

GC-FID-MS and X-ray Diffraction for the Detailed Evaluation of the Volatiles From *Senecio filaginoides*

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Abstract

The medicinal aromatic plant *Senecio filaginoides* DC, which is very widespread in the Patagonia region, was harvested at the vegetative, flowering, and fructification stages. The materials were extracted by hydrodistillation, yielding in average 0.34% v/w of essential oils with a pleasant sweet and greenish scent. A total of 56 components were identified by gas chromatography using flame ionization detection and mass spectra measurements, representing 96.1%–97.6% of the total oil. The sesquiterpenoid 10*a*H-furanoeremophil-1-one (**1**) appeared as a major constituent (16.2%–26.9%) of the oil. It could be isolated by cooling the oil at 4°C, thus yielding yellow crystalline solids. Its stereochemistry was assigned by single-crystal X-ray diffraction since previous studies identified the compound with different stereochemistries. The use of classical separation and analytical methodologies remains as a very useful strategy for the correct identification of compounds present in the volatile fraction of a plant and is a route for potential industrial applications.

Keywords

terpenoids, *Senecio filaginoides*, patagonia, essential oils, 10*a*H-Furanoeremophil-1-one, single-crystal X-ray diffraction

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Senecio (Asteraceae) is one of the largest genera worldwide, including about 3000 species, from which 270 species are reported in Argentina.¹ *Senecio filaginoides* DC is an aromatic and medicinal plant endemic to the Patagonia region. It is popularly known as *charcao*,² which is used in folk medicine for rheumatic pains, toothache, and in Mapuche birth ceremonies.³

Essential oils are the best-known and most-used products of an aromatic plant and their analyses have been improved since the introduction of gas chromatography (GC). GC with mass spectrometry (MS) allowed ecological analysis and plant systematics⁴ while the analytical evolution promoted strategies for the investigation of a plant volatile fraction, as mighty techniques have been introduced to allow the analyst to obtain valuable structural information of a compound in a short time period without prior isolation.⁵ Nevertheless, structures of main compounds present in the essential oils of some species still remain as incorrectly identified, with many of them with systematic value as eremophilane derivatives found in *Senecio* species.⁶ This shows that the need to have the pure compounds in hand is still a fact.⁷

Reports on the chemical composition of the essential oils of *S. filaginoides* are scarce and incomplete. Balzaretto et al⁸ identified only 9 components in the essential oil by GC-MS, with *a*-pinene and *a*-terpinene + *p*-cymene being the main constituents. Other studies were performed on nonpolar extracts

providing a furanoeremophilone as the main compound, which was identified as 10*β*H-furanoeremophil-1-one by Bohlmann et al,⁹ who named it as senberginone, and later Arancibia et al¹⁰ identified the 10*a*H-furanoeremophil-9-one isomer by nuclear magnetic resonance (NMR) spectroscopy. Two other furanoeremophilane-type sesquiterpenoids, 6*a*-acetyloxy-10*β*H-furanoeremophil-1-one and 6*a*-tigloyloxy-10*β*H-furanoeremophil-1-one, were also isolated from this species¹¹

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Table 1. Essential Oil Yields (mL/kg) of *Senecio filaginoides* Var. *Filaginooides* at 3 Plant Phenological Stages.

Phenological stage	Fresh plant material	Dried plant material ^a
Preflowering	6.3	10.2
Flowering	2.4	4.9
Postflowering	1.5	3.7
Average	3.4	6.3

^aAfter moisture removal from the vegetal material.

although Bohlman et al^{9,12} suggested the 6β orientation for these molecules by nuclear overhauser effect differences spectroscopy.

Since a detailed knowledge of the essential oils composition of *S. filaginoides* is unavailable, and due to the ambiguities found in the configuration of the furanoeremophilane derivatives, the aim of this work was to analyze by GCMS the essential oils composition obtained by hydrodistillation at different plant phenological stages and to unambiguously define the structure of its main sesquiterpenoid constituent.

