ELSEVIER

Journal of Chromatography A, 966 (2002) 233–238

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Short communication

Volatile components from *Anthriscus sylvestris* (L.) Hoffm.

Rein Bos[a,*], Albert Koulman[a], Herman J. Woerdenbag[b], Wim J. Quax[a], Niesko Pras[a]

[a]*Department of Pharmaceutical Biology, Groningen Research Institute of Pharmacy (GRIP), Groningen University Institute for Drug Exploration (GUIDE), A. Deusinglaan 1, 9713 AV Groningen, The Netherlands*

[b]*Groningen Research Institute of Pharmacy (GRIP), Groningen University Institute for Drug Exploration (GUIDE), A. Deusinglaan 2, 9713 AW Groningen, The Netherlands*

Received 29 March 2002; received in revised form 16 May 2002; accepted 17 May 2002

Abstract

The volatile components of fresh leaves and roots from *Anthriscus sylvestris* (L.) Hoffm., obtained through hydrodistillation, were analysed by GC and GC–MS. This was compared to dichloromethane extracts of both fresh and dried leaf and root material. The monoterpene fraction (69–70%) dominated, while β-phellandrene (39–45%) was the main component in both the leaf and the root oil. Other components in the leaf oil were β-myrcene (17%), sabinene (6.2%), *Z*-β-ocimene (5.4%) and benzene acetaldehyde (4.1%). In the roots we found *Z*-β-ocimene (16.9%) and α-pinene (4.6%) as other major components. These principle constituents of both essential oils were also present in the dichloromethane extracts of the fresh and dried leaves and the roots, although in much smaller percentages. Comparing hydrodistillation of fresh plant material with a dichloromethane extract, the latter yielded a considerably lower amount of constituents. In addition, air drying and freeze drying resulted in a significant loss of volatile constituents as compared to fresh material (dichloromethane extract).

Keywords: *Anthriscus sylvestris*; Plant materials; Essential oils; Monoterpenes; Terpenes; Volatile organic compounds

1. Introduction

The family of the Apiaceae is well known as a source of essential oils and a number of species are especially cultivated for it, like *Pimpinella anisum* and *Anthriscus cerifolium* [1]. The essential oils of the exploited Apiaceae members are often dominated by phenylpropanoid derivatives such as anethol, methylchavicol and 1-allyl-2,4-dimethoxybenzene.

In our recent phytochemical studies of the Apiaceae member *Anthriscus sylvestris*, we found that this species accumulates high amounts of deoxypodophyllotoxin and related lignans [2]. Lignans also derive from the phenylpropanoid pathway [3]. A logical question therefore would be if the high lignan content has an influence on the essential oil composition in comparison to that of the essential oils of related species in the Apiaceae family. *A. sylvestris* accumulates lignans in its roots. These compounds are not found in the leaves. In order to find a possible relationship we studied the essential oil composition of both leaf and root material.

Anthriscus sylvestris (L.) Hoffm., (commonly known as wild chervil or cow parsley) is an abundant weed in Northwest Europe, with a height of 0.15–0.80 m and flowers in May–June [4]. Due to

*Corresponding author. Tel.: +31-50-363-3354; fax: +31-50-363-3000.

E-mail address: r.bos@farm.rug.nl (R. Bos).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)00704-5

its high content of lignans, this species might form an interesting alternative for the production of podophyllotoxin [5].

Next to the composition of the essential oils obtained through hydrodistillation, we used other sample preparations and extraction methods to profile the volatile components in *A. sylvestris*. We compared apolar (dichloromethane) extracts of fresh and dried material. We also compared drying processes consisting of drying at room temperature or by lyophilisation.

In the literature no investigations have been found on the essential oils of the roots of *A. sylvestris*, and only three references about the essential oil of the leaves and flowers were found. The oldest study does not give any quantitative data, and in the two more recent studies only the emitted volatile compounds were investigated [6–8].

2. Experimental

2.1. Plant material

Whole flowering plants were collected near the city of Groningen on 15 June, 2001. Voucher specimens have been deposited in our institute (no. Asylv2001). The roots were separated from the aerial parts of the plants, from the aerial parts we only used the leaves. This material was either immediately subjected to hydrodistillation or dichloromethane extraction, or dried at room temperature for 64 h, or lyophilised until dry.

