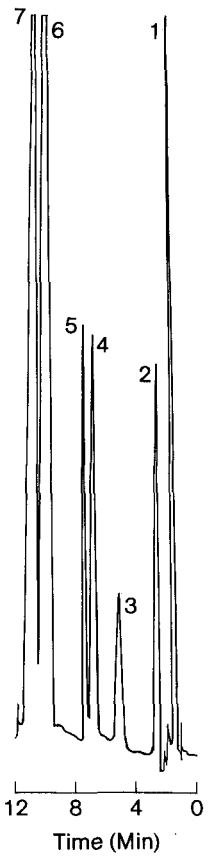

Figure 2

High performance liquid chromatogram of anthraquinones; conditions as in experimental.
1) Methanol; 2) dichloromethane; 3) rhein; 4) aloe emodin; 5) emodin; 6) physcion; 7) chrysophanol.

Flow rate: 2 ml/min.
Preparation of standard solutions: 0.05% W/V solutions of pure chrysophanol, physcion, emodin, aloe-emodin, and rhein in dichloromethane.

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Studies on the Essential Oil of *Valeriana celtica* L.

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Feline attractant

1 Introduction

Valeriana celtica L. [1–3] is a rare, wild-growing Alpine plant also known as "golden nard". It has an interesting history as a traditional household remedy against adverse effects related to the nervous system [4]. Soaps prepared from its roots show excellent nerve-strengthening properties [5].

It also protects linen and wool against insects. Moreover, *Valeriana celtica* L. exerts a strong attractant action on domestic cats (*Felix domestica*).

Valeriana celtica L. of the Valerianaicae with a chromosome number of 2n = c48 [2] or 2n : c72, c96 [3] is a typical Alpine plant. It grows in the Alps at altitudes between 1800 and 2800 m on flat humus-rich, non-lime containing soils, and sometimes on stony ground; the plant flowers in mid-summer (July-August). Very little is known about the composition of the essential oil of *Valeriana celtica* L. The present study deals with this aspect, in particular concerning the presence of feline attractants. The essential oil was obtained by steam distillation of the leaves of wild specimens of *Valeriana celtica* L. harvested in 1981 in Aosta Valley (Cogne) Italy at 2300 m above sea level. The leaves were collected after blooming of the plant because, according to the local inhabitants, the essential oil then posesses better properties.

A voucher has been deposited at the "Giardino botanico alpino Paradisia" Valnontey, Aosta (Italy).

The essential oil was analyzed as such by capillary gas chromatography–mass spectrometry and by the same technique after fractionation of the essential oil by straight phase HPLC facilitating identification of the components [6].

2 Experimental

2.1 Preparation of the Oil

100 g of dried leaves was pulverized and subjected to steam distillation in a modified Marcusson apparatus [7], yielding 0.22 g of a yellowish essential oil (0.22%).

2.2 Fractionation of the Oil

HPLC pre-separation of 10 mg of the essential oil was carried out on a silica column 25 cm × 0.46 cm packed with 5 μm spherical silica ROSiL (Alltech-Europe) installed in a Varian 5000 liquid chromatograph equipped with a Varichrom UV 50 detector operated at 220 nm. Four main fractions originating from a step elution program with 20 ml of hexane and 15 ml of methanol at a flow rate of 1 ml min^{-1} were collected in 10 ml test tubes provided with a stopper.

2.3 Gas Chromatographic Analysis

Capillary gas chromatography was performed on a HTS-OV-1 column (20 m × 0.3 mm ID, df: 0.3 μm) [8] installed in a Carlo Erba 4160 equipped with a cold on-column injector. Operating conditions were: injection temperature 30°C, column temperature ballistically programmed to 50°C and then to 250°C at 4° min^{-1}, detector FID at 300°C, carrier gas (hydrogen) flow rate 2 ml min^{-1}. Quantitation was carried out with a Varian CDS 111 integrator.

