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COMPOSITION OF TURPENTINE FROM *PINUS EDULIS* WOOD OLEORESIN

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Key Word Index—*Pinus edulis*; Pinaceae oleoresin; turpentine; terpenoids; sesquiterpenoids.

Abstract—Monoterpenoids and sesquiterpenoid hydrocarbons of *Pinus edulis* wood oleoresin were analyzed by chromatographic and spectroscopic methods. Monoterpenoid hydrocarbons (20·3%) were composed mainly of α-pinene, with camphene, β-pinene, 3-carene, sabinene, myrcene, limonene, β-phellandrene, *trans*-ocimene and terpinolene in secondary to trace amounts. Oxygenated terpenoids (0·28%) contained bornyl acetate and verbenone as major constituents, and linalool, camphor, terpinen-4-ol, citronellyl acetate, borneol, neral, α-terpineol, citronellol, nerol, and geraniol in smaller amounts. Oleoresin contained 1·1% of acetogenins, composed mainly of ethyl caprylate. Sesquiterpenoid hydrocarbons were high (5·7% in oleoresin) and were composed of germacrene D as a major constituent (36·6%), of γ-amorphene, α-copaene, and longifolene as secondary constituents (5–20%), and β-farnesene, α- and γ-murolenes, β_1-, γ-, δ-, and ε-cadinenes, α-amorphene, δ-guaiene, sibirene, α-cubebene, β-copaene, β-ylangene, sativene, cyclosativene, β-bourbonene, α- and γ-humulenes, caryophyllene, α-longipinene and longicyclene in smaller amounts. Composition of *P. edulis* and of *P. monophylla* turpentines was found to be similar, with percentage of ethyl caprylate being the best distinguishing criterion.

INTRODUCTION

Pinus edulis Engelm. belongs to the subsection Cembroides Engelm. (Sect. Parrya Mayr., Subgenus Strobus Lemm.), or pinyon pines [1]. It is a small, lower-elevation tree, growing mainly in Utah, Colorado, Arizona, and New Mexico, with few populations in adjoining areas of California, Texas, Oklahoma, and Wyoming. It is closely related to neighboring *P. monophylla* Torr. and Frem. in the west and *P. cembroides* Zucc. in the south. In the intermediate areas are pinyon pines of indefinite status which are treated either as introgressants or distinct varieties [1–6]. This report represents the continuation of efforts to find chemical differences between the turpentines of these pines, which could be used for chemosystematic treatment of intermediacy problems.

The composition of monoterpene hydrocarbons from wood oleoresin of *P. edulis* has been previously investigated by Schorger [7], Mirov and Iloff [8] and Zavarin *et al.* [9] and was found to be very similar to that of *P. monophylla* [9]. In the higher boiling fractions Mirov and Iloff [8] were able to identify ethyl caprylate, a compound responsible for the characteristic fragrance of *P. edulis* oleoresin. Ethyl caprylate occurred in roughly 20-fold smaller quantities in the oleoresin of *P. monophylla* [9] and was at the time the only promising chemical distinguishing character for the two species. Aside of ethyl caprylate, little was reported on the identity and amounts of other high-boiling constituents of this turpentine—a topic forming the subject of the present report.

RESULTS AND DISCUSSION

P. edulis oleoresin was collected near Santa Fe, New Mexico, and in the Indian Canyon, southwest of Duchesne, Utah. According to Lanner, no intergradation with *P. monophylla* is indicated in these localities [6]. After removal of acidic constituents by extraction with base, the volatile portion of the oleoresin was separated by distilla-

Table 1. Composition of monoterpenoid hydrocarbons from *Pinus edulis* and *Pinus monophylla**

Terpene	*P. edulis*	*P. monophylla*	R_t
α-Pinene	74·6	74·5	1·00
Camphene	0·4	1·0	1·40
β-Pinene	0·4	0·5	1·76
3-Carene	14·5	6·0	2·14
Sabinene	3·5	3·0	2·27
Myrcene	5·4	3·0	2·94
Limonene	tr	8·0	3·12
β-Phellandrene			3·61
Terpinolene	1·0	1·5	5·00
trans-Ocimene	0·2	2·5	5·17
Total monoterpenes in oleoresin	20·3	23·0	

* Percentages of individual compounds are based on total monoterpene weight. Sp. rotations $[\alpha_D^{25}]$ in CCl_4 solutions: α-pinene, +43·8°; 3-carene, +16·4°; limonene, −95·0°. R_t on β,β-oxydipropionitrile column with R_t (α-pinene) = 1·0.

tion at reduced pressure, and mono- and sesquiterpenoids as well as non-terpenoids of comparable volatility were analyzed by a combination of argentative liquid–solid chromatography and GLC. Separated and purified compounds were identified by IR spectroscopy and also, in a few instances, by NMR and MS. Quantitative composition of *P. edulis* turpentine was estimated by analytical GLC on the basis of peak integrals from chromatograms obtained by direct injection of fresh oleoresin; this checks on the possibility of some isolated compounds being artifacts (e.g. products of germacrene D interaction with silica gel [10]). The composition of the previously investigated turpentine of *P. monophylla* was also redetermined.