2.2. Isolation procedure

2.2.1. Essential oil

The essential oil samples were isolated from fresh material, leaves and roots, by hydrodistillation for 4 h in 500 ml water, according to the determination of the essential oil content in vegetable drugs, using the apparatus described in the *Nederlandse Farmacopee* [9]. Xylene (100 μl) was used as the collection liquid, and the oil samples were stored at −20 °C until analysed. The oil samples were diluted 50 times with cyclohexane prior to GC analysis; injected volume, 1.0 μl. For GC–MS analysis, the oil samples were separated into two fractions—with hydrocarbons and oxygen-containing compounds, respectively—by eluting 250 μl of oil on a Bakerbond SPE column, filled with 1 g of silica gel (Mallinckrodt BAKER, Deventer, The Netherlands, no. 7086-01), with subsequently 5 ml of *n*-hexane and 5 ml of diethyl ether. After gentle evaporation of the solvent of both fractions, 50 μl of each residue were diluted with 950 μl cyclohexane and then submitted to GC–MS analysis.

2.2.2. Dichloromethane extract

The dichloromethane (DCM) extracts from fresh material were isolated as follows: 10 g of cut (ca. 0.5 cm) plant material were extracted three times with 10 ml dichloromethane and dried over Na_2SO_4. The dichloromethane was evaporated carefully and the residue was dissolved in 5 ml of dichloromethane.

The dichloromethane extracts from the dried material were prepared as follows: 2.5 g of dried (at room temperature or lyophilised, respectively) material were extracted three times with 10 ml of dichloromethane. The dichloromethane was evaporated carefully and the residue was dissolved in 10 ml of dichloromethane.

2.3. Gas chromatography

GC analysis was performed on a Hewlett-Packard 5890 Series II gas chromatograph equipped with a 7673 injector and a Hewlett-Packard 3365 Series II Chemstation, under the following conditions: column, wall-coated open tubular (WCOT) fused-silica J&W DB-5 (30 m×0.249 mm, film thickness 0.25 μm; J&W Scientific, Folsom, CA); oven temperature program, 60–300 °C at 3 °C min^{-1}; injector temperature, 250 °C; detector (flame ionization detection, FID) temperature, 300 °C; carrier gas, He; inlet pressure, 17.5 p.s.i.; linear gas velocity, 30.8 cm s^{-1}; split ratio, 60:1; injected volume, 1.0 μl for the essential oils and the dichloromethane extracts of the dried material; 4.0 μl for the dichloromethane extract of the fresh material.

2.4. Gas chromatography–mass spectrometry

GC–MS (electron impact ionization, EI) was performed on a Unicam 610/Automass 150 GC–MS

system. The GC conditions were: column, WCOT fused-silica CP-Sil 5 CB (25 m×0.25 mm, film thickness, 0.25 μm; Chrompack, Middelburg, The Netherlands); oven temperature program, 50–290 °C at 4 °C min^{-1}; injector temperature, 260 °C; carrier gas, He; inlet pressure, 5 p.s.i.; linear gas velocity, 32 cm/s^{-1}; split ratio, 20:1; injected volume, 1.0 μl. MS conditions: ionization energy, 70 eV; ion source temperature, 250 °C; interface temperature, 280 °C; scan speed, 2 scans s^{-1}; mass range, 34–500 u (1 p.s.i.=6894.76 Pa).

2.5. Identification of the compounds

The identity of the components was assigned by comparison of their retention indices, relative to C_9–C_{17} *n*-alkanes, and mass spectra with corresponding data from reference compounds and from the literature [10,11]. The percentages of the components were calculated from the GC peak areas, using the normalization method.

3. Results and discussion

3.1. Essential oil composition

The yield of the essential oil sample distilled from the fresh leaves was 0.10% (v/w). We could identify 59 components in this oil, amounting to 93% of the total oil (see Table 1). The total percentage of the hydrocarbon fraction in the oil was 79%.

