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Chemical and Enantioselective Analyses of an Unprecedented Essential Oil from Ecuadorian *Aiouea montana*: A Natural Source of S-Methyl-O-2-phenylethyl Carbonothioate

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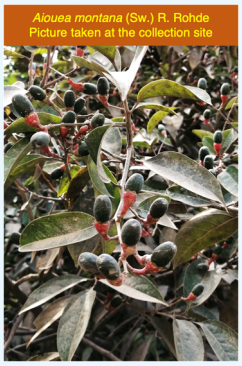
ABSTRACT: Fresh and dry leaves of *Aiouea montana* (Sw.) R. Rohde (Lauraceae) produced, in a quite high yield (0.88% and 1.60%, respectively), an unpleasantly smelling essential oil. The chemical composition was described in this study for the first time, detecting and quantifying 48 compounds. Major components of fresh and dry leaf essential oils were α-pinene (6.7−10.3%), β-pinene (2.8−3.8%), α-phellandrene (12.6−14.5%), α-copaene (3.1−15.7%), δ-cadinene (0.9−3.3%), and S-methyl-O-2-phenylethyl carbonothioate (58.5−33.3%). The dominant compound was already known in the literature by synthesis; however, it was unprecedented so far in nature. The carbonothioate was identified after purification and structure elucidation, by means of mass spectrometry, NMR spectroscopy, and FTIR spectrophotometry. The spectral results were identical to all data reported in the literature for the same molecule. Furthermore, the enantioselective analysis of the essential oil was conducted on a β-cyclodextrin-based stationary phase. Two chiral constituents, (+)-β-phellandrene and (1*R*,2*S*,6*S*,7*S*,8*S*)-(−)-α-copaene, were enantiomerically pure, whereas α-thujene, camphene, β-pinene, α-phellandrene, limonene, linalool, and germacrene D were scalemic mixtures. The different chemical and enantiomeric compositions suggested that enzymatic transformations could occur while drying.



1. INTRODUCTION

Ecuador is a tropical country, characterized by the presence of four very different climatic regions. It is well-known for possessing an outstanding biodiversity, and therefore, it has been included by the United Nations among the 17 "megadiverse" countries.[1] Every year, new taxa are discovered, and mainly for historical causes, most of the native and endemic botanical species are still practically unstudied.[2,3] For all these reasons, the biodiversity of Ecuador is an important heritage for the discovery of new natural products, mainly with a pharmaceutical focus but also from the ecological and biochemical points of view. Furthermore, the climatic change and the unwise exploitation of natural resources make the awareness about biodiversity an urgent need. Therefore, with the aim of contributing to the discovery of new natural products, our group has been studying the phytochemistry of the Ecuadorian biodiversity for more than 20 years.[4,5] In the past few years, our interest was focused on the description of new essential oils (EOs), with emphasis on their chemical and enantiomeric compositions, mainly from a biochemical point of view.[6−12] The present investigation was conducted in the same context, and it raised from the evidence that the leaves of *Aiouea montana* (Sw.) R. Rohde emitted a strong sulfurous odor all year long, suggesting the presence of uncommon volatile metabolites in high amounts. Furthermore, no bibliographical information was found in this respect, opening the way to possibly interesting results.

Aiouea montana (Sw.) R. Rohde is a quite common species, belonging to the family Lauraceae. Two important botanical libraries reported 49 and 75 synonyms, respectively, mainly belonging to genera *Cinnamomum*, *Phoebe*, *Laurus*, and *Ocotea*, among others.[13,14] The main synonym probably is *Cinnamomum triplinerve* (Ruiz and Pav.) Kosterm, of which *Aiouea montana* (Sw.) R. Rohde was considered an accepted name.[14] However, according to a recent study, practically all the neotropical *Cinnamomum* species phylogenetically differ from the Paleotropical taxa of the same genus, and they should be therefore renamed as *Aiouea* spp.[15] Due to the novelty of this taxon, *A. montana* is not present in the Catalog of Vascular Plants of Ecuador, where *C. triplinerve* is reported instead. According to the catalog, *C. triplinerve* is a native tree, growing in the coast, Andes, and Amazon regions of the country, between 0 and 2500 m above the sea level.[16] The plant has been described in the provinces of Bolívar, Carchi, Esmeraldas, Imbabura, Morona-Santiago, Napo, Pastaza, Pichincha, and Zamora-Chinchipe.[16] On the one hand, no bibliographical data were found about any essential oil from both *A. montana* and *C. triplinerve*. On the other hand, the chemical analyses

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published about the EOs from species corresponding to other synonyms showed a composition completely different from the one reported in the present study, confirming that some synonyms do not actually correspond to our species.[17,18] Finally, it should be mentioned that *trans*-3′-methylsulfonylallyl-*trans*-cinnamate, a sulfonated phenylpropanoid derivative, was obtained as a nonvolatile metabolite of *C. triplinervis* (probably an erroneous name for *triplinerve*) and *Phoebe brenesii* Standley.[19,20]

To the best of the authors' knowledge, the present investigation represented the first chemical and enantioselective analysis of an EO from *A. montana*, and it described S-methyl-O-2-phenylethyl carbonothioate for the first time in nature.

