Pergamon

Phytochemistry, Vol. 47, No. 6, pp. 1079–1083, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

PII: S0031-9422(97)00589-X

THE ESSENTIAL OIL COMPOSITION OF FRUITS FROM *SMYRNIUM PERFOLIATUM*

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(*Received* 28 *April* 1997)

Key Word Index—*Smyrnium perfoliatum*; Apiaceae; perfoliate alexanders; essential oil composition; germacrene D; 1β-acetoxyfuranoeudesm-3-ene; 1β-acetoxyfuranoeudesm-4(15)-ene; linderazulene.

Abstract—The essential oil composition of fruits from *Smyrnium perfoliatum* obtained by hydrodistillation has been investigated. A large proportion of the oil was composed of sesquiterpene hydrocarbons (60.5%) with germacrene D (45.2%) as main constituent. The fraction of oxygenated compounds (29.8%) was dominated by furanosesquiterpenoids (17.3%). Among them 1β-acetoxyfuranoeudesm-3-ene (9.0%), 1β-acetoxyfuranoeudesm-4(15)-ene (4.7%) and glechomafuran (1.3%) could be identified. Additionally, small amounts of the furanoazulene linderazulene were isolated and are found to be responsible for the deep violet colour of the oil.

INTRODUCTION

Smyrnium perfoliatum L. is a biennial umbellifer native to the Mediterranean region of South Europe extending to S. W. Asia. Due to its cultivation as a garden plant in former centuries, it is also naturalized in Western Europe (France, The Netherlands, Austria and G.B.). As its synonym *Smyrnium dioscoridis* suggests, *S. perfoliatum* is described in ancient literature as a medicinal plant. Regarding the composition of the essential fruit oil, there is only one publication in 1928 [1]. One monoterpene and one sesquiterpene hydrocarbon were isolated in addition to one oxygenated sesquiterpene, none of which has been identified; however, a dark violet colour of the oil was mentioned. The occurrence of sesquiterpene lactones in fruit and root extracts of *S. perfoliatum* was reported in 1987 [2]. Additionally, a recent paper deals with the essential oil composition of the epigeous and hypogeous parts of *S. perfoliatum* [3]. In the epigeous parts, aromadendrene, neryl isovalerate, γ-muurolene and α-santalene were found as major components, whereas α-pinene, aromadendrene and α-terpinyl valerate were described as dominant compounds in the hypogeous parts of the plant. As no further studies have been done concerning the composition of the essential fruit oil we have analysed the volatiles obtained from ripe fruits of *S. perfoliatum*.

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RESULTS AND DISCUSSION

Table 1 gives a complete analysis of the essential oil obtained by hydrodistillation of fruits from *S. perfoliatum*. Using capillary GC, 69 components were detected, out of which 57 compounds comprising 91.78% of the oil could be identified by means of mass and NMR spectroscopy. The oil is characterized by a high content of sesquiterpene hydrocarbons (60.68%), above all germacrene D (45.22%), whereas monoterpene hydrocarbons could only be detected in minor quantities. The fraction of oxygenated compounds (29.89%) is mainly composed of sesquiterpenoids, among them a large proportion of furanosesquiterpenoids (17.49%).

The most prominent furanosesquiterpenoid, compound **1** (Peak no. 64), has been identified as 1β-acetoxyfuranoeudesm-3-ene. Although the mass spectrum of this substance differs completely from data presented in [4], the respective ^{1}H NMR data are in agreement (Table 2), even if in literature the signals of several protons were not included and the solvent used was not mentioned. The structure could be verified by the ^{13}C NMR spectrum (Table 3) and the 2D-NMR data.

The mass spectrum of compound **2** (Peak no. 63), the second important furanosesquiterpenoid, clearly suggests that this substance is an isomer of **1**. Both mass as well as ^{1}H NMR spectral data (Table 2) of this constituent are in accordance with those published for 1β-acetoxyfuranoeudesm-3(15)-ene [5], which could be confirmed by the ^{13}C NMR spectrum (Table 3).

