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FURANOEREMOPHILANES AND OTHER CONSTITUENTS FROM *SENECIO CANESCENS**

SUSANA ABDO, MARIA DE BERNARDI,† GIULIO MARINONI,‡ GIORGIO MELLERIO,† SUSANA SAMANIEGO, GIOVANNI VIDARI†§ and PAOLA VITA FINZI†

Facultad de Ciencias, Escuela Politecnica de Chimborazo (ESPOCH), Casilla 4703, Riobamba, Ecuador; † Dipartimento di Chimica Organica, via Taramelli 10, 27100 Pavia, Italy; ‡Istituto Tecnico Industriale Statale "G. Cardano", via Verdi 19, 27100 Pavia, Italy

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Key Word Index—*Senecio canescens*; Compositae; sesquiterpenes; furanoeremophilanes; cacalohastine derivatives; dammarane triterpenes.

Abstract—Investigation of the aerial parts and roots of Ecuadorian *Senecio canescens* afforded in addition to known furanoeremophilanes the first natural furanoeremophilane hydroperoxide and two new cacalohastine derivatives, one of which is a dimer. The volatile fraction was analysed by GC and GC-mass spectrometry.

INTRODUCTION

Senecio canescens Humb. (tribe Senecioneae, Compositae) and related species belonging to the genus *Senecio* are important in Ecuadorian herbal medicine. The aerial parts of this plant are used against infections and rheumatism. For the latter disease topical application of leaves is recommended [1]. Nothing is known about the constituents of *S. canescens*.

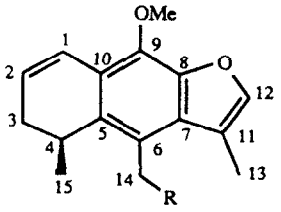
RESULTS AND DISCUSSION

The plant was collected on the highlands of the Andes in Central Ecuador (*ca* 3500 m) and divided into three parts: freshly cut green leaves, yellow-brown fibrous leaves and roots were separately soaked in hexane giving three extracts (E I–III). Each extract was repeatedly chromatographed on columns of silica gel and RP-18. Extract EI gave, in addition to a mixture of volatile compounds analysed by GC-mass spectrometry (vide infra), cacalohastine (**1**) [2, 3], dammaradienyl acetate (**12**) [4, 5] and germanicone (**13**) [6]. Extract EII afforded **1**, dehydrocacalohastine (**2**) [2, 3], maturinone (**3**) [7, 8], cacalonol (**4**) [9], cacalonol hydroperoxide (**5**), 6β-(2-methylbutanoyloxy)-9-oxo-1(10)-furanoeremophilene (**6**) [10], **12**, **13**, dammaradienone (**14**) [11, 12], spathulenol (**15**) [13], lupeol, α-amyrin, sitosterol and stigmasterol. Finally, compounds **1**, **2**, **4**, 14-angeloyloxy cacalohastine (**7**) [14], the dimeric sesquiterpenes **8** [3] and **9**, 13-acetoxydehydrocacalohastine (**10**) [3, 15], 13-hydroxydehydrocacalohastine (**11**) and sitosterol were isolated from extract EIII.

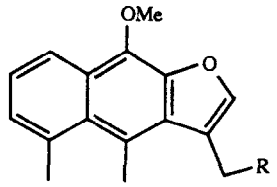
In the case of known compounds the structural assignments are based on the analytical data which were compared with those in the literature and reference compounds. Compounds **5**, **9** and **11** are new furanoeremophilanes. The assigned structures were based on their IR, mass, ^{1}H NMR spectra (Table 1), extensive decoupling experiments, ^{13}C NMR data (Table 2) and ^{1}H–^{13}C HETCOR experiments. The spectra of **5** were very similar to those of **4**. However, the EI mass spectrum of compound **5** showed a molecular ion at *m/z* 258 differing by 16 amu from that of **4**. The sharp OH stretching vibration at 3260 cm^{-1} and the signal of one proton in the ^{1}H NMR spectrum at δ 7.6, which could be exchanged by D_2O, indicated a hydroperoxy group. This attribution was confirmed by the positive reaction of **5** with potassium iodide–starch reagent and by the ions in the mass spectrum at *m/z* 241 and 225, corresponding to loss of OH and OOH fragments, respectively, from the molecular ion. Shift differences for the C-6 and C-14 signals in the ^{13}C NMR spectra of **5** and **4** (Table 2) were in agreement with the β- and γ-effects exerted by a hydroperoxy group replacing a OH group at C-6. As expected, reduction of compound **5** with Ph_3P gave **4**, thus confirming the structure. Compound **5** is probably an oxidation product of compound **2** and the direct precursor of alcohol **4**. The NMR spectra of **10** and **11** were similar. However, the intense OH band in the IR spectrum of **11** and the upfield shift of the signal attributed to H-13 in the ^{1}H NMR spectrum of **11**, with respect to that of **10**, clearly indicated that **11** must be the alcohol of acetate **10**. Acetylation of **11** under standard conditions gave **10** in quantitative yield, identical with the natural compound. The mass spectrum of **9** showed the molecular ion at *m/z* 580 ($C_{37}H_{40}O_6$) and fragment ions at *m/z* 481, 241 and 100, suggesting that **9** is a C_5-unsaturated ester of a furanoeremophilane dimer. The ester was identified as an angelate by the characteristic signals of the methyl (δ1.85 and 1.95) and methine (δ6.05) protons in the ^{1}H NMR spectrum (Table 1) and by the signals at δ 15.8 (*q*), 20.7 (*q*), 124.0 (*s*), 135.1 (*d*) and 167.8 (*s*) in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum of **9** showed the presence of two secondary methyl group at δ1.0 (J=7.0 Hz) and 1.07 (J=7.0