The monoterpene fraction (70%) dominated the essential oil sample. β-Phellandrene (38.8%), β-myrcene (16.7%), sabinene (6.2%), and *Z*-β-ocimene (5.4%) were the main components. The oxygen-containing monoterpene fraction represented 8.8% of the total oil with *trans*-sabinyl acetate as the main component (7.7%). The sesquiterpene fraction was smaller (8.6%) with germacrene-D (4.2%) and *E*,*E*-α-farnesene (2.5%) as main components (see Table 1).

The yield of the essential oil sample distilled from the fresh roots was 0.25% (v/w). A total of 50 components were identified, amounting to 80% of the total oil. The total percentage of the hydrocarbon fraction in the oil was 69%.

The essential oil sample was dominated by the monoterpene fraction (69%). β-Phellandrene (45.4%), *Z*-β-ocimene (16.9%), and α-pinene (4.6%) were the main components. The oxygen-containing monoterpene fraction represented 3.4% of the total oil with *trans*-sabinyl acetate as the main component (3.3%). The sesquiterpene fraction was smaller (3.2%) with germacrene-D (4.2%) as a main component.

As in other studies reported so far, beta-myrcene was identified by us as the main component of the oil as well. In contrast to the studies of Kurihara and Kukuch [6] and Valterova et al. [7], we did not find limonene. Another important difference is that in the study presented here β-phallendrene is the most abundant compound while this terpenoid was not found by any of the other studies or only as a minor component (<2%) [8]. In total we found 47 compounds that have not been previously reported from the essential oils of *A. sylvestris*.

The total percentage of essential oil in the roots was higher than in the leaves (0.25%). In general the profile of the root and leaf oils correspond, with only a few marked differences. The most pronounced difference is that in leaves β-myrcene was the second most predominant component, while this is *Z*-β-ocimene in the roots. Another difference is that the α-pinene concentration was 10 times higher in roots while the sabine concentration was almost 10 times higher in the leaves.

The essential oil of *A. sylvestris* is different from that of the related species *A. cerefolium*. Methylchavicol and 1-allyl-2,4-dimethoxybenzene, which are the most abundant constituents of the essential oil of the *A. cerifolium* leaves, could not be detected in the leaves of *A. sylvestris*. The main monoterpenes in *A. cerifolium* leaves (β-pinene and β-phellandrene) were also present in *A. sylvestris* [1].

There was no difference in the content of benzene acetaldehyde between the roots and leaves. This is the only major component in the essential oil that is biosynthetically related to lignans. The presence of lignans in the roots has no notable influence on the essential composition in comparison to the leaves, but might explain the absence of phenylpropanoids as main constituents in the essential oil of *A. sylvestris* compared to *A. cerifolium*, which contains only trace amounts of lignans.