Results and Discussion

Hydrodistillation of the aerial parts of *S. filaginoides* at 3 plant phenological stages gave colorless oils with an aromatic pleasant odor, in average yield 0.34% v/w, as summarized in Table 1, which showed a relative density of 0.8707 g/mL and a refraction index of 1.4776 at 20°C. A gas chromatography flame ionization detection mass spectrometry (GC-FID-MS) with a special system configuration was used, which provided 3 identification parameters from a single GC run¹³: linear retention indices (LRIs) in the polar and the nonpolar columns, as well as the mass spectrum of each compound. All experimental data were compared with those found in commercial libraries,^{14,15} in the literature,¹⁶⁻²⁰ or with those of our own laboratory-developed mass spectra library built-up from constituents of known oils.

We were able to identify 56 constituents in the essential oils, as shown in Table 2, accounting for 96.1%-97.6% of the total oil composition according to the plant phenological stage. The analysis revealed a dominance of 1-nonene (2.0%-4.7%), *a*-pinene (28.3%-40.5%), sabinene (1.5%-1.9%), β -pinene (4.7%-5.4%), δ -3-carene (1.8%-5.7%), (*Z*)- β -ocimene (3.5, 7.2%), limonene (1.1%-2.2%), β -phellandrene (1.2%-1.6%), and the sesquiterpenoid 10*aH*-furaneremophil-1-one (**1**) (16.1%-27.9%) (Figure 1). Many of the herein reported compounds were not previously found in this species. Another constituent showing the same [M]⁺ value and almost an identical MS fragmentation pattern as 10*aH*-furaneremophil-1-one (**1**) was detected in appreciable amounts (10.3%) during the flowering stage. It seems to be closely related to **1**, perhaps a diastereomer, but this fact could not be confirmed.

Other authors have also detected a furanoeremophilane in the nonpolar extracts of this species^{9,10} using NMR for the identification, but different structures were proposed to this compound.

Although GC was not used in these studies, this is a quite volatile molecule having a high LRI, just in the range where the assignment of exact structures turns more erratic.

Salmeron et al¹¹ also isolated 2 furanoeremophilane-type sesquiterpenoids: 6*a*-acetyloxy-10 β *H*-furaneremophil-1-one and 6*a*-tigloyloxy-10 β *H*-furaneremophil-1-one after extracting the aerial parts of *S. filaginoides* with petroleum ether followed by CC fractionation with hexane-cyclohexane mixtures. Their structures were elucidated from spectroscopic data. Meanwhile, from the roots and aerial parts of *S. pinnatus*,¹¹ 1*a*-hydroxy-6 β -angeloyloxy-10*aH*-furaneremophil-9-one was isolated and identified using the same methods.¹¹ Thus, both *Senecio* species gave furanoeremophilane derivatives with different C-10 stereochemistry than that specified herein. In addition, Bisht et al²¹ could confirm the 1,10 β -epoxy-6-oxofuraneremophilane configuration for the main *Senecio royleanus* constituent using X-ray diffraction (XRD) analysis. The furanoeremophil-1-one with the same C-10 stereochemistry as that herein determined was found by Bohlmann et al⁹ in *Senecio bergii* and *S. bracteolatus*, and by Bohlmann and Zdero¹² after extraction of the roots of *Smyrniium olusatrum* (Apiaceae) with nonpolar solvents. Mölleken²⁰ also isolated this compound from the essential oils obtained by hydrodistillation from the latter species. More recently, Papaioannou et al²² reported furanoeremophil-1-one as a major component of the stem and leaf oils from *Smyrniium olusatrum* (54.3% and 28.7%, respectively) without specifying its configuration.

Since 10*aH*-furaneremophil-1-one (**1**) was obtained from a room temperature solvent extraction and by hydrodistillation from *S. filaginoides* this fact ruled out the possibility that it could be a breakdown product formed during the heating procedure involved in the hydrodistillation methodology.