*Part 5 in the series 'Metabolites of Medicinal Plants'. For Part 4 see Jativa, C., Marinoni, G., De Bernardi, M., Vidari, G. and Vita Finzi, P. (1991) *J. Nat. Prod.* **54**, 460.

§Author to whom correspondence should be addressed.



R = H 1
R = $O_2C(Me)\overset{Z}{=}CHMe$ 7



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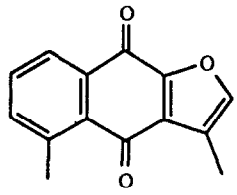
R = OAc 10



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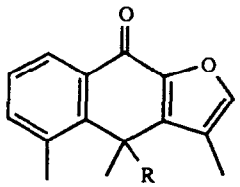
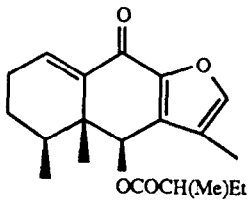


R = OH 4
R = OOH 5



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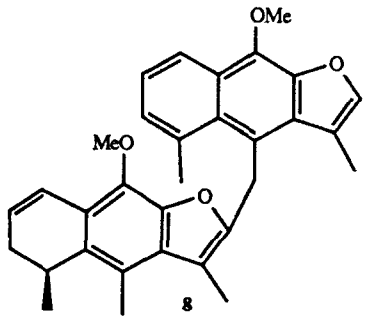

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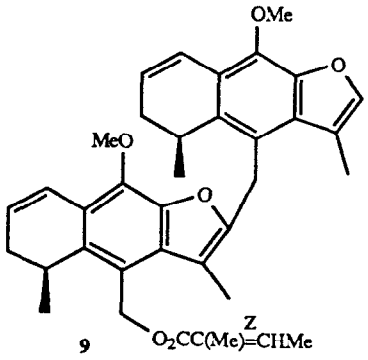

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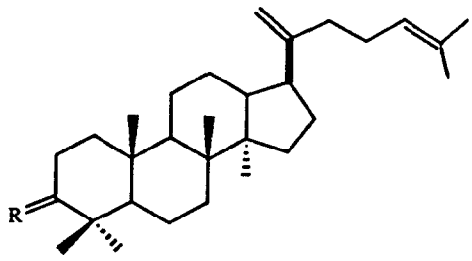
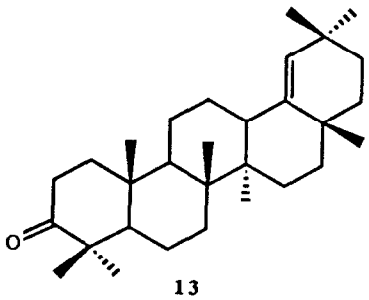


R = βOAc, αH 12
R = O 14



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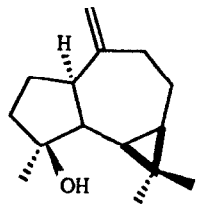

15

Hz), two methyl groups on furan rings at δ2.30 (*s*) and 2.37 (*d*, $J = 1.2$ Hz), two aromatic OMe groups (*s* at δ3.85 and 4.10), two pairs of olefinic protons at δ5.8–5.9 and 6.8–6.9, a methylene group at δ4.37 (AB *q*) between aromatic rings and one methylene (*s* at δ5.43) linked to the angeloyloxy residue. One of the methyl groups on the furan rings appeared as a singlet having no long range coupling with a proton at the α-position of the furan ring, and only one α-H of the furan ring was observed. The UV spectrum of **9** showed almost the same absorption curve as cacalohastines **1** and **7**. These results clearly indicated that **9** is the dimer between C-14 of compound **1** and C-12 of **7**.

