Journal of Chromatography, 518 (1990) 230–233
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 570

Note

Essential oil from *Thymus borgiae*, a new Iberian species of the *Hyphodromi* section

M. AMPARO BLAZQUEZ, ANA BONO and M. CARMEN ZAFRA-POLO*
Departament de Farmacologia i Farmacotècnia (Farmacognosia y Farmacodinamia), Facultat de Farmacia, Avda. Blasco Ibáñez 13, 46010 Valencia (Spain)
(First received March 12th, 1990; revised manuscript received May 16th, 1990)

The genus *Thymus* includes a wide range of species, many of which are used in folk medicine for their antimicrobial, antitussive and spasmolytic properties [1]. The taxonomic classification of this genus, however, is very complex and continuous efforts have been made to distribute its species correctly among the eight sections into which it is divided. From a taxonomic point of view, studies on the essential oils and flavonoids of the *Thymus* species can be useful as an aid in defining the species, in detecting hybridization in natural populations, in confirming the presence of geographical races and in confirming section limits.

Along these lines, and as a continuation of our studies on species from the *Hyphodromi* section [2,3], we report the qualitative and quantitative composition of the essential oil of *Thymus borgiae*, a new plant species recently described as an endemic population of thyme growing in Spain and included in the *Hyphodromi* section [4].

EXPERIMENTAL

Plant material

Aerial parts of *Thymus borgiae* growing in Guadalaviar (Teruel, Spain) were collected in July 1989 at the flowering stage. Voucher specimens were authenticated by Prof. B. Peris of the Department of Botany, Faculty of Pharmacy. The fresh plant material was submitted to hydrodistillation (3 x 100 g) in a modified Clevenger apparatus for 2.5 h, yielding 0.41 ± 0.03% (v/w) of a yellowish essential oil. The physical constants of the oil were $n_D^{20} = 1.569$, $[\alpha]_D^{20} = -2.78$ and $d_{20}^{20} = 0.936$.

Column chromatography (CC)

The oil (1 ml) was fractionated on a silica gel column using hexane in order to obtain the hydrocarbon fraction. The oxygenated compounds were then eluted with hexane–dichloromethane mixtures and dichloromethane. A total of ten fractions were obtained.

0021-9673/90/$03.50

Gas chromatography (GC)

GC was performed with a Konic 2000-C gas chromatograph equipped with a 25 m x 0.25 mm I.D. SE-52 (5% phenylmethylsilicone) high-performance capillary column. The column temperature was 60°C for 6 min, then increased at 5°C min to 150°C which was maintained for 10 min. The carrier gas was nitrogen at a flow-rate of 2 ml/min. The injector temperature was 225°C and the detector (flame ionization) temperature was 250°C. Splitless injection was used.

The whole oil and fractions obtained from column chromatography were analysed. The percentage of each component was determined by the peak area measured with a Spectra Physics 4290 electronic integrator.

Gas chromatography–mass spectrometry (GC–MS)

GC–MS analysis was carried out with a Hewlett-Packard 5995 B gas chromatograph–mass spectrometer with a membrane separator coupled to a Hewlett-Packard 9825 B data system. The chromatographic separations were done by a 12 m x 0.25 mm I.D. OV-1 (methylsilicone) high-performance capillary column. The same working conditions as used for GC were employed except that the carrier gas was helium at a flow-rate of 2 ml/min (splitless technique). Mass spectra were taken over the range m/z 28–400 with an ionizing voltage of 70 eV.

Identification of components

The individual compounds were identified by comparing their mass spectra with those of authentic samples or with data already available in the literature [5–7]. A number of components were also identified by GC with co-injection of authentic samples with the essential oil.

^{1}H NMR analysis

The 60 MHz ^{1}H NMR spectrum was recorded on a Hitachi Perkin-Elmer Model R-24 B instrument in $CDCl_3$ with tetramethylsilane as internal standard.

IR analysis

Spectra were recorded in carbon tetrachloride solution with a Perkin-Elmer Model 843 infrared spectrophotometer.

RESULTS AND DISCUSSION

The qualitative and quantitative composition of the essential oil from *Thymus borgiae* Rivas-Martinez, Molina & Navarro was determined by CC, GC and GC–MS (see Table I). The gas chromatogram shows the presence of 46 components, one of which represents about 60% of the total oil. GC and GC–MS analysis of the two fractions eluted from CC with hexane yielded the hydrocarbon fraction in which fourteen monoterpenes and twelve sesquiterpenes, which constitute 18.8% of the essential oil, were identified. Longifolene (0.5%), a sesquiterpene hydrocarbon, identified by comparison of its retention time and mass spectral data with those of pure standard, is reported here for the first time as a component of the essential oils from thyme.