As a definitive proposal of identification of the essential oil composition, we employed a specially built GC-FID-MS system having 2 columns of different polarity.¹³ In addition, simple separation and purification methods were used to obtain pure pale-yellow crystals of the main compound. Conventionally, isolation and purification of main compounds from plant extracts are performed by recrystallization, which is a low-cost and easy-to-handle strategy. Purified crystals were analyzed by single-crystal XRD to determine the tridimensional structure of the molecule. This allowed to ascertain the absolute stereochemistry of 10*aH*-furaneremophil-1-one (**1**) (Figure 2). This conclusion is of great value because this metabolite is a main constituent of the oil and eremophilanes are of taxonomic importance for this genus.

Conclusions

The present study is a contribution to the knowledge of *Senecio filaginoides*, which is a very abundant aromatic plant in the Patagonia region. The results highlight the great specificity of biosynthetic processes in nature, generating different stereochemical structures according to the species. The use of classical separation and analytical methods remains as a very useful strategy for the correct

Table 2. Percentage Composition of the Essential Oil Constituents of *Senecio filaginoides* Var. *Filaginoideis* at Different Plant Phenological Stages.^a

Compound	LRI ^b exp.	LRI ^b lit.	LRI ^c exp.	LRI ^c lit.	Percentage				References
					Preflowering	Flowering	Posflowering	References	
Oct-1-ene	790	788	840	842	-	0.1	0.4	0.4	14,15
Isovaleric acid	863	860	1670	1667	-	0.1	0.1	0.1	16
Non-1-ene	877	881	954	960	3.1	2.0	4.7	4.7	17
α -Thujene	925	928	1036	1026	0.1	0.4	0.1	0.1	16
α -Pinene	932	936	1043	1025	41.4	28.4	40.5	40.5	16
Camphene	946	950	1100	1069	-	0.1	0.1	0.1	16
<i>n</i> -Heptanol	960	959	1445	1452	0.9	-	-	-	14,15
Sabinene	970	973	1138	1122	1.5	1.9	1.5	1.5	16
Myrcene	975	989	1170	1161	0.7	1.0	1.0	1.0	16
β -Pinene	977	978	1133	1110	5.4	4.7	4.7	4.7	16
Isobutyl isovalerate	990	989	1194	1165	0.1	-	0.8	0.8	17
α -Phellandrene	1002	999	1191	1168	0.7	0.7	0.8	0.8	16
δ -3-Carene	1009	1008	1175	1147	1.8	2.4	5.7	5.7	16
α -Terpinene	1015	1017	1206	1179	-	0.1	0.2	0.2	16
<i>p</i> -Cymene	1017	1024	1286	1270	-	-	0.3	0.3	16
(<i>Z</i>)- β -Ocimene	1020	1029	1235	1235	7.2	3.5	6.9	6.9	16
Limonene	1024	1030	1221	1198	2.2	1.1	2.2	2.2	16
β -Phellandrene	1025	1030	1227	1209	1.2	1.6	1.2	1.2	16
1,8 cineol	1026	1031	1234	1221	0.1	0.3	0.5	0.5	16
(<i>E</i>)- β -Ocimene	1045	1048	1260	1250	0.5	0.2	0.3	0.3	16
Ethyl 5-methylhexanoate ^d	1048	1058	-	-	0.1	-	0.2	0.2	15
γ -Terpinene	1050	1060	1264	1245	0.2	0.2	0.3	0.3	16
<i>p</i> -Mentha-2,4(8)-diene	1084	1085	1268	1270	0.3	0.9	0.5	0.5	14,15
Terpinolene	1089	1087	1305	1282	-	0.2	0.2	0.2	16
Linalool	1090	1099	1549	1543	-	0.1	-	-	16
Isopentyl 2-methylbutanoate	1108	1100	1268	1273	-	-	0.3	0.3	14,15
Isopentyl isovalerate	1106	1102	1298	1292	0.5	0.1	0.1	0.1	14,15
2-Methylbutyl isovalerate	1108	1103	1307	1298	0.2	0.1	0.7	0.7	14,15
α -Camphonal	1133	1124	1507	1496	0.3	-	-	-	16
<i>p</i> -Mentha-1,5-dien-8-ol	1173	1166	1729	1718	0.3	-	0.1	0.1	14,15
Terpinen-4-ol	1179	1177	1614	1601	1.0	0.6	0.9	0.9	16
Isoamyl tiglate	1186	1191	1465	1470	-	-	0.1	0.1	14,15
α -Terpineol	1188	1190	1705	1694	0.7	0.2	0.6	0.6	16
2- <i>Z</i> -Hexenyl isovalerate ^d	1240	1241	-	-	-	-	0.2	0.2	14
Carvone	1242	1242	1745	1734	-	0.1	0.1	0.1	16
Hexyl isovalerate	1244	1247	1445	1437	-	-	0.2	0.2	15
3- <i>Z</i> -Hexenyl valerate ^d	1269	1279	-	-	-	-	0.2	0.2	14

