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THE ESSENTIAL OILS OF TWO CHEMOTYPES OF *PINUS SYLVESTRIS*

JEAN-CLAUDE CHALCHAT, RAYMOND-PHILIPPE GARRY, ANDRÉ MICHET and ALAIN REMERY

Laboratoire de Chimie Organique 2, U.E.R. de Recherche Scientifique et Technique, Université de Clermont II, B.P. 45,
63170 Aubiere, France

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Key Word Index—*Pinus sylvestris*; Pinaceae; essential oils; terpenes; cadinols; chemotypes.

Abstract—The essential oils of *Pinus sylvestris* from French Massif Central are described. Forty-six compounds have been identified by spectral methods. Two chemotypes are recognized.

INTRODUCTION

Pinus sylvestris L. is the most common pine in Europe and in Northern and Eastern Asia. In France, it is indigenous to the Massif Central, where it is often referred to as the Auvergne Pine. While the chemical composition of the monoterpene fraction of the essential oil of *Pinus sylvestris* has been well studied [1–5], the sesquiterpenoids have received little attention [6–9]. We have now examined twigs from two specimens, A from Puy-de-Dôme and B from Haute-Loire (two adjacent departments in Central France), subjected them to steam distillation and the volatile oils analysed. They represent two different types of native forest.

RESULTS AND DISCUSSION

Both oils, A and B, were analysed by GC on a capillary column and by mass spectrometry coupled to that column. Their per cent composition will be found in Table 1. The monoterpene fraction is predominant (A = 88.4%; B = 80.0%), which tallies with the literature [1]. The sesquiterpenes are a mixture of cadinenes and muurolenes. Oxygenated sesquiterpenes have not yet been identified in the essential oil of *Pinus sylvestris* leaves until now. We observed that an increase in the period of steam distillation results in an increase in the relative percentage of sesquiterpene alcohols at the expense of the sesquiterpenes, due to hydrolysis [10].

These two oils differ significantly in their Δ^3-carene content (A = 43.4%; B = traces) which is a chemotaxonomic character; the synthesis of this compound is presumably under genetic control [19, 20]. Oil A contains a particularly high proportion of this compound, which is coupled with a comparatively high percentage of terpinolene and correlatively smaller amounts of α- and β-pinenes, and vice versa for B (see Table 1). This occurs all the year round. Finally, we observed the presence, in small amounts, of myrcene and *trans*-β-ocimene in both oils.

EXPERIMENTAL

Plant material. The twigs of *Pinus sylvestris* were collected and kept at −20° before treatment. Twigs from each species (20–25 kg) were steam distilled for 6 hr. Essential oil (60 g) was extracted by decantation (yield = 0.3% in weight).

Separation. The essential oil of *Pinus sylvestris* (100 g) was prefractionated on a column of 200 g sodium silicoaluminate ($4\,Al_2O_3$, $2\,SiO_2$, Na_2O): hexane elution gave hydrocarbons (85 g), MeOH elution gave the oxygenated compounds (15 g). Each fraction was distilled on a spinning band column: monoterpenes and sesquiterpenes were isolated. The constituents present were separated: sesquiterpene hydrocarbons on an $AgNO_3$–silica gel column (1:9) and oxygenated products on silica gel. Monoterpenes were separated by prep. GC: Varian A 920, stainless steel column SF 96 (30%), (3 m × 8 mm), oven temp. 150–220°, H_2 200 ml/min.

Analysis. Isolated pure compounds were identified by retention time (R_t), ^1H and ^{13}C NMR, IR and MS. Other compounds were characterized from GC or CC fractions by GC/MS and GC retention. Analytical GC: FID, N_2 25 ml/min, temp. programme 50–220° at 2°/min, WCOT glass column (25 m × 0.3 mm) coated with CP Wax 51; GC/MS: 70 eV, WCOT glass column (50 m × 0.3 mm) coated with CP Wax 51, temp. programme 50–230° at 3°/min with He as a carrier gas. IR spectra were recorded in $CDCl_3$ soln. ^1H NMR spectra were measured in $CDCl_3$ at 60 or 220 MHz and ^{13}C NMR spectra were determined in $CDCl_3$ at 60 MHz, with TMS int. standard.

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REFERENCES

1. Ikeda, R. M., Stanley, W. L., Vannier, S. H. and Spitler, E. M. (1962) *J. Food Sci.* **27**, 455.
2. Jankov, L. K., Tzutzulova, A. M., Stojanova, B. and Nikolov, C. (1969) *Riv. Ital. Essence Profumi* **51**, 447.
3. Zafra, M. and Garcia-Peregrin, E. (1976) *J. Agric. Sci.* **86**, 1.
4. Maksimov, V. M., Deryuzhkim, R. I., Efimov, Y. P. and Kolesnikova, R. D. (1981) *Izv. Vyssh. Uchebn. Zaved., Lesn. Zh.* **5**, 91.
5. Hiltunen, R. (1976) *Ann. Acad. Sci. Fenn.* Ser. A **208**, 1.
6. Westfelt, L. (1967) *Acta Chem. Scand.* **21**, 159.
7. Juvonen, S. (1966) *Diss. Acta Bot. Fenn.* **71**, 1.
8. Norin, T. and Winell, B. (1972) *Acta Chem. Scand.* **26**, 2297.
9. Kolbe, M. and Westfelt, L. (1967) *Acta Chem. Scand.* **21**, 585.
10. Pauly, G., Gleizes, M. and Bernard-Dagan, C. (1973)