2. RESULTS AND DISCUSSION

2.1. Chemical Analysis. Fresh and dry leaves of *A. montana* afforded, by steam-distillation, an essential oil, whose distillation yield by weight was 0.88 ± 0.002% and 1.60 ± 0.005%, respectively, as mean values and standard deviations over four repetitions. Peculiarly, the EO was dominated by a very abundant compound, ranging from one-third to more than a half of the whole oil mass. This constituent was unknown to all the mass spectrometry (MS) libraries available to the authors, and after purification and structure elucidation, it was identified as S-methyl-O-2-phenylethyl carbonothioate (see Figure 1).

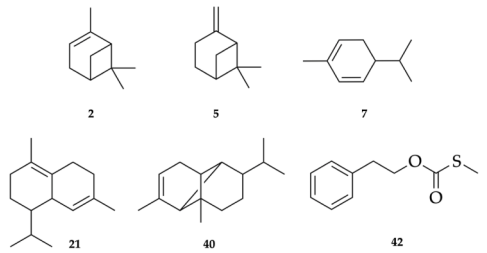

Figure 1. Chemical structures of the major components of EOs. The numbers refer to peak numbers in Table 1 and Figure 2.

On the one hand, the main components of fresh leaf EO (≥2.0%) were α-pinene (6.7%, peak 2), β-pinene (2.8%, peak 5), α-phellandrene (12.6%, peak 7), α-copaene (3.1%, peak 21), and S-methyl-O-2-phenylethyl carbonothioate (58.5%, peak 42). On the other hand, the volatile fraction of dry leaves was mainly composed of α-pinene (10.3%, peak 2), β-pinene (3.8%, peak 5), α-phellandrene (14.5%, peak 7), α-copaene (15.7%, peak 21), δ-cadinene (3.3%, peak 40), and S-methyl-O-2-phenylethyl carbonothioate (33.3%, peak 42). The compared gas chromatographic (GC) profiles are represented in Figure 2, whereas the qualitative and quantitative compositions are detailed in Table 1.

As clearly showed in Figure 2, *A. montana* EO presented a quite limited number of components, with peak 42 accounting for more than 50% of the whole oil's mass from fresh leaves and more than 30% from dry leaves. As previously mentioned, the corresponding compound had to be identified by preparative purification and structure elucidation, being absent in all the GC-MS libraries available to the authors (see Figure 3).

According to the mass spectrum, the molecular ion was not visible; however, the base peak of mass 104 *m*/*z* suggested the presence of a phenethyl group in the molecule. This group was confirmed by the ^{1}H NMR spectrum, where the proton signals of a monosubstituted aromatic ring could be observed between 7.23 and 7.35 ppm, whereas two coupled CH_2 signals corresponded to triplets at 4.45 and 3.00 ppm. Finally, a singlet, corresponding to a quite deshielded CH_3 signal, could be observed at 2.35 ppm. The ^{13}C NMR spectrum showed a total of height signals, corresponding to 10 carbon atoms, where two pairs of equivalent nuclei were part of the aromatic ring. According to DEPT-135, two carbon nuclei corresponded to quaternary atoms (171.7 and 137.3 ppm), two corresponded to CH_2 groups (67.8 and 35.2 ppm), five corresponded to aromatic CH groups (2 × 128.9, 2 × 128.6, and 126.7 ppm), and one corresponded to a CH_3 group (13.4 ppm). Of the two quaternary carbon atoms, one was typically aromatic (137.3 ppm), whereas the other one showed the characteristic chemical shift of an ester-like carbon atom (171.7 ppm). All these data could theoretically be consistent with the presence of both a phenethyl and a methyl organic moiety, linked to either an ester, thioester, carbonate, or thiocarbonate functional group. After bibliographically investigating the possible structures, all spectroscopical data (MS, NMR, and FTIR) resulted to be identical to S-methyl-O-2-phenylethyl carbonothioate (see Figure 1 and Supporting Information).[39] Additionally, the result of a HMBC experiment was consistent with the proposed structure and permitted excluding some ^{1}H and ^{13}C NMR signals, deriving from impurities (Supporting Information). To the best of the authors' knowledge, this is the second time that this compound is described in the literature and the first time it is found in nature. No applications or biological properties were reported for S-methyl-O-2-phenylethyl carbonothioate. Despite thiocarbonates being very uncommon secondary metabolites, other examples of this chemical group are known in plants. Among nonvolatile natural products, paederoside, an S-methyl carbonothioate iridoid from *Paederia scadens* (Lour.) Merr. and *Paederia fetida* L. (Rubiaceae), must be mentioned.[40] Some volatile thiocarbonates were also discovered in nature. This is for instance the case of *Tropaeolum majus* L., where O,S-diethyl carbonothioate was described, and *Ananas comosus* (L.) Merr., producing O,S-dimethyl carbonothioate.[41−43]

