

CHEMICAL COMPOSITION OF VOLATILES FROM CORTICAL OLEORESIN OF *PSEUDOTSUGA MENZIESII*

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Abstract—*Pseudotsuga menziesii* cortical oleoresin was found to contain 1.7% of oxygenated terpenoids and compounds of similar volatility composed of linalool, methylsalicylate, bornyl acetate, citronellol, geranyl acetate, methylthymol, citronellyl acetate, terpinen-4-ol, borneol, isopulegol, anethole, terpinen-4-ol acetate, camphor, geraniol, neryl acetate, and nerol. Sesquiterpenoid hydrocarbons were low (only 0.07%) and contained sibirane and longifolene as main constituents, with β -caryophyllene, γ -muurolene, γ -cadinene (identified by IR), and 20 additional compounds in small amounts. *p*-Cymen-8-ene was identified in monoterpene hydrocarbon fraction.

INTRODUCTION

IN AN earlier publication we reported on the geographic variability of cortical monoterpene hydrocarbons from *Pseudotsuga menziesii* (Mirb.) Franco (Douglas-fir), and concluded on the existence of four chemical races of this species, intergrading in several geographic areas.¹ Of these four races, three had their morphological corollaries in the var *menziesii* (Pacific coast) and var *glauca* (Beissn.) Franco (Rocky mountains), the latter treated as var *caesia* Aschers. and Graebn. (northern Rockies) and var *glauca* Schneider (southern Rockies) by botanists in continental Europe. The fourth race, growing in California's Sierra Nevada mountains, had no morphological equivalent but appeared to be closer to the Rocky Mountain populations on the basis of its monoterpene hydrocarbon composition.

While in this previous work, which involved the variability within, and intergradation between morphologically defined taxa, our reliance on monoterpene hydrocarbons (a class including only a dozen or so compounds) appeared satisfactory, the problem of relation of the Sierra Nevada populations to the other three Douglas-fir races suggested the desirability to maximize the number of chemical parameters. The present paper, which represents an effort in this direction, deals with the isolation and identification of higher-boiling constituents of Douglas-fir cortical turpentine.

The oxygenated monoterpene constituents of Douglas-fir needles are well discussed in the literature,²⁻⁵ but practically nothing on the cortical resin is available. The Douglas-fir sesquiterpenoids from either needles, cortex, or wood have hardly been investigated, and

¹ ZAVARIN, E. and SNAJBERK, K. (1973) *Pure Appl. Chem.* **34**, 411.

² GUENTHER, E. (1952) *The Essential Oils*, Vol. VI, pp. 210, Van Nostrand, New York.

³ SAKAIRI, T., MAARSE, H., KEPNER, R. E., JENNINGS, W. G. and LONGHURST, W. M. (1967) *Agric. Food Chem.* **15**, 1070.

⁴ MAARSE, H. and KEPNER, R. E. (1970) *Agric. Food Chem.* **18**, 1095.

⁵ VON RUDLOFF, E. (1972) *Can. J. Botany* **50**, 1025.

with the exception of isolation of germacrene D and gas chromatographs of wood and leaf oil sesquiterpenoids from *P. japonica* (Shirasawa) Beissner,^{6,7} no information is available on the essential oils from other *Pseudotsuga* species.

RESULTS AND DISCUSSION

The oleoresin sample used in the present study was obtained from trees growing near Fort Bragg, California. The monoterpene hydrocarbons analyzed by GLC to 36.5% α -pinene, 0.8% camphene, 26.8% β -pinene, 11.3% 3-carene, 7.9% sabinene, 3.0% myrcene, 4.4% limonene, 3.0% β -phellandrene, and 6.3% terpinolene and represented 30.1% of the oleoresin. This is in good accord with what would have been expected for a coastal California Douglas-fir.¹ Trace constituents included *p*-cymene and *p*-cymen-8-ene (*p*- α -dimethylstyrene). The latter is apparently new to Pinaceae. It was present to the amount of 0.024% in oleoresin and was identified by synthesis through dehydration of *p*-cymen-8-ol, using oxalic acid. Both *p*-cymen-8-ene and *p*-cymen-8-ol were reported to occur in foliage of many plant species, including *Chamaecyparis*.^{8,9} They also represent the main autoxidation products of terpinolene,¹⁰ which may bear relation to their biogenesis in view of the presence of terpinolene in sizeable amounts in the Douglas-fir cortical turpentine.