Table 1. Chemical composition of the essential oils of two chemotypes of *Pinus sylvestris* determined by GC (CP wax)

GC peak	Compounds	% in oil		Evidence	References
		A	B		
1	Santene	—	traces	MS, R_t	[11, 12]
2	Tricyclene	0.8	0.7	MS, R_t	[11, 12]
3	α-Pinene	22.2	41.1	MS, NMR	[11, 12]
4	Camphene	3.2	3.3	MS, NMR	[11, 12]
5	β-Pinene	3.8	18.4	MS, NMR	[11, 12]
6	Sabinene	0.5	0.3	MS, R_t	[11, 12]
7	Δ^3-Carene	43.4	traces	MS, NMR	[11, 12]
8	Myrcene	3.1	3.6	MS, NMR	[11, 12]
9	α-Terpinene	traces	traces	MS, R_t	[11, 12]
10	Limonene	0.9	2.6	MS, NMR	[11, 12]
11	β-Phellandrene	0.7	0.9	MS, R_t	[11, 12]
12	γ-Terpinene	1.1	0.1	MS, R_t	[11, 12]
13	*trans*-β-Ocimene	1.1	1.3	MS, R_t	[11, 12]
14	*para*-Cymene	0.3	0.2	MS, R_t	[11, 12]
15	Terpinolene	4.1	0.8	MS, R_t	[11, 12]
16	Hydrocarbon $C_{10}H_{14}$	traces	—	MS, R_t	
17	δ-Elemene	0.4	0.2	MS, R_t	[13]
18	α-Ylangene	0.1	0.1	MS, NMR	[14]
19	α-Copaene	0.1	0.3	MS, NMR	[14]
20	Longifolene	traces	—	MS	[13]
21	Bornyl acetate	0.3	0.1	MS, NMR	[12]
22	β-Caryophyllene	3.8	3.1	MS, NMR	[16]
23	4-Terpineol	1.1	0.8	MS, R_t	[15]
24	β-Guaiene	1.0	0.6	MS	[13]
25	β-Farnesene	0.1	0.4	R_t	[16]
26	γ-Muurolene	0.9	0.8	MS, NMR	[16]
27	α-Humulene	0.6	1.1	MS	[16]
28	Borneol	1.8	5.1	MS, R_t	[15]
29	α-Terpineol		0.7	MS, R_t	[15]
30	γ-Patchoulene	0.2	0.9	MS	[13]
31	γ-Cadinene	0.5	0.8	MS, R_t	[16]
32	α-Muurolene	0.3	0.1	MS, R_t	[16]
33	γ2-Cadinene	0.6	2.1	MS, NMR	[16]
34	δ-Cadinene	1.2	4.0	MS, NMR	[16]
35	4,10-Dimethyl-7-isopropyl[4.4.0]-bicyclo-1,4-decadiene	0.1	0.2	MS	[13]
36	α-Cadinene	0.1	0.3	MS, R_t, NMR	[17]
37	Calamenene	0.2	0.4	MS	[13]
38	Sesquiterpene alcohol $C_{15}H_{26}O$	0.1	0.1	MS	
39	Sesquiterpene alcohol $C_{15}H_{26}O$	—	0.1	MS	
40	Sesquiterpene alcohol $C_{15}H_{26}O$	traces	0.2	MS	
41	Sesquiterpene alcohol $C_{15}H_{26}O$	0.1	0.4	MS	
42	T-Cadinol	0.3	0.9	MS, NMR	[18]
43	δ-Cadinol			MS, NMR	[18]
44	T-Muurolol	0.3	0.9	MS, NMR	[18]
45	Sesquiterpene alcohol $C_{15}H_{26}O$	0.1	0.2	MS	
46	α-Cadinol	0.4	1.6	MS, NMR	[18]

Phytochemistry **12**, 1395.

11. Ryhage, R. and Von Sydow, E. (1963) *Acta Chem. Scand.* **17**, 2025.
12. Masada, Y. (1976) *Analysis of Essential Oils by GC/MS*, p. 109. Wiley, New York.
13. *Eight Peak Index of Mass Spectra* (1983). The Mass Spectrometry Data Centre, Nottingham.
14. Hunter, G. L. K. and Brogden, W. B., Jr. (1964) *J. Org. Chem.* **29**, 982.
15. Von Sydow, E. (1963) *Acta Chem. Scand.* **17**, 2504.
16. Hiltunen, R., Raisanen, S. and Von Schantz, M. (1980) *Planta Med. Suppl.* 112.
17. Vig., O. P., Bari, S. S., Sharma, M. L. and Dua, D. M. (1982) *Indian J. Chem.* **21B**, 145.
18. Borg-Karlson, A. K., Norin, T. and Talvitie, A. (1981) *Tetrahedron* **37**, 425.
19. Gleizes, M., Marpeau, A., Pauly, G. and Bernard-Dagan, C. (1982) *Phytochemistry* **21**, 2641.
20. Baradat, Ph., Bernard-Dagan, C., Fillon, C., Marpeau, A. and Pauly, G. (1972) *Ann. Sci. For.* **29**, 307.