(Continued)

Table 2. Continued

Compound	LRI ^b exp.	LRI ^b lit.	LRI ^c exp.	LRI ^c lit.	Percentage				References
					Preflowering	Flowering	Posflowering	Flowering	
Methyl (Z)-4-decenoate	1320	1323	1620	1623	-	-	0.1	0.1	14,17
β -Elemene	1387	1390	1603	1591	0.2	0.1	0.1	0.1	16
Ethyl (E)-dec-4-enoate	1391	1395	1680	1672	-	-	0.1	0.1	14,16
Methyl eugenol	1401	1402	2019	2006	-	-	0.2	0.2	16
β -Isocomene	1409	1407	1581	1571	0.3	0.1	0.1	0.1	14,15
Selina-4,11-diene ^d	-	-	1681	1688	0.2	0.2	0.2	0.2	17
<i>allo</i> -Aromadendrene	1455	1460	1658	1649	0.1	-	-	-	16
β -Selinene	1490	1486	1732	1717	0.3	-	0.1	0.1	16
Bicyclogermacrene	1512	1494	1742	1735	0.2	0.1	-	-	16
Kessane ^e	1538	1529	-	-	0.6	0.1	0.2	0.2	14
Spathulenol	1574	1577	2125	2127	0.2	0.2	0.1	0.1	17
Selina-3,11-dien-6 α -ol	1641	1642	1649	1656	0.3	0.1	0.1	0.1	14,15
Selin-11-en-4 α -ol	1659	1655	2249	2252	0.1	-	0.2	0.2	16
3,5,6,7,8,8 α -Hexahydro-4,8 α -dimethyl-6-(1-methylethenyl)-2(1H)-naphthalenone ^d	1775	1772	-	-	1.3	-	0.6	0.6	15
Furanoeremophil-1-one isomer ^d	1776	1751	2374	-	-	10.3	0.2	0.2	-
Dehydrofukinone ^d	1819	1792	-	-	-	1.7	1.3	1.3	18
Furanoeremophil-1-one isomer ^d	1875	-	-	-	2.6	2.1	0.6	0.6	-
10 α H-Furanoeremophil-1-one ^e	1890	-	2701	2706	20.7	27.9	16.2	16.2	19
Istanbulin B ^d	2315	-	-	-	-	2.1	0.4	0.4	20
Hydrocarbon monoterpenes					63.2	47.4	66.5	66.5	
Oxygenated monoterpenes					2.4	1.3	2.2	2.2	
Hydrocarbons sesquiterpenes					1.3	0.5	0.5	0.5	
Oxygenated sesquiterpenes					25.8	44.5	19.9	19.9	
Miscellaneous compounds					4.0	2.1	5.3	5.3	

Abbreviations: exp., experimental; lit., literature; LRI, linear retention index.

