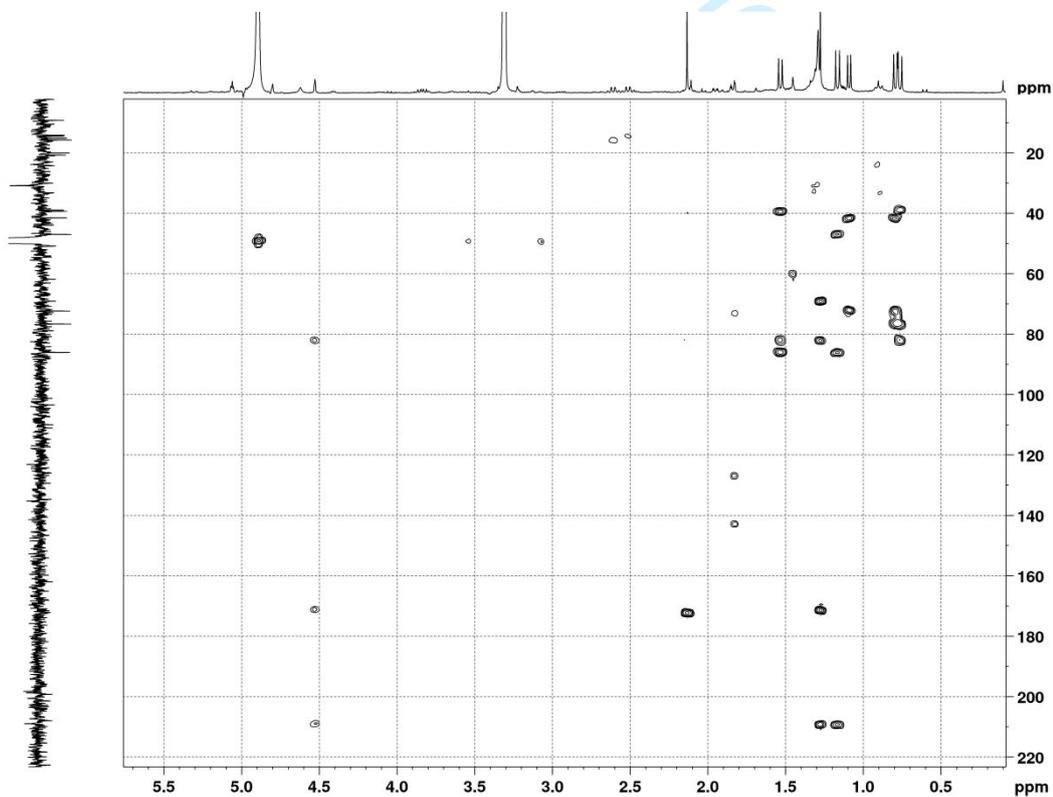


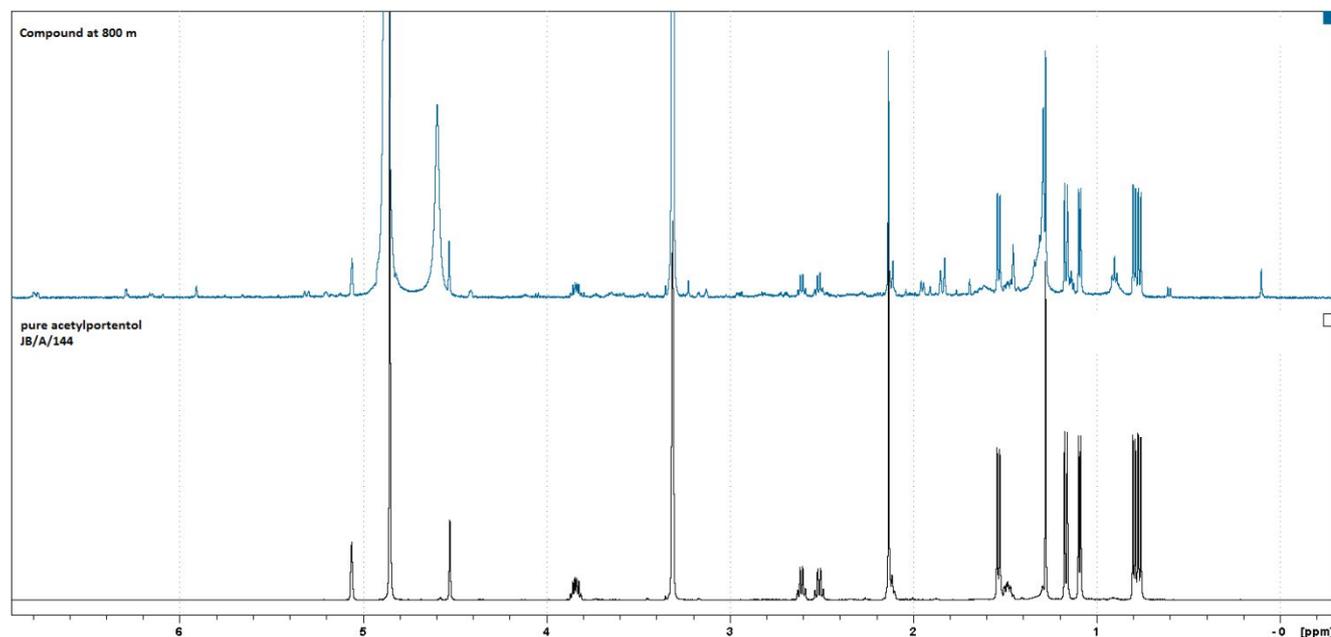
Figure S18. HMBC (MeOD 300/75 MHz) spectrum of compound **2**Figure S19. ^1H NMR (MeOD, 300 MHz) spectrum of compound **2** and **acetylportentol** from our chemical library

Table S1. Climatic parameters and statistical analysis of soil parameters

	Parameters	Collection sites		Test <i>t</i> (<i>p</i> -value)
		25 m 28°47'32.8"S 49°20'13.6"W	800 m 28°48'34.0"S 49°56'10.9"W	
Climatic parameters	Temperature (°C)	Average: 17.0 – 19.3 °C Maximum: 23.4 – 25.9 °C Minimum: 12.0 – 15.1 °C	Average: 13.8 – 15.8 °C Maximum: 19.4 – 22.3 °C Minimum: 9.2 – 10.8 °C	
	Solar irradiation	Minimum 13300 kJ m ⁻² day ⁻¹	Maximum 14680 kJ m ⁻² day ⁻¹	
	Annual rainfall (mm)	1.220 - 1.660	1.360 - 1.600	
	Air relative humidity (%)	82%	82%	
	% argile (m/v)	32.3 ± 1.53	18.3 ± 0.58	<0,001
Soil fertility	pH-water 1:1	4.5 ± 0.06	5.2 ± 0.20	0,004
