THE ESSENTIAL OIL OF *CUNILA INCISA* (LAMIACEAE) - A RICH SOURCE OF 1.8-CINEOLE

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Hydrodistillation of the aerial parts of *Cunila incisa* (Labiatae) yielded 1-1.3% of an essential oil. GC-FID, GC-MS and ^{13}C-NMR spectroscopy analysis indicated 1.8-cineole, sabinene, α-terpineole, γ-terpinene, terpinene-4-ol, p-cymene, and linalool as the main compounds.

Keywords: 1.8-cineole; *Cunila incisa*; essential oil; gas chromatography; Labiatae.

INTRODUCTION

The genus Cunila Royen ex. L., subfamily Nepetoideae, tribe Menthae[1], consists of 22 species, ten from Mexico[2] and twelve from the southern parts of South America (South Brazil, Argentina, Paraguay and Uruguay).

The here described member of this genus, *Cunila incisa* Bentham, is a shrub with a height up to 2m, which grows at the margins of the forest and roads in the highlands (300-1000 m altitude) of the Federal State of Rio Grande do Sul (RS) in South Brazil. Epling classified this species together with *Cunila angustifolia* in the botanical section *Incisae*, and reported their geographic distribution in the Brazilian Federal States of Minas Gerais, Santa Catarina and Rio Grande do Sul[3].

The Brazilian popular names for *C. incisa* as *"vassoura-cheirosa"* or *"erva-cheirosinha"* point to the intensive smell of its essential oil. A previous, preliminary study about the essential oil of *C. incisa* revealed the presence of large amounts of 1.8-cineole[4]. There were no literature data about the popular use of this plant, but in the regions where the here examined plant material was collected, the leaves are used against respiratory diseases.

In the present study, as a part of our current research project about the chemistry of eleven *Cunila* species from South Brazil, the chemical composition of the essential oil from *C. incisa* is described for the first time.

MATERIAL AND METHODS

Plant Material and Essential Oil Extraction. Aerial parts of *C. incisa* were gathered in October 1993 near Campestre da Serra (Sample I) and in May 1994 near Paraíso do Sul, Marupiara (Sample II) in the state RS. Voucher specimens (ICN 106355 and ICN 106357) have been deposited in the Botany Department Herbarium of the Federal University (UFRGS), Porto Alegre, RS, Brazil. The fresh aerial parts were submitted to hydrodistillation for 2 hours using a Clevenger type apparatus. The obtained essential oil was separated from water and dried over anhydrous sodium sulfate.

Gas-liquid chromatography (GC). Capillary GC was carried out on a Hewlett-Packard 5890 GC with a flame ionization detector (FID, 250°C) and a split/splitless injector (250°C, split 1:10) equipped with a glass insert. Nitrogen was used as carrier gas (1 ml/min). Peak areas and retention times were calculated with a Shimadzu C-R4A integrator. Separation of the compounds was achieved on a DB-WAX (J&W Scientific) fused silica capillary column (60 m x 0.25 mm x 0.25 μm) using a temperature program (40-220°C; 3°C/min). The injection volume was 1.0 μl of a 2% *v/v* solution of essential oil in pentane. Kovats retention indices (KI) of the compounds were determined relative to the retention times of a series of paraffin hydrocarbons (C_9-C_{26}) using a non-logarithmic scale[5].

Gas-liquid chromatography-mass spectrometry (GC-MS). GC-MS analysis was done with the NERMAG AUTOMASS (France, Paris) equipped with a split injector (250°C, split 1:10). The ionization energy was 70 eV, source temperature and interface temperature were 225°C and 220°C, respectively. The separation of the compounds was carried out with the same DB-WAX capillary column and temperature program as used in the GC analysis. Helium was used as carrier gas (1.0 bar). Chromatographic peaks were checked for homogeneity with the aid of the mass chromatograms of characteristic fragment ions.

^{13}C-Nuclear magnetic resonance spectroscopy. ^{13}C-Nuclear magnetic resonance spectra (^{13}C-NMR) were recorded in $CDCl_3$ solution (1:1 *v/v*) at 50 MHz on a Varian VLX-200 spectrometer with all shifts ref. to internal TMS. Following parameters were used: pulse width 45 degrees, acquisition time 1.5 sec for 64 K data table with spectral width of 250 ppm, number of accumulation was 10 000.