The composition of mono-terpenoid hydrocarbons from *P. edulis* shown in Table 1 agrees with data for material obtained near Fort Defiance, north-eastern Arizona [8, 9]. Apparently, only 3-carene and limonene occur in sufficiently different percentages in *P. monophylla* and *P. edulis* for chemical species differentiation. Even so, differences are small and offer promise only for assigning individual populations, rather than individual trees, to one or the other species.

Volatile acetogenins (Table 2) included ethyl caprylate and one additional ester. Of the two, ethyl caprylate was present in far larger amounts and in agreement with the earlier work [8, 9] represented the most promising compound for distinguishing *P. edulis* from *P. monophylla*. The structure of the other ester could not be determined as it was available in only a minute amount. In GLC it appeared at R_t = 0·39 on a Carbowax 20 M column (ethyl caprylate R_t = 0·48, menthol R_t = 1·0), and had a MW of 170 (MS). Its IR spectrum was simple, exhibiting typical saturated ester peaks at 1738, 1240, and 1170 cm^{-1}, in addition to bands characteristic of Me and CH_2 groups, and other weaker skeletal vibration bands. GLC comparison with synthetic materials indicated that it was not identical with ethyl caproate, methyl caproate, isoamyl isovalerate, or 3-methyl-2-butenyl isovalerate. No materials formed by the shikimic acid biosynthetic path (phenylpropanes) could be identified in the *P.*

Table 2. Composition of oxygenated monoterpenoids, methyl chavicol, and volatile acetogenins from *Pinus edulis* and *Pinus monophylla* oleoresins*

Volatile constituent	*P. edulis*	*P. monophylla*	R_t
Oxygenated monoterpenoids			
Total in Oleoresin:	0·28	0·36	
Linalool	0·5	10·4†	0·76
Camphor	0·5		0·76
Bornyl acetate	29·7†	31·4†	0·95
Terpinen-4-ol	1·9	tr	0·95
Citronellyl acetate	1·0	0·2	1·25
Borneol	7·4†	8·1	1·40
Neral	0·5		1·45
α-Terpineol	2·0		1·45
Verbenone	22·2†	11·5	1·54
Citronellol	3·0	4·1	1·80
Nerol	1·0		2·14
Geraniol	0·5	5·0	3·00
Methyl chavicol			
Total in Oleoresin:		0·041	1·30
Acetogenins			
Total in Oleoresin:	1·10	0·15	
Ethyl caprylate	95·0†	89·2†	0·49

Unknowns in P. edulis. In acetogenins: R_t = 0·39 (5·0%). In monoterpenoids, R_t = 0·51 (tr); R_t = 0·59 (0·5%); R_t = 0·65 (2·0%); R_t = 0·84 (1·0%); R_t = 1·25 (9·8%); R_t = 2·14 (8·5%) R_t = 2·38 (6·0%).

Unknowns in P. monophylla. In acetogenins: R_t = 0·39 (10·8%); R_t = 0·42 (tr). In monoterpenoids: R_t = 0·51 (tr); R_t = 0·59 (5·7%); R_t = 0·65 (tr); R_t = 0·70 (6·6%); R_t = 0·84 (tr); R_t = 1·20 (4·3%); R_t = 1·94 (4·0%); R_t = 2·38 (tr); R_t = 2·51 (tr); R_t = 2·62 (8·7%).

* Individual compounds are given in per cent of total weight of oxygenated monoterpenoids or acetogenins respectively. R_t are expressed in relation to longifolene, R_t = 1·0, using Carbowax 20 M column.

† Identified by IR and GLC. The rest were identified on the basis of GLC data only using OV-17 and Carbowax 20 M columns.

Table 3. Composition of sesquiterpenoids from *Pinus edulis* and *Pinus monophylla* oleoresins*

Sesquiterpenoid type	Constituents	*P. edulis*	*P. monophylla*	R_t
Acyclics	β-Farnesene	1·6*	7·6*	1·43
Cyclization 1/10				
Monocyclics	Germacrene D	36·3*	9·7	1·74
Bicyclics	α-Muurolene	0·6*	6·7*	1·80
	γ-Muurolene	2·7*	3·2*	1·56
	β_1-Cadinene	0·3*	0·2	2·36
	γ-Cadinene	4·6*	6·8*	2·07
	δ-Cadinene	1·8*	3·4*	2·07
	ϵ-Cadinene	0·2	tr	2·28
	α-Amorphene	0·4*		1·54
	γ-Amorphene	10·0*	11·7*	1·74
	Calamenene		0·3*	2·50
	α-Guaiene		1·4*	1·06
	δ-Guaiene	tr	0·7	1·18
	Sibirene	0·5	0·4	0·90
Polycyclics	α-Cubebene	0·8*	1·4	0·62
	α-Copaene	8·2*	11·7*	0·72
	β-Copaene	2·4*		1·07
	β-Ylangene	1·1	1·8	1·30
	Sativene	0·5*	1·2*	0·85
	Cyclosativene	4·0*	5·0*	0·72
	β-Bourbonene	0·5*	0·8	0·84
Cyclization 1/11				
Monocyclics	α-Humulene	0·5*	0·8	1·49
	γ-Humulene	0·2	2·4	1·88
Bicyclics	Caryophyllene	2·5*	2·9*	1·12
Polycyclics	Longifolene	18·4*	18·4*	1·00
	α-Longipinene	1·2*	1·0*	0·66
	Longicyclene	0·7*	0·5*	0·76
Total sesquiterpenoids in oleoresin		5·7	4·6	