^aCompounds listed in elution order from the nonpolar column.^bLRI DB-5 column.^cLRI DB-Wax column.^dTentative identification.^eMass spectrum in Supplemental Material.

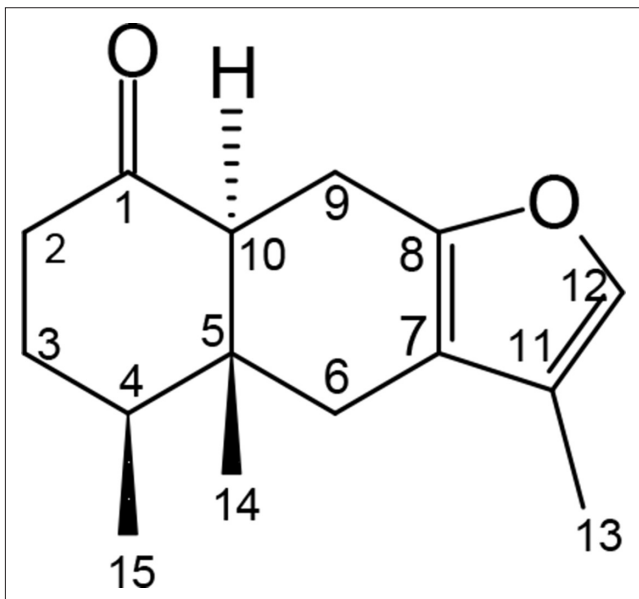


Figure 1. Formula of 10aH-furanoeremophil-1-one (**1**).

identification of compounds present in the volatile fraction of a plant. The pleasant scent of the essential oil of this plant suggests its potential use in the fragrance industry.

Experimental Section

Plant Material

The aerial parts were collected from the neighboring areas of Gualjaina River, some 30 km NE of Esquel, Futaleufú Department, Chubut Province, Argentina (42° 59' 46.5" S, 70° 46' 29.5" W) between October 2008 and February 2009. The plant material was identified as *Senecio filaginoides* DC var. *filaginoides*

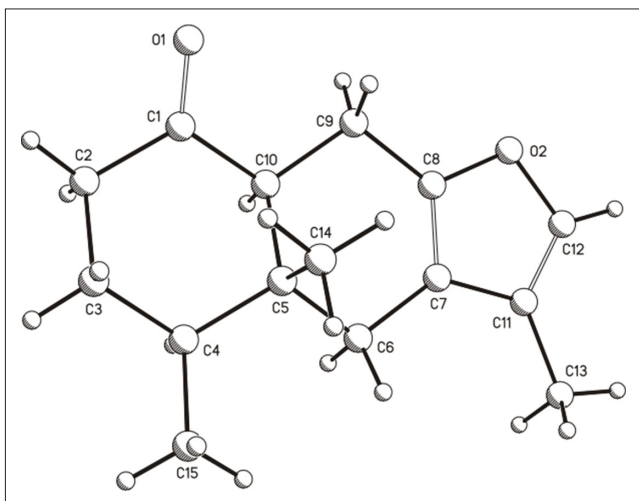


Figure 2. PLUTO plot of the X-ray crystal structure of (**1**).

by Lic. María Elena Arce and a voucher specimen is in deposit at Herbario Regional Patagónico under number HRP 6761.

Essential Oils Extraction

Essential oils were extracted from the air-dried grounded materials by hydrodistillation using a Clevenger-type apparatus for 4 hours following the method described in the Farmacopeia Argentina.²³ After cooling, settling, and drying over anhydrous sodium sulfate, the oils were recovered and stored at 4°C until analysis. The yields are expressed as mL essential oil/kg plant material in Table 1.