Analysis of the volatile constituents

Chromatographic separation of extract EI gave two non-polar fractions (F2 and F3) enriched in terpenes.

Table 1. ^{1}H NMR spectral data of compounds **4**, **5**, **9** and **11** (300 MHz, δ values, TMS = 0)

H	4*	5*	9†[a]	11†
1	8.07 *dd* (7.5; 1.5)	8.11 *ddd* (7.5; 2.0; 0.6)	6.85 *dd* (10.0; 3.0)	8.20 *dd* (8.5; 1.2)
1′	—	—	6.90 *dd* (10.0; 3.0)	—
2	7.37 *t* (7.5)	7.42 *t* (7.5)	—	7.30 *dd* (8.5; 7.0)
2′	—	—	5.8–5.32 *m*	—
3	7.48 *dd* (7.5; 1.5)	7.50 *ddd* (7.5; 2.0; 0.8)	2.1–2.25 *m* ($H\text{-}3_{ax}$,$H\text{-}3'_{ax}$)	7.22 *br d* (7.0)
3′		—	2.4–2.55 *m* ($H\text{-}3_{eq}$,$H\text{-}3'_{eq}$)	—
4	—	—	3.22 *br qu* (7.0)	—
4′	—	—	3.35 *br qu* (7.0)	—
12	7.72 *q* (1.2)	7.75 *q* (1.2)	7.34 *q* (1.2)	7.62 *s*
13	2.32 *d* (1.2)	2.32 *d* (1.2)	2.37 *d* (1.2)	4.95 *d* (5.5)
13′	—	—	2.30 *s*	—
14	1.84 *s*	1.85 *s*	4.37 AB*q* (15.0)	3.12 *s*
14′	—	—	5.43 *s*	—
15	2.83 *s*	2.78 *s*	1.0 *d* (7.0)	2.97 *s*
15′	—	—	1.07 *d* (7.0)	—
OMe	—	—	3.85 *s*; 4.10 *s*	4.12 *s*
OCOR	—	—	1.85 *qu* (1.5) 1.95 *dq* (7.5; 1.5) 6.05 *qq* (7.5; 1.5)	—
OH	4.95 *s*	7.6 *s*	—	1.77 *t* (5.5)

Coupling constants (Hz) are given in parentheses. *qu* = quintet.
*Solvent = Me_2CO-d_6.
†Solvent = $CDCl_3$.
[a]Signals for protons H-1, H-1′; H-4, H-4′ and H-15, H-15′ can be interchanged.

Table 2. ^{13}C NMR data of furanoeremophilanes **4**, **5** and **11** (75.47 MHz, δ values)*†

C	4‡	5‡	11§
1	125.4 (1)	125.7 (1)	120.4 (1)
2	128.2 (1)	128.6 (1)	123.8 (1)
3	137.9 (1)	137.7 (1)	128.3 (1)
4	145.1[a](0)	144.0[a](0)	130.9 (0)
5	138.6[b](0)	138.2[b](0)	122.7[a](0)
6	71.8 (0)	83.2 (0)	135.3[b](0)
7	132.6[b](0)	134.2[b](0)	126.8[a](0)
8	147.2[a](0)	146.9[a](0)	136.7[b](0)
9	172.9 (0)	172.9 (0)	143.2 (0)
10	144.6[a](0)	142.0[a](0)	128.6[a](0)
11	121.9 (0)	121.7 (0)	121.2[a](0)
12	147.0 (1)	146.9 (1)	145.2 (1)
13	9.1 (3)	8.8 (3)	57.1 (2)
14	27.8 (3)	24.7 (3)	19.8 (3)
15	21.8 (3)	21.3 (3)	26.7 (3)
OMe	—	—	61.1 (3)

*The numbers in parentheses indicate the number of hydrogens attached to the corresponding carbon and were determined from DEPT experiments.
†Assignments are based on ^1H–^{13}C chemical shift correlated 2D NMR spectroscopy.
‡Solvent: Me_2CO-d_6.
§Solvent: $CDCl_3$.
[a,b]Assignments in the same vertical column may be interchanged.