TABLE I. OXYGENATED MONOTERPENOIDS FROM *Pseudotsuga menziesii* CORTICAL OLEORESIN

Class	Compound	%
Acyclics, 47.9%	Citronellol	10.5
	Linalool	27.6
	Geraniol	1.0
	Nerol	tr*
	Citronellyl acetate	3.3
	Geranyl acetate	5.0*
	Neryl acetate	0.5*
Monocyclics, 10.9%	Terpinen-4-ol	2.7
	Terpinen-4-ol acetate	2.0*
	Methylthymol	4.0
	Isopulegol	2.2
Bicyclics, 14.9%	Borneol	2.2
	Bornyl acetate	11.0
	Camphor	1.7
Nonterpenoids, 26.7%	Methylsalicylate	24.7
	Anethole	2.0
Total in oleoresin, 1.7%		

* Identified by retention volumes only, using two columns, but identifications deemed reliable in view of co-occurrence with other compounds of related structure in the same source.

Oxygenated monoterpenoids and related compounds of similar volatility amounted to 1.7% of oleoresin (Table I), and about half of them were ubiquitous acyclic alcohols and their acetates. A high proportion of acyclics was reported by Sakai *et al.* and Maaise

⁶ YOSHIKAWA, K., OHYA, Y., SAKAI, T. and HIROSE, Y. (1969) *Tetrahedron Letters* 2263.

⁷ HIROSE, Y. (1969) *Paper No. 28 Div. of Agr. and Food Chem. 58th Nat. ACS Meeting*, New York.

⁸ THOMAS, A. F. (1965) *Perf. Essential Oil Rec.* **56**, 301.

⁹ CHENG, Y. S. and VON RUDLOFF, E. (1970) *Phytochemistry* **9**, 2517.

¹⁰ KILIN, F., FARROW, H. and ROJAHN, W. (1965) *Diagnotico Rept.* **12**, 3.

et al^{3,4} for oxygenated monoterpenoids of mature needles of Douglas-fir, although the percentage composition of these acyclics appeared to be different. Among bicyclics the presence of camphor, borneol, terpinene-4-ol, and their acetates, was expected because of their probable biogenetic derivation from, respectively, bornane-2- and 1-*p*-menthene-4-carbonium ions, the ions giving rise to camphene and sabinene which are both present in the same oleoresin. Methylthymol has been previously identified in *Abies cortex*¹¹ and *Pinus* foliage.¹² Isopulegol is not uncommon in plant kingdom, although apparently it has not yet been isolated from *Pinaceae*. It commonly co-occurs with citronellal¹³ and easily forms from it under the influence of many chemical reagents, e.g. silica gel at $T \geq 100^\circ$,¹⁴ it has also been reported as one of the products of the action of *Pseudomonas aeruginosa* on citronellal.¹⁵ The presence of isopulegol in *P. menziesii* probably relates to the higher percentages of citronellol and its acetate in cortical turpentine.

¹³ GUENTHER, E. (1952) *The Essential Oils*, Vol. II, pp. 209. Van Nostrand, New York.

TABLE 2. SESQUITERPENE HYDROCARBONS FROM *Pseudotsuga menziesii* CORTICAL OLEORESIN

Class		Sesquiterpene†	%
Acyclics, 0.2%		β -Farnesene	0.2*
Cyclization 1/10, 58.8%	Bicyclic, 48.1%	α -Muurokene	5.8†
		γ -Muurokene	4.6*
		β_1 -Cadinene	1.8†
		γ -Cadinene	7.0*
		δ -Cadinene	0.7†
		ϵ -Cadinene	1.2†
		Calamenene	1.1
		Sibirene	22.5*
		Selina-3,7(11)-diene	0.9
		α -Guaiene	1.3
		δ -Guaiene	1.2
	Polycyclic, 10.7%	Sativene	2.2†
		Cyclosativene	4.5†
		α -Copaene	2.6
		β -Copaene	1.3
		α -Cubebene	0.1
Cyclization 1/11, 38.7%	Monocyclic, 11.2%	α -Humulene	3.1
		β -Humulene	8.1
	Bicyclic, 8.0%	β -Caryophyllene	7.1*
		α -Himachalene	0.8
		β -Himachalene	0.1
	Polycyclic, 19.5%	Longifolene	18.0*
		α -Longipinene	0.8†
		Longicyclene	0.7†
Unknowns, 2.3%		CBX-166	2.3
Total in oleoresin, 0.07%			

* Identified by IR

† Positions of α -himachalene/ β -ylangene, β -himachalene/ α -bisabolene, ϵ -cadinene/ β -bisabolene, and β_1 -cadinene/*cis*- α -bisabolene overlapped on Carbowax 20 M and Silicone OV 17 columns used. Structure assignment was made here on the basis of co-occurrence and ubiquitousness.

‡ Identified by GLC retention times only, but identification judged acceptable in view of co-occurrence with compounds identified by IR, ubiquitousness, and characteristic position on GLC.

¹¹ OGNYANOV, I. and TSANKOVA, E. (1966) *Richt. Aromen, Koerpfl.* **16**, 39.

¹² LEE, CH. J., SNAJBERK, K. and ZAVARIN, E. (1974) *Phytochemistry* **13**, 179.