Isolation of 10aH-Furanoeremophil-1-One (**1**)

After storing the essential oil at 4°C overnight, a pale-yellow crystalline residue separated. The solid was separated by filtration and washed with cold *n*-hexane. It was purified by slow recrystallization using a *n*-hexane/AcOEt (9:1) mixture. The crystals were insoluble in dichloromethane, petroleum ether (35°C-38°C), and *n*-hexane, although freely soluble in ethyl acetate and ethanol. Melting point: 75°C-76°C, The MS is shown in Supplemental Figure S1.

Gas Chromatography With Flame Ionization Detection and Mass Spectra Analysis

The essential oils were analyzed by GC-FID-MS using a Perkin-Elmer Clarus 500 instrument with a modified configuration, equipped with a single split/splitless injector connected by a flow splitter to 2 capillary columns: a polyethylene glycol MW ca. 20 000 Da column and a 5%-phenyl-95%-methyl silicone column, both 60 m × 0.25 mm id with 0.25 μm of fixed phase (J&W Scientific). The polar column was connected to an FID, whereas the nonpolar column was connected simultaneously to an FID and to a quadrupole mass detector (70 eV) by a MS-Vent system. Helium was used as the carrier gas (flow rate: 1.87 mL/min). The column temperature was programmed according to the gradient: 90°C for 5 minutes, increasing at a rate of 3°C/min-230°C and maintained for 13 minutes. The injector temperature was 255°C. Both FID temperatures were 240°C, and the temperatures for the transference line and the ionic source were set at 180 and 150°C, respectively. Mass range (*m/z*) and scan time were 40-350 Da and 1 second, respectively. The manual sample injection volume was 0.2 mL of the oils and the split ratio was 80:1. A mixture of aliphatic hydrocarbons (C₆-C₂₄, Sigma Aldrich) in *n*-hexane was coinjected to calculate the LRI using a generalized equation.

Compound identification was done comparing the LRI relative to C₈-C₂₄ *n*-alkanes, obtained in both columns, with those of reference compounds and from the literature.¹⁶⁻²⁰ Additionally, each experimental MS was compared with those from commercial libraries^{14,15} and from our own data basis. The percentage composition of the essential oil components was calculated by peak area

normalization of FID responses without considering corrections for response factors. The lowest response obtained from both columns for each component was considered.

Single-Crystal XRD Study of 10aH-Furanoeremophil-1-One (**1**)

A crystal measuring $0.42 \times 0.24 \times 0.22$ mm was mounted on a glass fiber and data were collected on an Enraf Nonius Bruker CAD4 diffractometer. The crystal was orthorhombic, space group $P2_12_12_1$, with cell dimensions $a = 8.339(2)$ Å, $b = 10.956(1)$ Å, $c = 14.525(2)$ Å, $V = 1326.9(4)$ Å³, $\rho_{\text{calc}} = 1.163$ g/cm³ for $Z = 4$, $C_{15}H_{20}O_2$, MW = 232.31, and $F(000) = 504$ e. A total of 1009 reflections were collected using graphite-monochromated Cu K α radiation ($\lambda = 1.54184$ Å) within the 6.09 – 59.88° θ range for $0 \leq h \leq 9$, $0 \leq k \leq 12$, $2 \leq l \leq 16$. The structure was solved by direct methods using the SIR2004 software. For the structure refinement, the non-hydrogen atoms were treated anisotropically, and the hydrogen atoms were refined isotropically. The unique reflections were 968, the observed reflections were 934, and final discrepancy indices, refining 166 parameters, were $R_F = 3.3\%$ and $R_w = 9.2\%$. The final difference Fourier map was essentially featureless, with the highest residual peak and hole having residual densities of 0.086 and -0.102 e/Å³, respectively. Crystallographic data, excluding structure factors, have been deposited at the Cambridge Crystallographic Data Centre under number 1414005. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK. Fax: +44-(0)1223, 336033 or e-mail: deposit@ccdc.cam.ac.uk.

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Declaration of Conflicting Interests

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Supplemental Material

Supplemental material for this article is available online.

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