After the major constituent was identified, the chemical composition of fresh leaf EO was compared to the one from dry leaves, previously dehydrated under gentle conditions. Whereas the qualitative profiles were practically identical, the quantitative compositions were very different. On the one hand, most components manifested a growth in their percent amount after drying, with α-copaene showing the greatest increase (from 3.1% to 15.7%). On the other hand, S-methyl-O-2-phenylethyl carbonothioate reduced significantly, passing from 58.5% to 33.3%. These results suggested that drying is not a safe process if a high content of S-methyl-O-2-phenylethyl carbonothioate is needed. Furthermore, this phenomenon was not probably due to a physical process, such as evaporation, since lower boiling point compounds increased, whereas the heavier S-methyl-O-2-phenylethyl carbonothioate reduced.

2.2. Enantioselective Analyses. The enantioselective analyses, carried out on a β-cyclodextrin-based stationary phase, enabled determination of the enantiomeric distributions and enantiomeric excesses (*e.e.*) of nine chiral terpenes and terpenoids in both volatile fractions. Two EO constituents, (*S*)-(+)-β-phellandrene and (1*R*,2*S*,6*S*,7*S*,8*S*)-(−)-α-copaene,

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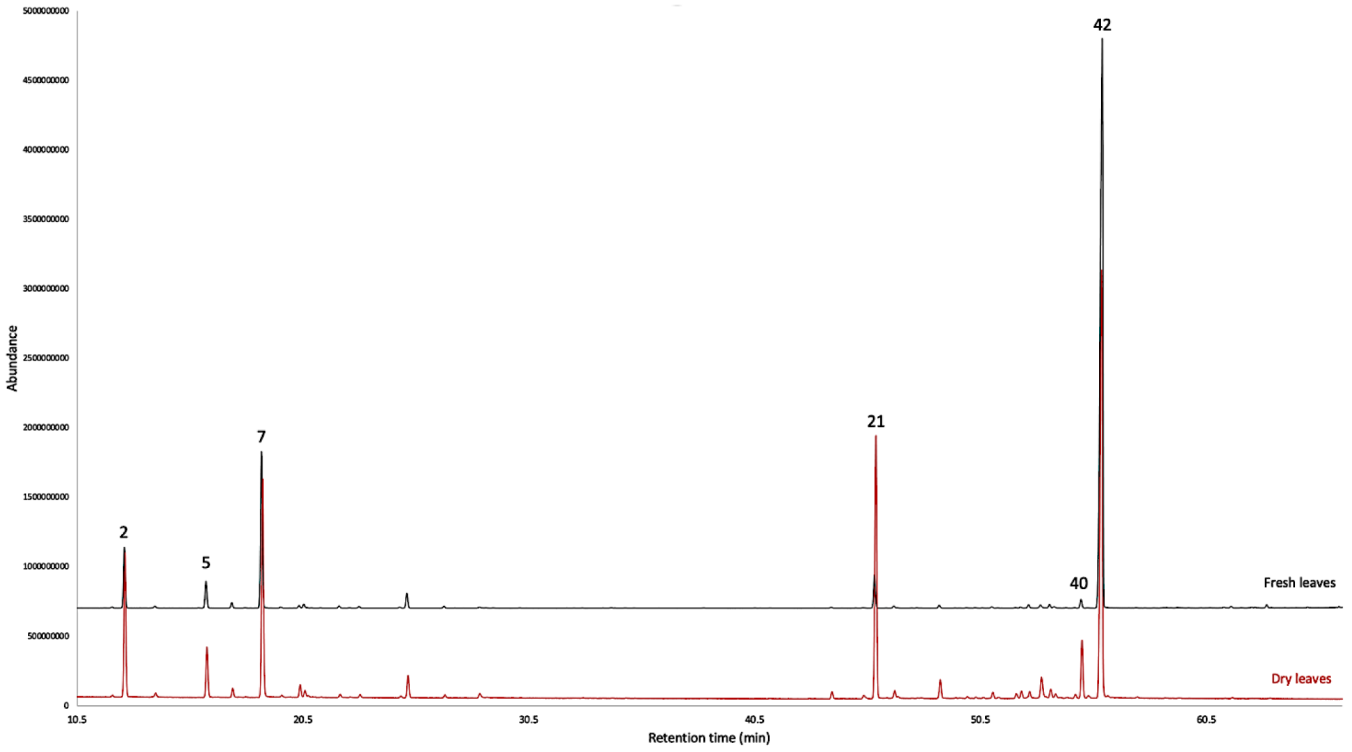