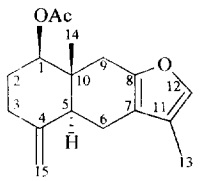
Table 1. Composition of the essential oil of *Smyrnium perfoliatum* (fruits)

Peak no.	Compound	R_I	%	Method of identification
1	2-Butanone	953	0.11	GC, MS
2	Pentan-2-one	976	0.05	GC, MS
3	*n*-Pentanal	982	0.19	GC, MS
4	α-Pinene	1037	0.19	GC, MS
5	*n*-Hexanal	1080	0.04	GC, MS
6	**β-Pinene**	**1102**	**4.44**	**GC, MS**
7	Sabinene	1112	0.03	GC, MS
8	Myrcene	1150	0.05	GC, MS
9	Limonene	1186	0.06	GC, MS
10	2-Methylbutanol	1195	0.09	GC, MS
11	Isoamyl alcohol	1196	0.08	GC, MS
12	γ-Terpinene	1234	0.02	GC, MS
13	α-Cubebene	1469	0.13	GC, MS
14	δ-Elemene	1482	0.08	GC, MS
15	α-Ylangene	1485	0.08	GC, MS
16	α-Copaene	1508	0.44	GC, MS
17	β-Bourbonene	1537	0.42	GC, MS
18	Benzaldehyde	1542	0.07	GC, MS
19	β-Cubebene	1558	0.70	GC, MS
20	Linalol	1568	0.07	GC, MS
21	β-Ylangene	1576	0.25	GC, MS
22	**β-Elemene**	**1590**	**1.88**	**GC, MS**
23	β-Gurjunene	1617	0.24	GC, MS
24	**β-Caryophyllene**	**1623**	**1.13**	**GC, MS**
25	γ-Elemene	1665	0.71	GC, MS
26	α-Humulene	1699	0.50	GC, MS
27	γ-Muurolene	1720	0.71	GC, MS
28	α-Terpineol	1729	0.09	GC, MS
29	**Germacrene D**	**1746**	**45.22**	**GC, MS**
30	Bicyclosesquiphellandrene	1748	0.31	GC, jlMS
31	α-Muurolene	1755	0.76	GC, MS
32	**Bicyclogermacrene**	**1766**	**1.54**	**GC, MS**
33	Geranyl acetate	1784	0.08	GC, MS
34	**δ-Cadinene**	**1788**	**1.80**	**GC, MS**
35	γ-Cadinene	1791	0.32	GC, MS
36	β-Sesquiphellandrene	1794	0.14	GC, MS
37	Methyl salicylate	1798	0.11	GC, MS
38	Cadina-1,4-diene	1813	0.10	GC, MS
39	α-Cadinene	1823	0.10	GC, MS
40	**Germacrene B**	**1861**	**3.03**	**GC, MS**
41	Isofuranogermacrene	1898	0.17	GC, MS
42	epi-Cubebol	1916	0.29	GC, MS
43	1,5-Epoxysalvial-4(14)-ene?	1950	0.25	MS
44	Cubebol	1965	0.33	GC, MS
45	Caryophyllene oxide	2008	0.20	GC, MS
46	Salvial-4(14)-en-1-one?	2030	0.09	MS
47	Germacra-1(10)*E*,5E-dien-4β-ol	2064	0.67	GC, MS
48	$C_{15}H_{26}O$ (*m/z*: 109, 93, 161, 41, 55)	2075	0.16	MS
49	Epi-cubenol	2079	0.12	GC, MS
50	$C_{15}H_{22}O$ (*m/z*: 107, 121, 135, 67, 93)	2089	0.17	GC, MS
51	β-Elemenone	2099	0.56	GC, MS
52	Spathulenol	2128	0.47	GC, MS
53	$C_{15}H_{22}O$ (*m/z*: 107, 135, 136, 67, 91)	2165	0.19	MS
54	τ-Cadinol	2167	0.38	GC, MS
55	τ-Muurolol	2182	0.76	GC, MS
56	δ-Cadinol	2191	0.25	GC, MS
57	**α-Cadinol**	**2219**	**1.54**	**GC, MS**
58	**Germacrone**	**2223**	**4.49**	**GC, MS**
59	$C_{15}H_{18}O_2$ (*m/z*: 43, 230, 187, 109, 145)	2333	0.56	MS
60	$C_{15}H_{20}O_2$ (*m/z*: 119, 159, 202, 43, 120)	2345	0.28	MS