They were thoroughly investigated by capillary GC-mass spectrometry. The results from these analyses are summarized in Table 3 where indication of the sample from which each component has been identified is reported. The identities of the compounds were based on mass spectral data in agreement with those of commercial libraries [16] and/or by direct comparison with data obtained from authentic samples. Moreover the fragmentation pattern of each terpene was automatically compared with the mass spectra of the literature. To this purpose, for a quick scanning, only a few selected ions of the reference spectra were considered and added to the files of our personal computer [17]. The retention time of standard compounds was also used for identification. Twenty-six compounds could be identified in this way, the great majority being sesquiterpenes. β-Selinene, β-bisabolene, α-muurolene and β-gurjunene were the main constituents of F2, while β-sesquiphellandrene and β-bisabolene were the most abundant components of the F3. Furthermore, two diterpenes were identified in the F2.

Furanoeremophilanes are widespread in the Senecioneae. The compounds isolated from *S. canescens* indicate a relationship to the 'cacalioid' genera such as *Roldana* and *Paracalia* (syn. *Cacalia* auct. mult. non L.). Chemically *S. canescens* seems very well differentiated from the few other Ecuadorian *Senecio* species which, to our knowledge, have been investigated so far. Thus *S. teretifolius* [18], *S. scytophyllus* [18] and *S. smithii* [19] do not contain cacalohastine derivatives. However, more species

Table 3. Volatile components of *Senecio canescens*

Compound	Fraction detected	Identification methods*
Octadecene	F2	a, b
α-Copaene	F2	a, b
γ-Ylangene	F2	a, b
β-Bourbonene	F2 F3	a, b, c
α-Cubebene	F2	a, b
α-Zingiberene	F2	a, b
β-Sesquiphellandrene	F2 F3	a, b, c
Tetradecene	F2	a, b
β-Chamigrene	F2	a, b
β-Cubebene	F2 F3	a, b, c
Aromadendrene	F2	a, b
β-Gurjunene	F2	a, b
α-Gurjunene	F2 F3	a, b, c
γ-Cadinene	F2	a, b
β-Himachalene	F2	a, b
β-Farnesene	F2	a, b
β-Selinene	F2	a, b
α-Muurolene	F2 F3	a, b, c
β-Bisabolene	F2 F3	a, b, c
δ-Cadinene	F2	a, b
Calamenene	F3	a, c
Pentadecene	F2	a, b
Pentadecano	F2	a, b
4,10-Dimethyl-7-isopropyl-bicyclo [4.4.0]-1,4-decadiene	F2	a, b
Neophytadiene	F2	a, b
Sandaracopimaradiene	F2	a, b

*Identification methods: a, GC-MS; b, retention index in methylsilicone SE-30; c, retention index in phenylmethylsilicone HP5.

need to be investigated before chemotaxonomic conclusions can be drawn.

EXPERIMENTAL

Plant material. Senecio canescens was collected in September 1985 and November 1986 on the slopes (3600 m) of Mont Ilinizas, South of Quito, Ecuador. The plant was identified by Dr José Cuatrecasas (Smithsonian Institution of Washington DC U.S.A.). A voucher specimen is deposited in the Departamento de Quimica, Facultad de Ciencias, ESPOCH (Riobamba, Ecuador).