Table 1
Composition of the essential oil from *Anthriscus sylvestris*

	RI[a]	Fresh leaves ess. oil[b] (%)	Fresh leaves DCM[b] (%)	Air dried leaves DCM (%)	Freeze dried leaves DCM (%)	Fresh roots ess. oil (%)	Fresh roots DCM (%)	Air dried roots DCM (%)	Freeze dried roots DCM (%)
Tricyclene	921	t[c]					t	t	
α-Thujene	925	0.3				0.3	0.1		
α-Pinene	932	0.5		0.1		4.6	1.0		
Phenyl acetaldehyde	945	t	0.1			0.1	t	0.7	0.1
Camphene	946	0.1				0.1	t		
Isopropyl benzene	956	0.2				0.1		0.1	
Benzaldehyde	961	t				t			
Sabinene	970	6.2	1.0	0.3		0.8	1.9		
β-Pinene	975	0.4	0.1			0.2	0.2	0.3	0.2
β-Myrcene	990	16.7	3.0	1.8	0.3	0.3	2.5	2.2	0.6
α-Phellandrene	1004	1.3				0.3	0.4	0.3	0.1
Δ^3-Carene	1010	0.1	0.2	0.1		t	1.5	0.8	0.3
α-Terpinene	1017	0.1				0.1	t		
p-Cymene	1022	t					0.2	0.1	t
β-Phellandrene	1027	38.8	2.3	0.6	0.2	45.4	15.1	10.8	2.6
Z-β-Ocimene	1038	5.4	1.7	0.6	0.1	16.9	4.0	4.3	1.1
Benzene acetaldehyde	1043	4.1	0.9	0.3	0.1	3.8	1.5	1.3	0.4
γ-Terpinene	1055	t				0.1			
cis-Sabinene hydrate	1068	t				t			
Terpinolene	1087	0.1				0.2	0.2	0.1	0.1
trans-Sabinene hydrate	1097	t				t	t		
Undecane	1100	0.1		t		t			
cis-Pinene hydrate	1121	0.1				t			
allo-Ocimene	1130	0.2				t	0.1	0.1	
trans-Pinocarveol	1139	0.6		0.1	0.2	0.1	0.1		
trans-Pinene hydrate	1140	t							
Terpinen-4-ol	1177	0.3				t	t		
α-Terpineol	1187	t					t		
cis-Piperitol	1193	t				t	0.1		
Dodecane	1200	t					t		
trans-Piperitol	1205	t				t	t	0.1	
2-Phenylethyl acetate	1256	t	0.6	0.2	0.1	t	0.2	0.2	
Bornyl acetate	1284	t	0.1				0.1		
trans-Sabinyl acetate	1290	7.7	2.7	1.1	0.4	3.3	2.4	t	
Unknown (166/82)	1296	t	0.1			t	0.2	0.1	
Tridecane	1300						0.1	t	
Isoamylbenzyl ether	1309	t				t			
n-Nonanyl acetate	1311	0.1					t		
E,E-2,4-Decadienal	1314	0.1						t	
cis-Sabinyl acetate	1325	t				t	0.2	0.1	
α-Ylangene	1372	t				t	0.1	t	
β-Bourbonene	1383	t				t	0.1		
β-Cubebene	1390	0.1				t	0.2		
β-Elemene	1391	0.1	0.1			0.1		0.1	
Tetradecane	1400	t				t			
E-Caryophyllene	1418	0.2	0.1	0.1		t	0.1	t	
α-Humulene	1454	t				0.1			
β-Chamigrene	1475	0.1				0.1		0.3	0.1
Germacrene-D	1477	4.2	1.8	1.5	0.8	2.1	0.3	0.2	0.2
γ-Himachalene	1480	t				0.5	0.4	0.2	0.2
α-Farnesene	1491	1.2	0.6	0.6	0.3	t	0.2	0.1	0.1
Bicyclogermacrene	1494	t	0.1			0.1	0.1	0.1	0.1

Table 1. Continued

	RI[a]	Fresh leaves ess. oil[b] (%)	Fresh leaves DCM[b] (%)	Air dried leaves DCM (%)	Freeze dried leaves DCM (%)	Fresh roots ess. oil (%)	Fresh roots DCM (%)	Air dried roots DCM (%)	Freeze dried roots DCM (%)
α-Muurolene	1499	0.1	0.1	0.1		t	0.2		
Pentadecane	1500	t				t			
E,E-α-Farnesene	1505	2.5				0.1	t		
Δ-Cadinene	1521	0.1				0.1	0.1	0.1	0.1
E-γ-Bisabolene	1527	t					0.3		0.2
Cadina-1,4-diene	1527	t				0.2		0.5	
Germacrene D-4-ol	1574	0.2	0.1	0.1		t	0.2	0.2	0.2
epi-α-Cadinol	1643	0.1				t	t		
epi-α-Muurolol	1645	0.2	0.1			t	0.1		
% Identified		92.6				80.1			
Grouped components									
Monoterpene hydrocarbons		70.2	8.3	3.5	0.6	69.3	27.2	19.0	5.0
Oxygen-containing monoterpenes		8.6	2.8	1.2	0.6	3.4	2.9	0.2	
Sesquiterpene hydrocarbons		8.6	2.8	2.3	1.1	3.2	2.1	1.6	1.0
Oxygen-containing sesquiterpenes		0.5	0.2	0.1		t	0.3	0.2	0.2
Others			1.7	0.2	0.2		2.0	2.4	0.5
Oil yield		0.10				0.25			

[a] Retention index relative to C_9–C_{17} *n*-alkanes on the DB-5 column.
[b] Relative proportions of the essential oil constituents and the DCM extracts were expressed as percentage obtained by peak-area normalization, all relative response factors being taken as one.
[c] Trace (<0.05%).