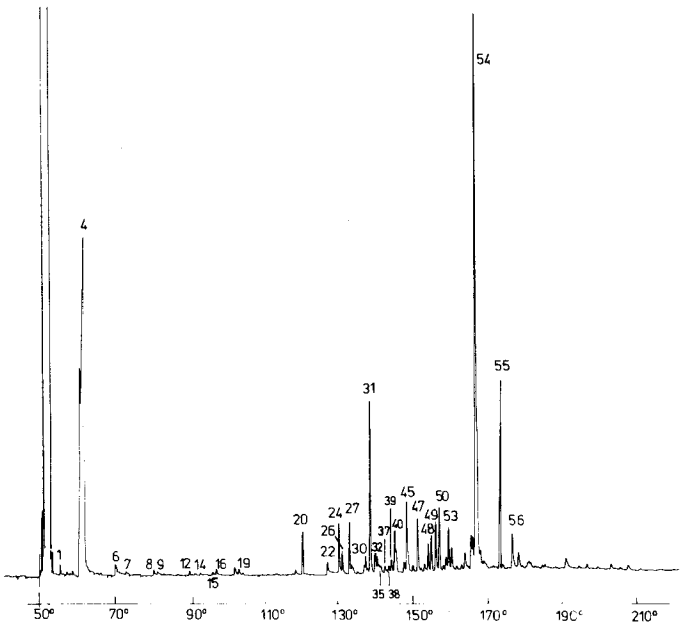

Figure 1

Chromatogram of the total essential oil of *Valeriana celtica* L.

2.4 Gas Chromatography–Mass Spectrometry

GC-MS were obtained on a Finnigan 4000 system equipped with a Data General Nova 3 computer. The same column and conditions as reported for the GC analysis were used to obtain comparable results, except that helium was used as carrier gas (flow rate 2 ml min^{-1}). The column was directly connected to the ion source *via* 1 m OV-1 coated fused silica tubing connected to the glass column with a polyimide seal [9].

Table 1

Identified compounds in the essential oil of *Valeriana celtica* L.

Peak no.	Compound			%[2]
1	Furfural			
2	Toluene			
3	Ethyl valerate			
4	Isovaleric acid			15.2
5	Benzaldehyde			
6	Valeric acid			1.0
7	4-Methylvaleric acid			
8	*p*-Cymene			
9	Ocimene			
10	Angelica lactone (*t*)			
11	Fenchyl alcohol (*t*)			
12	Fenchone			
13	Unknown			
14	Tujone			
15	Camphor			
16	Propyl isovalerate			
17	α-Terpineol			
18	Chrysanthenyl acetate			
19	Sabinyl acetate (*t*)		19	
20	Nepetalactone		20	1.3
21	Geranyl acetate			
22	Benzyl isovalerate		22	
23	α-Ionone		23	
24	β-Patchoulene		26	1.60
25	Sesquiterpene hydrocarbon			
26	β-Elemene		26	
27	Cedradiene		27	1.8
28	Sesquiterpene hydrocarbon		28	
29	β-Caryophyllene			
30	β-Gurjunene		204	
31	Seychellene		204	5.9
32	α-Guaiene			
33	α-Patchoulene		204	
34	Sesquiterpene hydrocarbon		204	
35	β-Ionone		192	
36	Cadinene		206	
37	Alloaromadendrene		204	
38	γ-Patchoulene		204	
39	α-Muurolene		204	1.0
40	Selina-4(14),7(11)-diene		204	1.7
41	Unknown		218	
42	Sesquiterpene alcohol		220	
43	Sesquiterpene alcohol		228	
44	Sesquiterpene hydrocarbon		204	
45	Patchoulane (*t*)		206	3.4
46	Sesquiterpene alcohol		220	
47	Sesquiterpene alcohol		220	2.9
48	Sesquiterpene alcohol		222	2.0
49	Sesquiterpene alcohol		220	2.2
50	Unknown		218	2.9
51	Daucalene		198	
52	Isodaucalene		198	
53	Sesquiterpene alcohol		222	2.0
54	Patchouli alcohol		222	26.2
55	Unknown	m.w.	236	6.0
56	Unknown		218	9.7