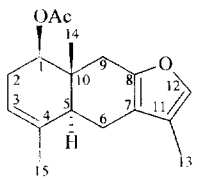
Table 1. *Continued*

Peak no.	Compound	R_I	%	Method of identification
61	$C_{15}H_{18}O_2$ (*m/z*: 172, 187, 108, 230, 43)	2594	0.59	MS
62	**Furanosesquiterpene* (*m/z*: 199, 214, 274, 43, 172)**	**2655**	**1.01**	**MS**
63	**1β-Acetoxyfuranoeudesm-4(15)-ene**	**2663**	**4.65**	**NMR, MS**
64	**1β-Acetoxyfuranoeudesm-3-ene**	**2677**	**9.04**	**NMR, MS**
65	**Glechomafuran**	**2871**	**1.28**	**NMR,MS**
66	Furanosesquiterpene* (*m/z*: 199, 232, 172, 147, 41)	2902	0.28	MS
67	Furanosesquiterpene* (*m/z*: 232, 108, 109, 91, 43)	2965	0.43	MS
68	Furanosesquiterpene* (*m/z*: 232, 108, 43, 109, 41)	3011	0.28	MS
69	Linderazulene	3062	0.35	NMR, MS
	Total		96.20	

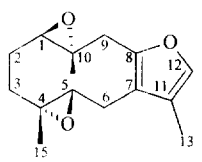
* Positive reaction on TLC after spraying with 0.25% dimethylaminobenzaldehyde in acetic acid–phosphoric acid (EP-reagent).



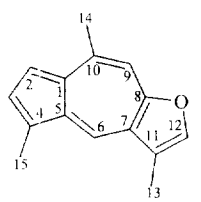
1
1β-acetoxyfuranoeudesm-4(15)-ene



2
1β-acetoxyfuranoeudesm-3-ene



3
glechomafuran



4
linderazulene

Compound **3** (Peak no. 65) was identified as glechomafuran by comparing its ^1H and ^{13}C NMR spectral data (Tables 3 and 4) with the respective data given in [6–8], however in [7] the signals at δ 121.9 and 116.0 were wrongly assigned. As the HMBC demonstrates, the signal at δ 116.0 is coupled with the doublet at δ 3.45 (H-9) while the signal at δ 121.9 shows no connectivity to H-9. Consequently C-7 must be assigned to the signal at δ 121.9 and C-11 to the signal at δ 116.0. The mass spectrum is comparable to the data presented in [8], although the intensive fragments at *m/z* 43, 108 and 109 were not mentioned in the paper.

Additionally, small amounts of compound **4** (Peak no. 69) were isolated and identified as linderazulene. This substance, which is known as a pigment of gorgonians [9], has been found in a higher plant for the first time. It is responsible for the deep violet colour of the oil. In addition, we have detected the same compound in some oils of *Curcuma zedoaria* as a trace constituent [10].

With the exception of **4** all the furanosesquiterpenoids in this study are known constituents of other *Smyrnium* species. Compound **1** has been found in fruits of *S. galaticum* [4], while **2** and **3** have been isolated from fruits of *S. olusatrum* previously [5].

Comparing these results with those recently published by Tirillini *et al.* [3], it is very surprising that there is no similarity, since the investigations of herbs, roots and fruits of other *Smyrnium* species [10] exhibited only quantitative differences between the respective essential oil compositions. It is particularly astonishing, that furanosesquiterpenoids have not been detected in any of the investigated organs, as the presence of this group of substances has been described for all species of the genus *Smyrnium* [11–15]. Concerning *S. perfoliatum*, furanodiene has been found as a constituent of the roots previously [2].

EXPERIMENTAL

Plant material and isolation of the volatile compounds. The fruits of *Smyrnium perfoliatum* were harvested in July 1992 from Kirklareli in Turkey. A voucher specimen has been deposited in the Herbarium of the Department of Pharmaceutical Biology, University of Hamburg. The ripe fruits were crushed and subjected to hydrodistillation for 6 h [16] yielding 0.65% of a deep violet coloured oil. Before detailed GC and GC–MS analysis, the oil was prefractionated by silica gel dry CC using *n*-pentane and a mixture of *n*-pentane and Et_2O (8:2) as eluents [17].