Figure 2. GC-MS profile of *A. montana* EOs on a 5%-phenyl-methylpolysiloxane stationary phase. The peak numbers refer to Table 1.

were enantiomerically pure, whereas all the others chiral compounds were present as scalemic mixtures. Among the components of these mixtures, (*S*)-(+)-α-phellandrene showed the highest enantiomeric excess (about 98%). Other enantiomers, although corresponding to quantitatively important compounds, were not included in this analysis for being inseparable on this chiral selector, or for the unavailability of their enantiomerically pure standards. The detailed results of the enantioselective analyses are reported in Table 2.

Another piece of evidence for a possible biochemical cause in the previously discussed different compositions between fresh and dry leaves is represented by the results of these enantioselective analyses. In fact, according to Figure 4 where the enantiomeric excesses of some chiral compounds are compared, we can observe that whereas the abundance of some enantiomers was unaffected by drying, the abundance of others significantly changed, such as in the cases of (*R*)-(+)-limonene and (*R*)-(−)-linalool. Since common physicochemical phenomena like evaporation or spontaneous oxidation are not enantiospecific, the most probable cause for a selective change in the enantiomeric excess is an enzymatic transformation.

3. CONCLUSIONS

The leaves of the Neotropical species *Aiouea montana* (Sw.) R. Rohde produced a high yield, sulfur smelling EO, corresponding to 0.88% and 1.60% by weight of fresh and dry plant materials, respectively. About a half and more than one-third of the weight of fresh and dry leaf oil was constituted by S-methyl-O-2-phenylethyl carbonothioate, whose presence in nature was described here for the first time. Since this plant is quite common in Central and South America and due to its high distillation yield, the EO is an interesting source of this uncommon and still poorly studied thiocarbonate. The compared chemical and enantioseletive analyses, on both fresh and dry leaf volatile fractions, showed an important reduction of the carbonothioate amount and a relevant change in some enantiomeric excess after drying. Therefore, the dehydration of *A. montana* is not a safe process, and the leaves should preferentially be freshly distilled if a higher content of S-methyl-O-2-phenylethyl carbonothioate is desired.

4. EXPERIMENTAL SECTION

4.1. Plant Material. The leaves of *A. montana* were collected on October 6, 2021, from many trees, distributed within the distance of about 300 m around a central point, with coordinates 3°49′56″S and 79°28′41″W. The altitude of this area was about 1780 m above the sea level. The taxonomical identification was carried out by one of the authors (N.C.), and a botanical specimen is conserved at the herbarium of the Universidad Técnica Particular de Loja, with voucher code 14997. About 400 g of the whole fresh plant material was distilled the same day of collection, whereas the remaining leaves were dried at 35 °C for 48 h and stored in a dark fresh place until use. This study was conducted with permission of the Ministry of Environment, Water, and Ecological Transition of Ecuador, with MAATE registry number MAE-DNB-CM-2016−0048.

4.2. Distillation and GC Sample Preparation. Both fresh and dry leaves were preparatively steam-distilled, in four repetitions (for 4 h each), using the same Dean−Stark apparatus previously described in the literature.[10] Fresh leaf repetitions were conducted over 100 g portions, whereas dry leaves were distilled as aliquots of 180 g each. The EO obtained from fresh leaves was a clear colorless liquid, whereas the one distilled from dry plant material was slightly yellowish. Both volatile fractions were characterized by a strong, unpleasant, sulfureous smell. The organic layers, initially