	P (mg/dm ³)	3.9 ± 0.98	12.1 ± 2.31	0,005
	K (mg/dm ³)	146.7 ± 4.16	537.3 ± 16.17	<0,001
	Al (cmolc/dm ³)	1.9 ± 0.26	0.7 ± 0.75	0,060
	Ca (cmolc/dm ³)	3.1 ± 0.17	7.5 ± 2.06	0,064
	Mg (cmolc/dm ³)	2.2 ± 0.17	3.1 ± 0.51	0,041
	Organic matter (%)	2.2 ± 0.06	4.4 ± 0.26	<0,001

Cu (mg/dm ³)	0.8 ± 0.06	2.3 ± 0.12	<0,001
Fe (mg/dm ³)	209 ± 14.18	33.3 ± 5.51	<0,001
Zn (mg/dm ³)	2.2 ± 0.10	5.7 ± 1.72	0,025
Mn (mg/dm ³)	81 ± 13.16	54.1 ± 9.70	0,046
Humidity	17.61 ± 1.27	30.54 ± 4.01	<0,001

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Table S2. Densitometric data (absorbance units, AU correlated to Rf) for compounds (cpd) detected in all extracts of *Calea pinnatifida*. All data were obtained after integration of each densitogram for the 24 extracts (4 replicates for each extracts). The plant was extracted using solvents of increasing polarities (*n*-hexane (HEX), dichloromethane (DCM), ethyl acetate (EA)).