GC. A Varian series 3700 gas chromatograph equipped with an FID and a DB-Wax fused-silica capillary column (30 m × 0.25 mm, film thickness: 0.25 μm) was employed. Oven temp. was programmed from 45° to 220° with a heating rate of 3° min^{-1}. Injector and detector temp. were 220°; carrier gas was 0.9 ml min^{-1} N_2. Calculation of the percentages was based on peak areas obtained with a HP 3396 A integrator without response factor correction.

Compounds **1**–**4** were isolated by prep. GC using a

Table 2. ^{1}H NMR spectral data of compounds **1** and **2** in comparison to the literature (δ-values)

H	**1**[a,*]	literature [5]‡	**2**[a,†]	literature [4]‡
1	4.83 *dd* (1a,2e = 4.4)	4.83 *dd* (4.5, 12)	4.88 *dd* (1a,2a = 10.4, 1a,2e = 6.1)	4.92 *dd* (4,11)
2	1.47 *ddt* (2a,2e = 13.8, 2a,3a = 12.0, 2a,1a = 12.0, 2a,3e = 4.4)	1.58 *dddd*	1.98–2.10 *m*	—
2′	1.81–1.86 *m*	1.90 *dq*	2.36–2.44 *m*	—
3	1.90 *dt* (3a,3e = 13.8, 3a,2e = 4.4)	2.25 *m*	5.34 *br s*	5.27 *br d* (11)
3′	2.09 *ddd* (3e,2e = 2.5)	2.25 *m*	—	—
5	1.77–1.83 *m*	2.33 *dd* (3, 12)	2.24–2.37 *m*	—
6	2.28 *m*	2.40 *dd* (5, 12)	2.58 *m*	—
6′	2.28 *m*	2.47 *dd* (5, 12)	2.58 *m*	—
9	2.45 *d* (9a,9e = 16.1)	2.41 *d* (16)	2.33 *d* (9a,9e = 16.1)	2.08 *d* (12)
9′	2.76 *d*	2.57 *d* (17)	2.50 *d*	2.27
12	7.02 *br s*	7.04 *br s*	7.04 *br s*	7.08 *br s*
13	1.79 *d* (13,12 = 1.2)	1.92 *d* (1)	1.93 *d* (13,12 = 1.0)	1.94 *d* (1)
14	0.82 *s*	0.81 *s*	0.86 *s*	1.34 *s*
15	4.65 *d* (15,15′ = 1.6)	4.79 *d* (1)	1.69 *d* (15,3 = 1.2)	1.70 *s*
15′	4.77 *d*	4.92 *d*	—	—
2ac	1.64 *s*	2.06 *s*	2.07 *s*	2.14

Coupling constants in parentheses in Hz.
[a] all protons were assigned by COSY, NOESY, HMBC, and HMQC.
* in C_6D_6.
† in $CDCl_3$.
‡ solvent not mentioned.

Table 3. ^{1}H NMR spectral data of compounds **3** and **4** in comparison to the literature (δ-values)

H	**3**[a,*]	literature [6]†	literature [7]†	**4**[a,‡]	literature [9]§
1	2.37 *dd* (1a,2a = 10.3, 1a,2e = 1.9)	2.86 *d* (11)	2.86 *dd* (10.5, 2)	—	—
2	1.66–1.72 *m*	1.5–2.2 *m*	2.14 *m* (1,2a = 2)	6.95 *d* (2,3 = 3.2)	7.04 *d* (4)
2′	0.98–1.07 *m*	1.5–2.2 *m*	1.49 *m* (1,2b = 10.5)	—	
3	1.85 *m*	1.5–2.2 *m*	2.31 *m*	7.11 *d*	7.25 *d*
3′	0.98–1.07 *m*	1.5–2.2 *m*	1.38 *m*	—	—
5	2.77–2.85 *m*	3.16 *q* (9.5, 2.5)	3.20 *dd* (10, 2.5)	—	—
6	2.18 *m*	2.56 *d* (17)	2.36 *ddd* (18, 10, 1.5)	8.14 *s*	8.10 *s*
6′	2.77–2.85 *m*	3.03 *d* (17)	3.03 *br d* (18)	—	—
9	2.36 *d* (9a,9e = 15.8)	2.30 *m*	2.57 *br d* (17)	7.03 *s*	7.28 *s*
9′	3.45 *d*	3.45 *d* (15)	3.41 *d* (17)	—	—
12	6.83 *br s*	7.06 *br s*	7.08 *br s*	7.37 *br s*	7.13 *s*
13	1.59 *d* (13,12 = 1.3)	1.81 *d* (1.5)	1.93 *d* (1.5)	2.15 *d* (13,12 = 1.3)	2.33 *s*
14	0.97 *s*	1.30 *s*	1.29 *s*	2.55 *s*	2.75 *s*
15	0.92 *s*	1.25 *s*	1.18 *s*	2.44 *s*	2.64 *s*