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Table 1. Qualitative and Quantitative Chemical Composition of *A. montana* EO on a 5%-Phenyl-methylpolysiloxane Stationary Phase

				fresh leaves		dry leaves		
N°	compounds	LRI[a]	LRI[b]	%	σ	%	σ	reference
1	α-thujene	926	924	0.1	0.02	0.1	0.01	21
2	α-pinene	934	932	6.7	1.54	10.3	0.12	21
3	camphene	950	946	0.3	0.04	0.4	0.01	21
4	sabinene	975	975	trace	-	trace	-	22
5	β-pinene	979	974	2.8	0.44	3.8	0.06	21
6	myrcene	993	988	0.6	0.07	0.7	0.01	21
7	α-phellandrene	1009	1008	12.6	1.46	14.5	0.26	23
8	α-terpinene	1020	1020	0.1	0.01	0.1	0.01	24
9	*p*-cymene	1030	1030	0.2	0.03	0.8	0.01	25
10	limonene	1032	1032	0.4	0.04	0.5	0.03	24
11	β-phellandrene	1034	1034	trace	-	0.1	0.02	26
12	1,8-cineol	1036	1036	trace	-	trace	-	27
13	(*E*)-β-ocimene	1052	1052	0.2	0.02	0.2	0.01	28
14	γ-terpinene	1062	1062	0.1	0.01	0.2	0.01	27
15	*p*-mentha-2,4(8)-diene	1084	1085	0.1	0.07	trace	-	21
16	terpinolene	1089	1086	1.4	0.16	1.6	0.03	21
17	linalool	1109	1107	0.2	0.02	0.3	0.01	29
18	phenyl ethyl alcohol	1129	1127	0.2	0.04	0.7	0.03	30
19	α-terpineol	1206	1207	trace	-	trace	-	31
20	α-cubebene	1348	1348	0.1	0.03	0.5	0.01	21
21	α-copaene	1377	1374	3.1	0.66	15.7	0.35	21
22	β-cubebene	1390	1387	0.3	0.03	0.6	0.01	21
23	β-elemene	1391	1389	trace	-	0.1	0.01	21
24	phenyl ethyl isobutanoate	1405	1403	trace	-	trace	-	32
25	(*E*)-β-caryophyllene	1421	1417	0.3	0.05	1.3	0.03	21
26	aromadendrene	1440	1439	trace	-	trace	-	21
27	α-guaiene	1440	1437	trace	-	0.3	0.01	21
28	2-phenyl ethyl butanoate	1446	1447	0.1	0.02	0.2	0.02	33
29	*trans*-muurola-3,5-diene	1451	1451	trace	-	trace	-	21
30	α-humulene	1459	1459	0.2	0.02	0.5	0.01	34
31	*allo*-aromadendrene	1462	1458	trace	-	trace	-	21
32	*trans*-cadina-1(6),4-diene	1475	1475	0.1	0.01	trace	-	21
33	dauca-5,8-diene	1475	1471	trace	-	0.4	0.01	21
34	γ-muurolene	1479	1478	0.1	0.03	0.5	0.01	21
35	germacrene D	1485	1480	0.4	0.05	0.6	0.02	21
36	β-selinene	1493	1489	trace	-	1.6	0.03	21
37	bicyclogermacrene	1499	1500	0.3	0.13	trace	-	21
38	premnaspirodiene	1500	1505	trace	-	0.7	0.08	21
39	γ-cadinene	1518	1513	0.1	0.01	0.2	0.01	21
40	δ-cadinene	1523	1522	0.9	0.16	3.3	0.06	21
41	zonarene	1528	1528	trace	-	0.2	0.01	21
42	S-methyl-O-2-phenylethyl carbonothioate	1538	-	58.5	4.18	33.3	3.73	§
43	α-cadinene	1543	1544	trace	-	0.1	0.01	35
44	germacrene D-4-ol	1584	1583	0.1	0.06	trace	-	36
45	spathulenol	1586	1584	trace	-	trace	-	37
46	γ-eudesmol	1637	1633	0.1	0.02	trace	-	26
47	α-cadinol	1666	1662	0.1	0.01	trace	-	38
48	(*E*)-sesquilavandulyl acetate	1734	1739	0.1	0.05	trace	-	21
	monoterpenes			25.6		33.3		
	oxygenated monoterpenoids			0.2		0.3		
	sesquiterpenes			5.9		26.6		
	oxygenated sesquiterpenoids			0.4		0.0		
	others			58.8		34.2		
	total			90.9		94.4		

[a]Calculated linear retention index (LRI). [b]Reference linear retention index (LRI); % = percent by weight of EO; σ = standard deviation; § = identification by MS, ^{1}H NMR, and ^{13}C NMR; trace = < 0.1%.