Rf of start and end positions of the peak		0.10-0.12	0.13-0.16	0.18-0.29	0.30-0.35	0.35-0.41	0.42-0.47	0.45-0.53	0.53-0.56	0.60-0.65	0.65-0.71	0.69-0.79	0.75-0.79	0.74-0.84	0.78-0.84
Rf of the maximal height of the peak		0.1	0.14	0.25	0.33	0.39	0.44	0.5	0.55	0.64	0.67	0.73	0.77	0.8	0.85
Extract	altitude	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11	Peak 12	Peak 13	Peak 14
HEX 25	25	830	0	2333	420	2574	0	1277	752	1222	0	7708	0	0	15302
HEX 25	25	717	0	2046	426	2493	0	1471	623	1478	0	7861	0	0	17014
HEX 25	25	1153	0	3048	739	3646	0	2516	1602	2711	0	9709	0	0	18727
HEX 25	25	1130	0	3924	799	3882	0	2447	1332	2596	0	9759	0	0	18517
mean		957.5	0	2837.75	596	3148.75	0	1927.75	1077.25	2001.75	0	8759.25	0	0	17390
Std deviation		217.62	0.00	837.81	201.27	717.70	0.00	644.92	466.33	761.25	0.00	1127.46	0.00	0.00	1587.33
HEX 800	800	1281	880	2626	0	1088	1297	502	4976	2576	0	12832	0	0	14288
HEX 800	800	1331	872	2837	0	1263	971	837	4949	2123	0	12834	0	0	14657
HEX 800	800	1143	849	2718	0	1024	965	438	4899	1874	0	12959	0	0	15799
HEX 800	800	853	476	2226	0	1473	714	308	4712	1146	0	13514	0	0	17350
mean		1152	769.25	2601.75	0	1212	986.75	521.25	4884	1929.75	0	13034.75	0	0	15523.5
Std deviation		214.60	195.94	264.97	0.00	201.20	239.00	225.44	119.02	597.87	0.00	324.98	0.00	0.00	1377.11
DCM 25	25	1736	0	1003	2099	4045	0	288	1415	0	330	0	330	3924	3999
DCM 25	25	1886	0	912	2001	3984	0	214	1507	0	498	0	499	4298	5176
DCM 25	25	2011	0	1017	2121	4357	0	289	1462	5395	0	0	510	4682	5304
DCM 25	25	2173	0	1464	2219	4378	0	310	1802	0	688	0	688	5149	5664
mean		1951.5	0	1099	2110	4191	0	275.25	1546.5	1348.75	379	0	506.75	4513.25	5035.75
Std deviation		185.59	0.00	247.75	89.45	205.50	0.00	42.07	174.43	2697.50	291.94	0.00	146.24	524.79	721.38
DCM 800	800	1708	0	1392	0	0	818	746	3113	0	0	0	746	8283	7212
DCM 800	800	871	0	1225	0	0	508	266	2814	0	0	0	342	6979	6013
DCM 800	800	607	0	1793	0	0	1173	357	3376	0	0	0	262	7826	6868