Coupling constants in parentheses in Hz.
[a] all protons were assigned by COSY, NOESY, HMBC, and HMQC.
* in C_6D_6.
† in $CDCl_3$.
‡ in acetone-d_6.
§ in CCl_4.

Table 4. ^{13}C NMR spectral data of compounds **1**–**4** (δ-values)

C	1	2	3	4
1	80.1	77.1	67.5	133.7
2	28.3	29.0	24.1	116.9
3	34.0	119.7	36.6	132.2
4	147.4	134.6	58.9	136.6[a]
5	44.1	44.2	60.9	127.1[a]
6	20.7	21.2	24.8	124.9
7	115.7	116.6	116.2	121.2
8	149.1	150.1	147.0	158.8
9	36.0	35.0	38.5	111.4
10	40.2	37.5	60.3	139.5
11	119.3	119.4	121.9	119.7
12	137.8	137.8	136.7	139.1
13	8.1	8.1	8.4	7.6
14	12.0	11.4	17.4	24.3
15	109.0	20.8	16.2	13.2
1ac	169.7	169.8	—	—
2ac	20.6	20.6	—	—

Measured in C_6D_6. All signals were assigned by HMBC, and HMQC.

[a] signals may be interchanged.

Varian aerograph 1700 equipped with a stainless steel column (1.95 m × 0.5 mm) packed with 20% silicon GE SE-30 on 80–100 mesh Volaspher A2 connected with an outlet splitter/FID (split-ratio: 10:1). The column was held isothermal at 190°, the N_2 flow rate was 190 ml min^{-1}.

GC–MS. A Hewlett-Packard 5890 A series II; MSD 5970B GC–MS system equipped with a HP-UX series 9000, Mod.340 data system was used. A DB-Wax fused-silica capillary column (60 m × 0.25 mm, film thickness: 0.25 μm) was directly led into the ion source (220°), temp. program as above, flow: 0.9 ml min^{-1} He, ionisation voltage: 70 eV (E.I.-mode).

Component identification. The identification of the individual constituents was performed by computer search of the Wiley/NBS Registry of Mass Spectral Data, the TNO Library of Mass Spectral Data, and a user generated library with retention indices and mass spectral data of authentic reference substances. The structures of compounds **1**–**4** were determined by ^1H and ^{13}C NMR spectroscopy, including COSY, HMBC, HMQC, and NOE measurements.

1β-*Acetoxyfuranoeudesm*-3-*ene* (**1**). ^1H NMR: Table 2; ^{13}C NMR: Table 4; EIMS (70 eV, 220°) *m/z* (rel. int.): 274 $[M]^+$ (57), 214 (88), 199 (100), 185 (52), 171 (27), 159 (33), 135 (28), 108 (56), 91 (38), 79 (28), 43 (76).

1β-*Acetoxyfuranoeudesm*-4(15)-*ene* (**2**). ^1H NMR: Table 2; ^{13}C NMR: Table 4; MS data are in agreement with [5].

Glechomafuran (**3**). ^1H NMR: Table 3; ^{13}C NMR: Table 4; EIMS (70 eV, 220°) *m/z* (rel. int.): 248 $[M]^+$ (39), 161 (13), 135 (17), 109 (44), 108 (81), 91 (27), 79 (31), 77 (22), 65 (13), 55 (15), 43 (100), 41 (26), 39 (19).

Linderazulene (**2**). ^1H NMR: Table 3; ^{13}C NMR: Table 4; EIMS *m/z* (rel. int.): 210 $[M]^+$ (85), 209 (100), 195 (29), 166 (12), 165 (41), 152 (17), 82 (11), 45 (20).

Acknowledgements—We thank Dr Wulf Schultze for the GC–MS analysis.

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