It should be noted that with the hydrodistillation method used, major rearrangements of sensitive terpenes, such as sabinene and sabinene hydrate may occur, due to the presence of plant acids being released into the boiling water.

3.2. Dichloromethane extract

The number of identified components in the dichloromethane extracts of leaf material was much smaller compared to the number of compounds in the essential oil obtained through hydrodistillation, 21 versus 59, respectively. The compounds present in the essential oil at low or trace amounts did not appear after the extraction with dichloromethane, with the exception of phenyl ethyl acetate. In addition, the number of compounds in the dichloromethane extracts of dried material was much lower. Lyophilisation resulted even in more loss of volatile constituents than drying at room temperature. There are also quantitative differences between these extracts. For instance the extract of the freeze-dried material does not show β-phellandrene as the main component.

In the fresh, air dried at room temperature and freeze-dried root material, we could identify, respectively, 46, 31, and 18 components. In these three root extracts, β-phellandrene (15.1%, 10.8%, and 2.6%) is the main component. Other components in the fresh root extract were *Z*-β-ocimene (4.0%), β-myrcene (2.5%), sabinene (1.9%), Δ^3-carene (1.5%), and *trans*-sabinylhydrate (2.4%). The other main components of both the extracts obtained through air drying at room temperature and lyophilisation of root material were β-phellandrene (10.8% and 2.6%), *Z*-β-ocimene with 4.3 and 1.1% together with β-myrcene with 2.2% and 0.6%, respectively. In comparison with the root oil the amount of components in the dichloromethane extract from the fresh roots is almost the same, although the same remarks can be made as for the leaves.

4. Conclusion

In general, it can be concluded that from dried plant material only qualitative information about the main volatile components can be obtained.

Comparing hydrodistillation of fresh plant material with a dichloromethane extract, the latter yielded a considerably lower amount of constituents.

In addition, air drying and freeze drying resulted in a significant loss of volatile constituents as compared to fresh material (dichloromethane extract).

References

[1] J.H. Zwaving, D. Smith, R. Bos, Pharm. Weekbl. 106 (1971) 182.
[2] A. Koulman, R. Bos, M. Medarde, N. Pras, W.J. Quax, Planta Med. 67 (2001) 858.
[3] N.G. Lewis, L.B. Davin, in: U. Sankawa (Ed.), Polyketides and Other Secondary Metabolites Including Fatty Acids and Their Derivatives, Elsevier, Amsterdam, 1999, p. 639.
[4] T.G. Tutin, V.H. Heywood, N.A. Burges, D.M. Moore, D.H. Valenine, S.M. Walters, D.A. Webb (Eds.), Flora Europeae, Vol. 2, University Press, Cambridge, 1968, p. 326.
[5] W. van Uden, J.A. Bos, G.M. Boeke, H.J. Woerdenbag, N. Pras, J. Nat. Prod. 60 (1997) 401.
[6] T. Kurihara, M. Kikuchi, Yakugaku Zasshi 99 (1979) 602.
[7] I. Valterová, G. Nehlin, A.-K. Borg-Karlson, Biochem. System Ecol. 25 (1997) 477.
[8] A.K. Borg-Karlson, I. Valterova, L.A. Nilsson, Phytochemistry 35 (1993) 111.
[9] Anonymous, in: 6th edn, Nederlandse Farmacopee, Staatsuitgeverij, The Hague, 1966, p. 72, 2nd printing.
[10] D. Joulain, W.A. König, The Atlas of Spectral Data of Sesquiterpene Hydrocarbons, E.B.-Verlag, Hamburg, 1998.
[11] R.P. Adams, Identification of Essential Oil Components by Gas Chromatography–Mass Spectroscopy, Allured, Illinois, 1995.