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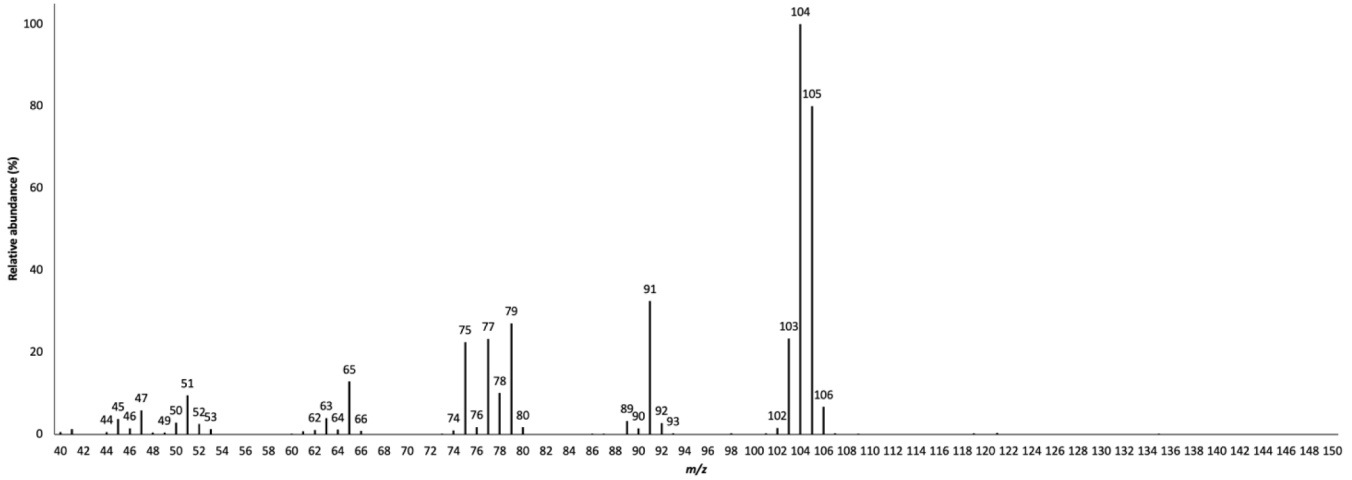

Figure 3. Electron ionization mass spectrum of S-methyl-O-2-phenylethyl carbonothioate (peak 42).

Table 2. Enantioselective Analyses of Some Chiral Terpenes from *A. montana* EO on a 2,3-Diethyl-6-*tert*-butyldimethylsilyl-*β*-cyclodextrin Chiral Selector[a]

		fresh leaves		dry leaves	
LRI	enantiomers	composition (%)	*e.e.* (%)	composition (%)	*e.e.* (%)
915	(1*S*,5*R*)-(+)-*α*-thujene[b]	17.1	65.7	11.1	77.8
918	(1*R*,5*S*)-(−)-*α*-thujene[b]	82.9		88.9	
922	(1*R*,4*R*)-(−)-camphene	48.6	2.9	48.1	3.8
937	(1*R*,4*S*)-(+)-camphene	51.4		51.9	
950	(1*R*,5*R*)-(+)-*β*-pinene	93.0	85.6	93.1	86.2
960	(1*S*,5*S*)-(−)-*β*-pinene	7.5		6.9	
1019	(*R*)-(−)-*α*-phellandrene	0.9	98.2	1.2	97.6
1022	(*S*)-(+)-*α*-phellandrene	99.1		98.8	
1063	(*S*)-(+)-*β*-phellandrene	100.0	100.0	100.0	100.0
1060	(*S*)-(−)-limonene	38.0	23.9	44.8	10.4
1075	(*R*)-(+)-limonene	62.0		55.2	
1182	(*R*)-(−)-linalool	93.8	87.6	89.0	78.1
1195	(*S*)-(+)-linalool	6.2		11.0	
1320	(1*R*,2*S*,6*S*,7*S*,8*S*)-(−)-*α*-copaene	100.0	100.0	100.0	100.0
1461	(*R*)-(+)-germacrene D	34.6	30.7	32.0	36.0
1468	(*S*)-(−)-germacrene D	65.4		68.0	

[a]LRI = calculated linear retention index; *e.e.* = enantiomeric excess. [b]Tentative enantiomer identification according to ref[44].

lighter than the water phase, tended to precipitate over time and had to be carefully recovered during distillation. After separation, the EOs were dried over anhydrous sodium sulfate and stored at −15 °C until use. For all GC analyses, about 10 mg of exactly weighted EOs were diluted with 1 mL of cyclohexane, containing *n*-nonane as the internal standard (0.7 mg/mL). Both solvent and internal standards were analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). In all GC analyses, 1 μL of the sample was injected.