Fractions 3–10 eluted with hexane–dichloromethane mixtures yielded the ox-

TABLE I

COMPONENTS IDENTIFIED IN THE ESSENTIAL OIL OF *THYMUS BORGIAE*

Peak	Compound	Retention time on SE-52 (min)	Peak area (%)[a]	Identified by
1	α-Thujene	3.56	0.2	GC; GC–MS
2	α-Pinene	3.61	0.2	GC; GC–MS
3	Camphene	3.73	1.6	GC; GC–MS
4	Δ^3-Carene	3.97	0.1	GC–MS
5	Sabinene	4.09	0.1	GC–MS
6	β-Pinene	4.41	0.2	GC; GC–MS
7	Myrcene	4.69	1.1	GC; GC–MS
8	α-Phellandrene	4.79	0.4	GC–MS
9	α-Terpinene	4.90	0.6	GC; GC–MS
10	*p*-Cymene	5.42	0.1	GC; GC–MS
11	Limonene	5.53	0.4	GC; GC–MS
12	1,8-Cineol	5.94	1.7	GC; GC–MS
13	*cis*-β-Ocimene	6.40	0.1	GC–MS
14	γ-Terpinene	7.20	0.2	GC; GC–MS
15	Terpinolene	7.87	1.2	GC; GC–MS
16	Linalol	9.32	1.0	GC; GC–MS
17	Camphor	10.32	3.9	GC; GC–MS
18	Pulegone	10.56	t	GC–MS
19	Borneol	10.76	1.1	GC; GC–MS
20	Terpinen-4-ol	11.33	0.1	GC; GC–MS
21	α-Terpineol	11.79	0.1	GC; GC–MS
22	Bornyl acetate	12.22	0.2	GC; GC–MS
23	Thymol	15.26	0.5	GC; GC–MS
24	Carvacrol	15.93	59.7	GC; GC–MS IR; ^{1}H NMR
25	α-Copaene	17.63	0.3	GC; GC–MS
26	β-Bourbonene	17.97	t	GC–MS
27	Longifolene	18.18	0.5	GC; GC–MS
28	Caryophyllene	18.68	3.9	GC; GC–MS
29	Unidentified	19.23	0.3	—
30	*allo*-Aromadendrene	19.60	0.1	GC; GC–MS
31	α-Humulene	19.79	0.2	GC; GC–MS
32	α-Cubebene	20.37	0.2	GC–MS
33	Sesquiterpene alcohol	20.45	2.7	GC–MS
34	Unidentified	20.58	0.1	—
35	Germacrene B	20.86	2.6	GC; GC–MS
36	Germacrene D	20.91	3.8	GC; GC–MS
37	Calamenene	21.13	0.1	GC; GC–MS
38	Unidentified	21.63	0.3	—
39	δ-Cadinene	21.75	0.5	GC; GC–MS
40	Calacorene	22.07	t	GC; GC–MS
41	Caryophyllene epoxide	23.03	2.0	GC–MS
42	Aromadendrene epoxide	23.10	1.2	GC–MS
43	Sesquiterpene alcohol	23.21	0.3	GC–MS
44	Sesquiterpene alcohol	23.45	0.9	GC–MS
45	Eudesmol	23.61	0.2	GC–MS
46	Cadinol	24.98	0.4	GC; GC–MS

[a] t = Traces (<0.1%).

ygenated fraction, with carvacrol as the main constituent (59.7%). The isolation of this compound was possible by preparative thin-layer chromatography on silica gel from fraction 8 [solvent, toluene–ethyl acetate (93:7); $R_F = 0.55$ with a red reaction to vanillin–sulphuric acid reagent]. Its IR, ^{1}H NMR and mass spectral data were similar to those obtained for an authentic sample.

In conclusion, the essential oil of *Thymus borgiae* can be considered, for other species of this genus, to be a typical phenolic essential oil. Another phenolic compound (thymol, 0.5%) and its phenolic precursors *p*-cymene (0.1%) and γ-terpinene (0.2%) are also present in this essential oil.

Although traditionally the *Thymus* essences belonging to the *Hyphodromi* section are not considered as phenolic essential oils, when these species grow at altitudes of 1600–1700 m, such as occurs with *Th. borgiae*, a strong increase in carvacrol is observed. Thus, the essential oils from *Th. bracteatus* and *Th. godayanus*, two species with great botanical affinities with one another and with *Th. borgiae*, have percentages of carvacrol ranging from 16.9% [8] to 17.3% [3], respectively. However, when these plants are collected at lower altitudes, this oxygenated monoterpene only appears in trace amounts in *Th. bracteatus* and 4.4% in *Th. godayanus*. This can explain the high percentage of carvacrol found in the essential oil of *Th. borgiae* and its inclusion in the *Hyphodromi* section. Studies on the flavonic content of this taxon are in progress to establish a definitive taxonomic confirmation.

REFERENCES

1 C. O. Van den Broucke, *Fitoterapia*, 54 (1983) 171.
2 M. A. Blázquez, M. C. Zafra-Polo and A. Villar, *Planta Med.*, 55 (1989) 198.
3 M. A. Blázquez and M. C. Zafra-Polo, *Pharmazie*, 44 (1989) 651.
4 S. Rivas-Martinez, A. Molina and G. Navarro, *Opusc. Bot. Pharm. Complutensis*, 4 (1988) 107.
5 A. Cornu and R. Massot, *Compilation of Mass Spectral Data*, Heyden, London, 1975.
6 H. C. Noever de Brauw, J. Bouwman, A. C. Tas and G. La Vos, *Compilation of Mass Spectra of Volatile Compounds in Food*, TNO Zeist, Utrecht, 1979.
7 E. Stenhagen, S. Abrahamsson and F. W. McLafferty, *Registry of Mass Spectral Data*, Wiley, New York, 1974.
8 R. Morales, *Taxonomía de los Géneros Thymus (Excluida la Sección Serpyllum) y Thymbra en la Península Ibérica, Ruizia*, Vol. III, Serv. Public. del C.S.I.C., Madrid, 1986.