Extraction and isolation. Green young leaves (1 kg), fibrous leaves (480 g) and roots (1.3 kg) were finely ground, then separately extracted with hexane, yielding three crude extracts, EI (11.5 g), EII (5.6 g) and EIII (26 g), respectively. The extract EI was suspended in MeOH, filtered and concd. The residue was chromatographed on silica gel (0.2–0.5 mm) with hexane–EtOAc gradient mixtures, yielding fractions A–H. Silica gel CC of fr. A (345 mg) with hexane–Et_2O gave long chain alkanes (8 mg) and F2 and F3, enriched in terpenes, which were analysed by GC-MS (*vide infra*). Crystallization (MeOH) of fr. C yielded **1** (100 mg), while silica gel CC (hexane–Et_2O mixtures) of fr. G (260 mg) followed by crystallization, afforded **12** (6.2 mg) and **13** (3 mg). Ten fractions (1–10) were obtained by silica gel CC of extract EII with a hexane–EtOAc–MeOH gradient elution. Long chain alkanes were identified (IR, ^{1}H NMR) in fr. 1. Sitosterol, stigmasterol, **1**–**3** were identified in fr. 2 (TLC and GC-MS). Crystallization of fr. 3 gave **4** (25 mg), while silica gel CC (hexane–Et_2O) of fr. 4 afforded more **3** and **4**, and **5** (4 mg). Dry silica gel (hexane–Et_2O, 15:1) and RP-18 (MeOH–EtOAc, 7:1) CC of fr. 6 yielded **13** (15 mg) and **14** (10 mg). Compound **12** (18 mg) was isolated from fr. 7. Finally three consecutive CC (A: silica gel, hexane–Et_2O, 6.5:1; B: RP18, MeOH–EtOAc, 10:1; C: RP18, H_2O–MeOH gradient) of fr. 9 gave spathulenol (**15**) (12 mg), lupeol (15 mg), α-amyrin (25 mg) and **6** (8 mg). Fr. I–XII were obtained by elution of extract EIII over silica gel (0.2–0.5 mm) with a hexane–EtOAc–MeOH gradient. Fr. I contained long chain alkanes (IR); crystallization of fr. II and III from MeOH gave **2** (800 mg) and **1** (2 g), respectively. Four consecutive silica gel CC of fr. IV with hexane–Et_2O mixtures afforded more **2** (100 mg) and **1** (480 mg), **7** (20 mg) and **8** (15 mg). Compound **9** (2 mg) was isolated from fr. V. Separate crystallization of frs. VI–XI gave, respectively, a long chain alkyl ketone (120 mg), **4** (35 mg), **10** (50 mg), a long chain alkyl alcohol (300 mg), sitosterol (2 mg) and **11** (25 mg).

6-Hydroperoxy-6-desoxycacalonol (**5**). Pale yellow solid, mp 161–162°; IR ν_{max}^{KBr} cm^{-1}:3260 (OH), 1655 (C=O), 1610, 1585, 1540, 1470, 1417, 1230, 960, 825; UV λ_{max}^{EtOH} nm (log ε): 308 (3.67); ^{1}H NMR Table 1; ^{13}C NMR Table 2; EIMS (probe) 70 eV, *m/z* (rel. int.): 258 $[M]^+$ (8), 241 $[M-OH]^+$ (11), 225 $[M-OOH]^+$ (100).

14-Angeloyloxy-12-(cacalohastin-14-yl)cacalohastine (**9**). IR ν_{max}^{Film} cm^{-1}: 1720 (COOR), 1600, 1340, 1220, 1150, 1120, 1080, 1030, 990, 760; UV λ_{max}^{EtOH} nm (log ε): 286 (4.52); ^{1}H NMR Table 1; EIMS (probe) 70 eV, *m/z* (rel. int.): 580 $[M]^+$ (37), 481 [M

$-C_4H_7CO_2]^+$ (12), 241 (34), 240 (40), 239 (82), 232 (15), 226 (15), 225 (66), 100 $[C_4H_7CO_2H]^+$ (100), 85 (23), 83 (18), 82 (21), 55 (83), 54 (21), 53 (14), 43 (12), 41 (15).

13-*Hydroxy*-3,4-*dehydrocacalohastine* (**11**). Crystals, mp 121–122° (CH_2Cl_2–hexane), IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1620, 1590, 1390, 1370, 1210, 1160, 1110, 1050, 750; UV λ_{max}^{EtOH} nm (log ε): 321 (4.10), 331 (4.09), 348 (4.07); ^1H NMR Table 1; ^{13}C NMR Table 2; EIMS (probe) 70 eV, *m/z* (rel. int.): 256 $[M]^+$ (100), 225 $[M-CH_2OH]^+$ (9), 224 (18), 223 (99), 195 (6), 167 (9), 165 (15), 152 (22), 119 (15).

Analysis of volatile fractions F2 and F3. F2 and F3 obtained by CC were analysed by GC-MS. The capillary column was an SE-30 15 m length, 0.25 mm i.d., 0.25 μ film thickness for F2 and an HP5 25 m length, 0.2 mm i.d., 0.33 μ film thickness for F3. The columns were directly introduced into the ion source. The electron impact mode (EI) was used. Chromatographic conditions: injector 200°, transfer line 260°; column oven program at 60° for 1 min then increased at a rate of 4° min^{-1} to 250° for 10 min for F2; column oven program at 60° for 1 min then increased at a rate of 2° min^{-1} to 150°, then 6° min^{-1} to 250° for 10 min for F3. Helium was the carrier gas, 35 cm sec^{-1} linear velocity. Mass spectra were acquired over 30–620 amu range at 1 sec $decade^{-1}$ with ionizing electron energy 70 eV, electron current 0.5 mA, ion source 200°. The samples were dissolved in CH_2Cl_2 and injected (1 μl) with an injection split 1:30.

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