DCM 800	800	1503	0	1862	0	0	861	350	3416	0	0	0	239	9212	8180
mean		1172.25	0	1568	0	0	840	429.75	3179.75	0	0	0	397.25	8075	7068.25
Std deviation		518.55	0.00	308.59	0.00	0.00	272.05	214.85	278.42	0.00	0.00	0.00	236.65	930.82	896.35
EA 25	25	462	0	437	1282	2917	0	530	650	0	0	1697	1650	2202	6665
EA 25	25	511	0	503	1522	3429	0	880	597	0	0	0	1331	2539	4660
EA 25	25	480	0	394	1306	3190	0	660	403	0	0	0	1159	1881	3751
EA 25	25	510	0	515	1472	3742	0	781	710	0	0	0	1515	1661	7291
mean		490.75	0	462.25	1395.5	3319.5	0	712.75	590	0	0	424.25	1413.75	2070.75	5591.75
Std deviation		23.96	0.00	56.98	119.37	350.84	0.00	151.45	132.94	0.00	0.00	848.50	214.33	383.14	1662.91
EA 800	800	1564	0	1861	0	1733	0	1084	4793	0	0	0	2681	7512	7494
EA 800	800	1197	0	1401	0	1264	0	589	3705	0	0	0	1474	6471	6554
EA 800	800	1120	0	1083	0	1340	0	652	4079	0	0	0	1197	8107	7817
EA 800	800	983	0	1100	0	1146	0	607	3903	0	0	0	1291	5979	7357
mean		1216	0	1361.25	0	1370.75	0	733	4120	0	0	0	1660.75	7017.25	7305.5
Std deviation		248.31	0.00	363.78	0.00	254.35	0.00	235.50	473.96	0.00	0.00	0.00	689.82	967.59	536.83

Table S3. NMR spectroscopic data of compound 1

	δ_C (ppm) ^a	δ_C (ppm) ^{b,*}	δ_H (ppm) ^a	δ_H (ppm) ^b	HMBC (H \rightarrow C) ^{a,b}	COSY ^{a,b}	NOESY ^{a,b}
1	205.1	204.4					
2	127.9	124.7	6.71	5.98	1; 3; 4	3	15; 3; 6
3	148.2	147.5	6.04	4.9	1; 4; 14	2; 4	14; 6; 2
4	29.3	27.7	3.13	2.8	3; 4; 14	3; 5b; 14	14; 5a; 7
5	41.2	40	1.46	0.93	3; 4; 6	4; 5b; 6	14; 5b; 7; 4
			1.83	1.2	4; 6; 7; 11; 14	4; 5a; 6	14; 5a; 6
6	78.6	75.9	4.64	4.36	8; 11; 12	5a; 5b	5b; 7; 3; 9
7	42.5	41	2.86	2.46	11; 12; 13	13	4; 5a; 6; 13b
8	75.4	73.5	5.64	6.01	9; 6	7; 9	7; 13b
9	75.7	73.9	5.64	5.67	21; 8	8	6; 15
10	80.8	78.8					

1	11	137	135					
2	12	171	168.3					
3	13	127	125.2	6.25	6.18	7; 11; 12	7	13b
4				5.98	5.22	7; 12	7	7; 13a
5	15	23.7	23.4	1.34	1.07	1; 9; 10		2; 9
6	14	19.9	19.2	1.11	0.57	3; 4; 5	4	3; 4; 5a; 5b
7	16	166.8	165.5					
8	17	127.9	126.5					
9	18	142	140.5	6.16	5.65	19	19; 20	19; 20
10	19	20.3	20.3	1.75	1.92	16; 17; 18	18; 20	18
11	20	16	15.4	1.92	1.97	16; 17; 18	18; 19	18
12	21	171.6	169.8					
13	22	20.4	19.7	2.01	1.63	21		

a: measured in MeOD, b: measured in C₆D₆, *Inverse detection (HSQC, HMBC).

References

van den Berg RA, Hoefsloot HCJ, Westerhuis JA, Smilde AK, van der Werf MJ. 2006. Centering, scaling, and transformations: Improving the biological information content of metabolomics data. *BMC Genomics*. 7: 1–15.