4.3. Qualitative (GC-MS) and Quantitative (GC-FID) Chemical Analyses. The qualitative analyses were conducted by GC-MS, with a Trace 1310 GC coupled with an ISO 7000 single quadrupole MS, both from Thermo Fisher Scientific (Walthan, MA, USA). The oven was equipped with a 5%-phenyl-methylpolysiloxane capillary column (HP-5 from Agilent Technologies, Santa Clara, CA, USA). The column was 30 m long with 0.25 mm internal diameter and 0.25 μm phase thickness. The carrier gas was helium; it was set at the constant flow of 1 mL/min and purchased form Indura S.A. (Guayaquil, Ecuador). The injector was operated in spilt mode (40:1) and heated at the constant temperature of 230 °C. The analyses were conducted according to the following thermal program: 50 °C for 10 min, followed by a first ramp of 2 °C/min until 155 °C and a second ramp of 15 °C/min until 230 °C. The final temperature was maintained for 0.5 min. For what concerns the mass spectrometer, both the transfer line and electron ionization (EI) ion source (70 eV energy) were set at 230 °C. The MS was operated in SCAN mode, with a mass range detection of 40−400 *m*/*z*. All the EO components, except peak 42, were identified by comparison of each linear retention index (LRI) and EI mass spectrum with data from the literature (see Table 1). The LRIs were calculated according to Van den Dool and Kratz, based on a mixture of *n*-alkanes in the range of C_9−C_{22} from Sigma-Aldrich.[45]

The quantitative analyses were carried out under the same GC instrument, column, carrier gas and flow, injector temperature, and oven conditions as the qualitative ones, but using a flame ionization detector (FID) instead of MS. The injector was operated at the split ratio of 10:1. All the EO components, including peak 42, were quantified calculating each relative response factor versus isopropyl caproate, according to their combustion enthalpy.[46,47] The transformed

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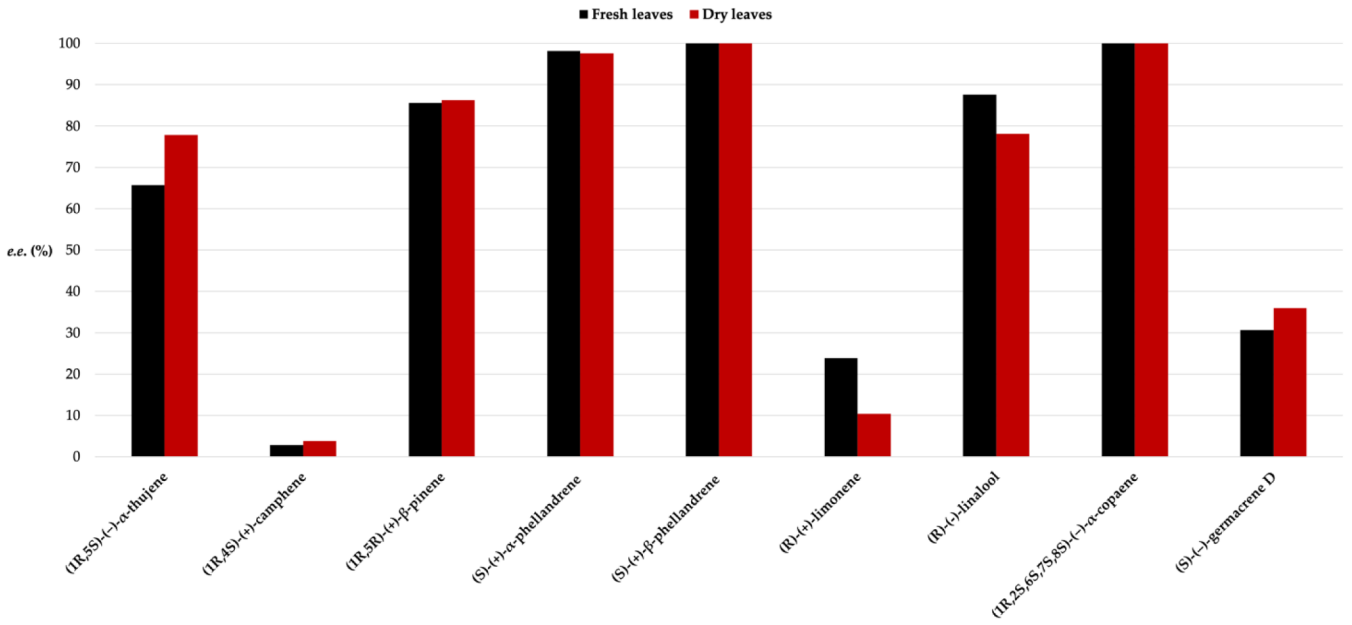

Figure 4. Compared enantiomeric excess (*e.e.*) of the analyzed enantiomers in fresh (black) and dry (red) leaves EOs.

integration areas were applied to a six-point calibration curve (R^2 = 0.998), which was traced as described in the literature, using isopropyl caproate as the calibration standard.[48] The standard was synthetized by the authors and purified until 98.8% (GC purity).