RESULTS AND DISCUSSION

Hydrodistillation of the fresh aerial parts of *C. incisa* gave a colorless essential oil with a smell very similar to that of the essential oil of *Eucalyptus globulus*. The yield was 1.0% (*v/w*) for sample I and 1.3% (*v/w*) for sample II, respectively. Figure 1 shows a typical gas chromatogram obtained from sample II. The compounds were identified by comparison of their mass spectra with those of the internal mass spectra library[6] and by comparison of the Kováts indices with those of standards and literature data[7,8]. Furthermore, the ^{13}C-NMR spectra of the essential oil were considered for the identification of the main compounds (GC concentration above 1%). The ^{13}C-NMR spectra signals have been assigned based on reported individual spectra of each compound[9], considering their chemical shifts and signal intensities. The results of the qualitative and quantitative analysis by GC-FID, GC-MS, and ^{13}C-NMR of the constituents are shown in table 1 in order of elution from the DB-Wax column. A total of 37 compounds were identified including 67-75% oxygenated monoterpenes, 23-27% monoterpenes, 1-1.8% sesquiterpenes, 0.4-0.8 oxygenated sesquiterpenes, and 0.3-0.5% other components.

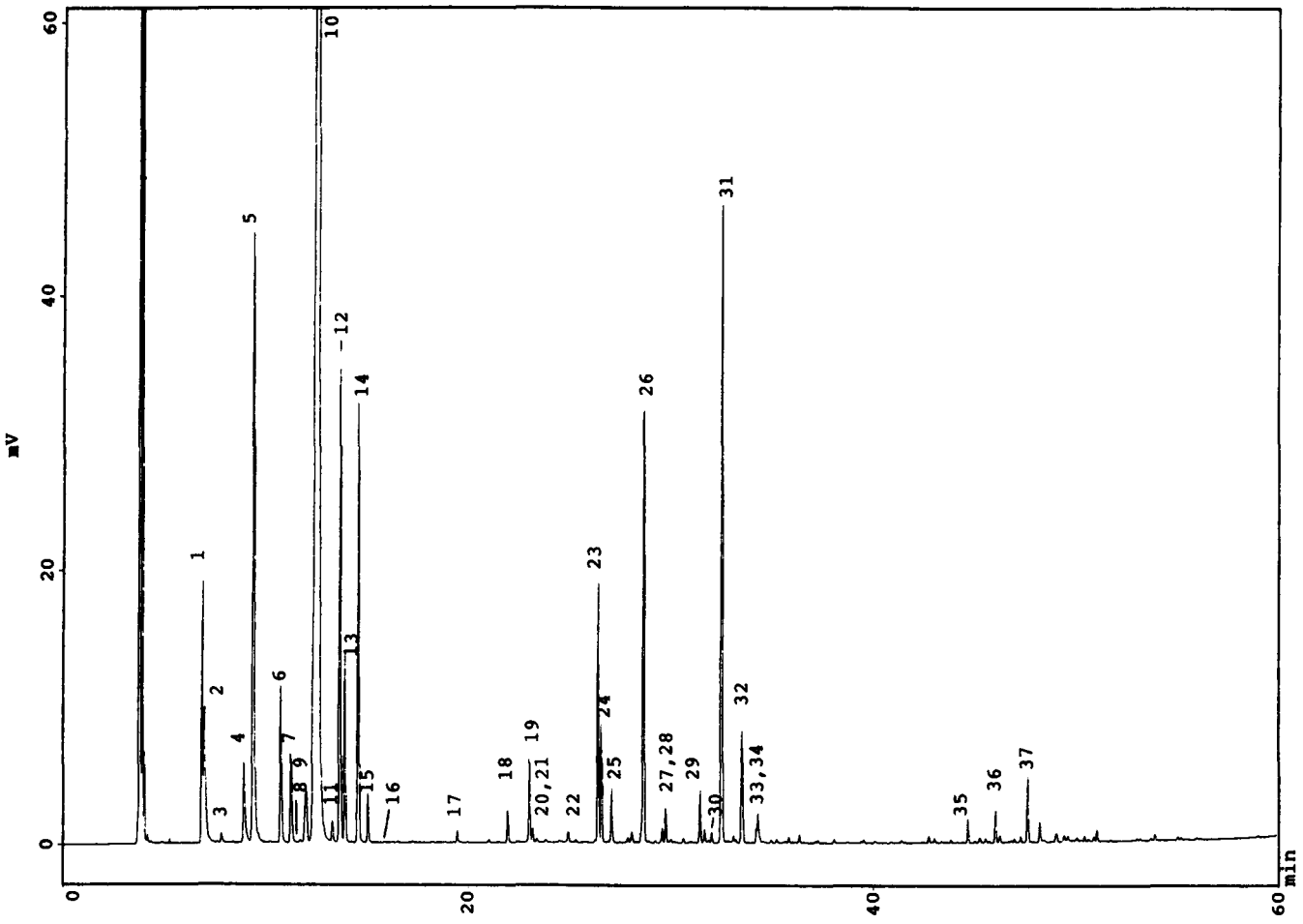

Figure 1. *Gas chromatogram of the essential oil of Cunila incisa (Sample II) on a DB-WAX (J&W Scientific; 60 m x 0.2 mm x 0.2 μm) fused silica capillary column. See Table 1 for peak identification.*