¹³ GUENTHER, E. (1952) *The Essential Oils*, Vol. II, pp. 209. Van Nostrand, New York.

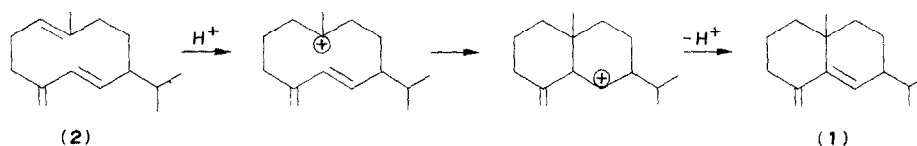
¹⁴ CHANG, T. C., WASHIO, S. and UEDA, H. (1970) *Agric. Biol. Chem.* **34**, 1734.

¹⁵ HAYASHI, T. and TATSUMI, CH. (1970) *Nippon Nogetakagaku Kaishi* **44**, 556.

Non-terpenoids were relatively high and included methyl salicylate and anethole. Methyl salicylate has not been reported heretofore from Pinaceae, although it is not uncommon otherwise. Sakai *et al.*³ Maaise *et al.*⁴ and von Rudloff⁵ do not mention it as a foliage oil constituent. However, in an earlier work Johnson and Cain¹⁶ reported the presence of 0.07% of salicylic acid (probably a product of hydrolysis) in the sodium hydroxide extract of leaves-and-twigs steam distillate from the same species. Anethole has been previously reported in many leaf oils, particularly of Umbelliferae.¹⁷ Recently it has been isolated from commercial Finnish "pine oil," the product of steam distillation of *Pinus sylvestris* L. and *Picea abies* (L.) H. Karst.¹⁸ foliage.

The per cent of sesquiterpene hydrocarbons was disappointingly low (0.07%) Most of these (Table 2) represented sibirene (1) and the ubiquitous longifolene, with some cadinenes, muurolenes and β -caryophyllene. The latter has been previously reported by Sakai *et al.* in the needles of Douglas-fir.³

Sibirene (selina-4(14)-5-diene) was originally isolated from the wood of *Pinus sibirica* Ledeb.,^{19,20} we recently identified it by IR in *Abies balsamea* (L.) Mill. cortical oleoresin.¹² The same compound has also been identified by GLC in *Abies sibirica* Ledeb. cortex, in *Larix sibirica* Ledeb. in *Pinus sylvestris* L. and in *Picea obovata* Ledeb. wood.²¹ It appears to occur in increased amounts in oleoresins containing higher percentages of cadinenes/muurolenes.^{12,21} This might be indicative of its biosynthesis from germacrene D (2) by the pathway below. Germacrene D, isolated recently from *P. japonica*, has been postulated as precursor of γ -isomers of cadalene-type⁷ sesquiterpenoids.



EXPERIMENTAL

Cortical oleoresin was collected from the young Douglas-fir trees in the Fort Bragg, California area. Methodology of the oleoresin analysis followed the procedure described in our earlier paper on *Abies balsamea* cortical oleoresin.¹² Artifact formation during analysis was checked by GLC using untreated oleoresin.

IR spectrum of sibirene reported between 600 and 3500 cm^{-1} in order of decreasing peak intensity and with peaks in parentheses ranked together according to the strongest peak: 2930, 2960, 2870, 2840, 890, (1450, 1460, 1435), 1375, 1640, 840, 1360, 3070, 1660, 1025, 1168, 860, 1154, 1350, 1042, 1296, 940, 1255, 1330, 1220, 1285, 1100, 1120, 1200, 1320, 1186, 980, 970, 1080, 928, 817, 667, 1780, 800, 620, 730, 770. R_f : Carbowax 20 M 0.90 (to longifolene), silicone OV 17 2.57 (to menthol).

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¹⁶ JOHNSON, C. H. and CAIN, R. A. (1937) *J. Am. Pharm. Assoc.* **26**, 406.

¹⁷ GUENTHER, E. (1952) *The Essential Oils*, Vol. II, pp. 508, Van Nostrand, New York.

¹⁸ JUUVONEN, S. and HUOVINEN, K. (1972) *Farm. Aikak.* **81**, 80.

¹⁹ PENTKOVA, V. A., MOTIL, O. and HEROUT, V. (1961) *Coll. Czech. Chem. Commun.* **26**, 1362.

²⁰ PENTKOVA, V. A., KASHITANOVA, N. K., REZVUKHIN, A. I. and KOLIPOVA, E. I. (1966) *Khim. Priro. Soed.* 239.

²¹ PENTKOVA, V. A., DUBOVINKO, ZH. V., VOL'SKII, L. N., VASILUK, S. M., CHIRKOVA, M. A. and SHMIDT, E. N. (1968) *Izv. Sibir. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk*, pp. 114.