4.4. Enantioselective Analysis. The enantioselective analyses were carried out by GC-MS, in the same instrument, MS parameters, and injector temperature as the qualitative ones. The enantioselective column was characterized by a stationary phase, based on 2,3-diethyl-6-*tert*-butyldimethylsilyl-*β*-cyclodextrin as a chiral selector (MEGA S.r.l., Legnano, Italy). The elution was conducted according to the following thermal program: 50 °C for 1 min, followed by a ramp of 2 °C/min until 220 °C, that was maintained for 10 min. The carrier gas (helium) was set at the constant pressure of 70 kPa. The detected enantiomers were identified by injection of enantiomerically pure standards, purchased from Sigma-Aldrich, except for *α*-thujene, whose identification was tentatively conducted according to the literature.[44]

4.5. Purification and Spectroscopic Analyses of S-Methyl-O-2-phenylethyl Carbonothioate. Preliminarly, the best separation conditions were analytically determined on thin layer chromatography (TLC), using Merck silica gel 60 F_{254} aluminum foils from Sigma-Aldrich (phase thickness 200 *μ*m). After drying, the eluted TLC plates were exposed to UV light (254 and 366 nm) and treated with a mixture of sulfuric acid and vanillin, in order to detect the separated compounds, as previously described in the literature.[11] The best separation was achieved by eluting with a mixture of petroleum ether/diethyl ether at a 98:2 ratio. After that, the major component of the EO was purified by preparative layer chromatography (PLC), using Merck PLC silica gel 60 F_{254} plates, also purchased from Sigma-Aldrich. The preparative plates were 200 mm of width × 100 mm height × 1 mm phase thickness. About 30 mg of fresh leavf EOs, previously dissolved in 500 *μ*L of dichloromethane, were applied to the plate, subsequently eluted with the same mobile phase described for the analytical TLC. Finally, the dry eluted PLC plate was exposed to 254 nm UV light, and the most intense appearing band, located at Rf = 0.30, was scratched from the glass surface. The recovered stationary phase was packed inside a small glass column and eluted with 5 mL of pure diethyl ether. After careful solvent evaporation at reduced pressure (rotavapor *R*-210 from Büchi Labortechnik AG, Flawil, Switzerland), 12.5 mg of a clear colorless oil was obtained, corresponding to about 41.7% by weight. The product, analyzed by GC-MS in the same EO conditions, resulted to correspond to peak 42 (see Table 1) in a quite pure form. The whole sample was therefore submitted to nuclear magnetic resonance (NMR) spectroscopy and Fourier transform infrared spectrophotometry (FTIR). On the one hand, all NMR analyses were conducted with a 500 MHz Bruker spectrometer (Bruker, Billerica, MA, USA), where ^{1}H, ^{13}C, DEPT-135, and HMBC experiments were performed in $CDCl_3$ solution. The NMR spectrometer was locked to the solvent signal, corresponding to 7.26 ppm for ^{1}H NMR and 77.36 ppm for ^{13}C NMR. On the other hand, the FTIR analysis was carried out neat, through a Thermo Fisher Scientific spectrophotometer model Nicolet iS10 (Walthan, MA, USA). All the solvents were of analytical grade, purchased from Sigma-Aldrich. Raw spectral data are available in the Supporting Information.

*4.5.1. S-Methyl-O-2-phenylethyl Carbonothioate (**42**).* Colorless, sulfur-smelling oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 2.35 (s, 3H), 3.00 (t, J = 7.3 Hz, 2H), 4.45 (t, J = 7.3 Hz, 2H), 7.24−7.28 (m, 3H), 7.32−7.35 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.4 (CH_3), 35.2 (CH_2), 67.8 (CH_2), 126.7 (CH), 128.6 (2 × CH), 128.9 (2 × CH), 137.3 (C), 171.7 (C); FTIR (neat) cm^{-1} 1705, 1140, 700; EIMS (see Figure 3).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c02993.

Chemical structure of S-methyl-O-2-phenylethyl carbonothioate (Figure S1); EIMS spectrum of S-methyl-O-2-phenylethyl carbonothioate (Figure S2); FTIR spectrum of S-methyl-O-2-phenylethyl carbonothioate (neat, Figure S3) ; ^{1}H NMR spectrum (500 MHz) of S-

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methyl-O-2-phenylethyl carbonothioate in $CDCl_3$ (Figure S4); ^{13}C NMR spectrum (100 MHz) of S-methyl-O-2-phenylethyl carbonothioate in $CDCl_3$ (Figure S5); DEPT-135 experiment of S-methyl-O-2-phenylethyl carbonothioate in $CDCl_3$ (Figure S6); HMBC experiment of S-methyl-O-2-phenylethyl carbonothioate in $CDCl_3$ (Figure S7) (PDF)

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Notes

The authors declare no competing financial interest.

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