Comparison of the chemical composition of the essential oils I and II (Table 1), which have been collected in different regions and at different harvesting times, shows that there are no significant qualitative differences. The main volatile compound was 1.8-cineole (50-60%), confirming our previous communication based on thin layer chromatographic analysis[4]. Furthermore, sabinene (7.2-8%), α-terpineole (4-6.8%), γ-terpinene (3.5-5%), terpinene-4-ol (3.8-4.5%), p-cymene (3-4%), and linalool (2.2-4.5%) could be detected in higher concentrations in both samples.

The high content of 1.8-cineole could be an explanation for the popular use of the aerial parts of *C. incisa* against respiratory diseases. As it was demonstrated previously, this compound poses significant secretolytic and secretomotoric properties[10,11] Furthermore, bronchospasmolytic[11], antiphlogistic[11,12] and antiseptic[13] actions have been reported.

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Table 1. Compounds identified in the essential oil of *Cunila incisa* (Labiatae)

Peak No. in Fig. 1	Compound	Linear Kováts Index on DB-WAX	Mol Weight	GC Peak Area % Sample I	GC Peak Area % Sample II	Identification Method
1	**α-Thujene**	1008	136	1.87	2.01	a,b,c
2	**α-Pinene**	1012	136	2.26	1.78	a,b,c
3	Camphene	1045	136	0.26	0.10	a,b
4	**β-Pinene**	1089	136	1.01	1.12	a,b
5	**Sabinene**	1106	136	8.04	7.23	a,b,c
6	**β-Myrcene**	1146	136	1.01	1.54	a,b,c
7	α-Terpinene	1161	136	0.9	1.02	a,b
8	2.3-Dehydrocineole	1175	152	0.05	0.02	a,b
9	Limonene	1183	136	0.75	0.90	a,b
10[d]	**1.8-Cineole (Eucalyptol)**	1199	154	59.31	50.72	a,b,c
11	β-Z-Ocimene	1219	136	0.005	0.20	a,b
12	**γ-Terpinene**	1228	136	3.42	5.00	a,b,c
13	β-E-Ocimene	1235	136	0.012	1.61	a,b
14	**p-Cymene**	1251	134	2.96	3.92	a,b,c
15	α-Terpinolene	1262	136	0.4	0.43	a,b
16	Butanoic acid, 3-methyl, 3-methylbutylester	1284	172	0.12	0.01	a
17	Cyclohexenol	1370	100	0.041	0.17	a,b
18	Z-Linalooloxide, furanoid	1429	170	0.71	0.29	a,b
19	Cyclic monoterpene alcohol	1454	154	0.55	0.77	a
20	E-Linalooloxide, furanoid	1458	170	0.24	0.14	a,b
21	Sesquiterpene	1474	204	0.05	0.03	a
22	Sesquiterpene	1499	204	0.2	0.01	a
23	**Linalool**	1536	154	4.48	2.26	a,b,c
24	Cyclic monoterpene alcohol	1540	154	0.72	1.05	a
25	Cyclic monoterpene alcohol	1552	154	0.27	0.5	a
26	**Terpinen-4-ol**	1591	154	3.79	4.53	a,b,c
27	Benzoic acid methyl ester	1614	136	0.1	0.12	a,b
28	Cyclic monoterpene alcohol	1619	154	0.17	0.3	a
29	Linalylpropanoate	1661	210	0.34	0.45	a,b
30	Sesquiterpene	1676	204	0.04	0.1	a
31	**α-Terpineol**	1689	154	3.92	6.75	a,b,c
32	Germacrene D	1715	204	0.26	1.21	a,b
33	Sesquiterpene	1734	204	0.38	0.11	a,b
34	Sesquiterpene	1736	204	0.04	0.3	a
35	1.6.10-Dodecatrien-3-ol-3,7,11-trimethyl	2031	222	0.19	0.2	a
36	Ledol	2072	222	0.11	0.3	a
37	(-)-Spathulenol	2121	220	0.27	0.52	a
Total				**99.25**	**97.72**	

a) GC-MS; b) Kovats index on DB-WAX; c) ^{13}C-NMR; d) β-Phellandrene was detected by GC-MS as a trace compound at the beginning of the peak of 1.8-cineole

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