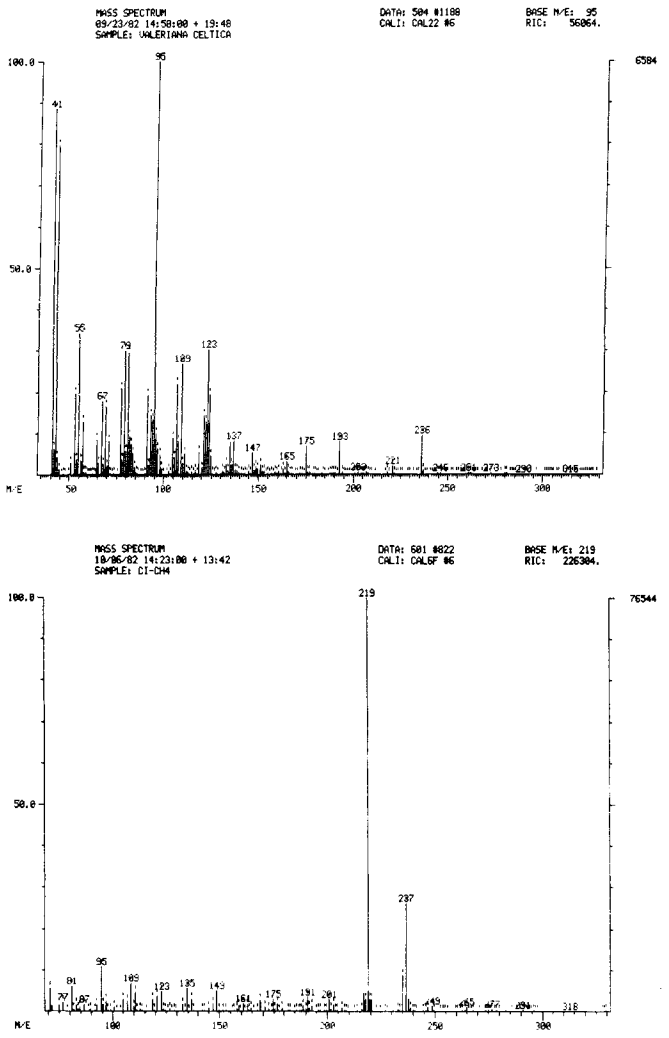

Figure 2

EI and CI (CH_4) mass spectrum of unidentified sesquiterpenoid accounting for 6% of the essential oil of *Valeriana celtica* L.

3 Results and Discussion

Figure 1 shows the chromatogram of the total essential oil. Isovaleric acid (peak 4), seychellene (peak 31), patchouli alcohol (peak 54), and an oxygenated sesquiterpene with molecular weight 236 (peak 55) are the main constituents comprising 53.4% of the oil. The total essential oil was fractionated by straight phase HPLC in four fractions. Each fraction was analyzed by capillary gas chromatography–mass spectrometry. The first fraction contained the sesquiterpene hydrocarbons. 17 of them were isolated and 13 identified. Seychellene (5.9% in total oil), cedradiene (1.8%), selina-4-(14),7-(11)diene (1.7%), β-patchoulene (1.6%), and α-muurolene (1.0%) were the main constituents.

The fraction 2 (**Fig. 2**) was mainly composed of ketones and esters. The principal constituents are: fenchone, tujone, chrysanthenyl acetate, geranyl acetate, and two sesquiterpenoids.

Fraction F_3 presented as main constituents 2 sesquiterpene compounds (MW 206 and 218 respectively) and a 236 molecular weight compound representing 6.0% of the total oil. The EI and CI mass spectra of this unknown compound are shown in Figure 2. The last fraction contained the principal components of the oil: isovaleric acid (15.2% in total oil) and patchouli alcohol (26.2%). Also nepetalactone (1.3%) was identified in this fraction.

The off-line combined technique of HPLC/CGC offers several advantages for essential oil analysis, *e.g.* fast pre-separation of small sample amounts (10 mg); no losses of volatiles by evaporation of the mobile phase; in on-column injection, the eluates can be injected as such; reduction of artifacts or alteration of the oil. As a result of this investigation 55 compounds have been classified by their mass spectral pattern; 37 were identified and 4 assigned tentatively.

The data are listed in **Table 1** together with the percentages for the compounds accounting for more than 1%. Two compounds of the essential oil of *Valeriana celtica* L. are known to posess feline attractant properties, namely isovaleric acid and nepetalactone. Nepetalactone is the main component of many *Nepeta* species, particularly in *Nepeta cataria* or Catnip. Its action on the olfactory sense of domestic cats was proven by *Waller et al.* [10].

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