* Percentages of individual sesquiterpenoids are expressed on the basis of their total weight. Asterisked compounds have been identified by isolation and IR methods as well as by their retention times on OV-17 and Carbowax 20-M columns, the rest by GLC alone, R_t values for Carbowax column are tabulated with R_t (longifolene = 1·0). Specific rotations [α_D^{25}] in carbon tetrachloride solutions: longifolene, +42·5°; α-muurolene, −79·5°.

edulis oleoresin, whereas ubiquitous methyl chavicol made its appearance in *P. monophylla*, albeit in small amounts.

Oxygenated monoterpenoids (Table 2) amounted to only 0·28% of *P. edulis* oleoresin, with bornyl acetate, borneol, and verbenone representing about 60% of these materials (0·08, 0·02, and 0·06% in oleoresin, respectively). Bornyl acetate was present in about the same amount in *P. monophylla*. The chief difference between the two species was the higher verbenone and lower linalool and geraniol contents of *P. edulis* oleoresin. Unfortunately, the low oxygenated monoterpenoid content of *P. edulis* and *P. monophylla* oleoresins makes these distinctions only marginally practical for chemosystematic purposes. Verbenone was previously reported in amounts between 0·018% to traces in several *Pinus* oleoresins [11]; its occurrence in *P. edulis* can be of chemo-ecological importance, as it was several times identified as an important component of bark beetle pheromones [12, 13].

The sesquiterpenoid hydrocarbon fraction of *P. edulis* oleoresin amounted to nearly 6% of the oleoresin and was composed of at least 25 compounds discernible by GLC methods, of which 20 were isolated and spectroscopically identified (Table 3); over-all, the composition of sesquiterpenoids was close to that of *P. monophylla*. α-Amorphene [14], γ-amorphene [15], β_1–cadinene, β-bourbonene, α-humulene, and germacrene D are apparently newcomers to *Pinus* oleoresins.

Amorphenes are stereo-isomers of cadinenes and muurolenes [16], and are biosynthetically related to β-bourbonene and germacrene D [10, 16]. The high percentage of germacrene D (2·1% in oleoresin), which together with β-farnesene and α-muurolene percentages represented the only promising sesquiterpene parameters for *P. edulis–P. monophylla* differentation was particularly noteworthy.

EXPERIMENTAL

Oleoresin, 522 g, was collected using previously described methods [9]. Acidic constituents were removed by extraction with base and the volatile portion was separated by distn at red pres. Segregation of volatile portion into monoterpenoid hydrocarbons, oxygenated monoterpenoids and sesquiterpenoid hydrocarbons, and isolation of individual compounds followed the procedure described earlier except for the slightly different GLC parameters [17, 18]. Analytical GLC: Monoterpenoid hydrocarbons—10% β,β-oxydipropionitrile on Chromosorb P 100/120, column 305 × 0·32 cm (o.d.); oxygenated monoterpenoids and sesquiterpenoid hydrocarbons: (a) 1% Carbowax 20 M on Chromosorb G 100/120, column 1530 × 0·32 cm (o.d.); (b) 1% Silicone OV-17 on Chromosorb G 100/120, column 915 × 0·32 cm (o.d.). Preparative GLC: Monoterpenoid hydrocarbons: 1·5% Silicone OV-17 + 0·1% Igepal on Chromosorb G 100/120, column 1830 × 0·64 cm (o.d.); oxygenated monoterpenoids and sesquiterpenoid hydrocarbons: 1% Carbowax 20 M on Chromosorb G 100/120, column 3048 × 0·64 cm (o.d.).

Isolation of Germacrene D. Turpentine sample was dissolved in an equal vol of light petrol and shaken for 2 hr with an excess of a 50% aq. $AgNO_3$. Precipitate was filtered, washed with petrol and dried. Germacrene D was recovered in a 95% purity (GLC) by treating the Ag complex with excess dil. aq. NH_3 and extracting the sesquiterpenoid with CCl_4. An alternative method consisted of dissolving turpentine in MeOH and allowing the resulting soln to stand overnight at ambient temperature in the presence of an excess of $AgNO_3$. Since germacrene D and γ-amorphene peaks overlapped in GLC, the analysis of the turpentine before and after this treatment allowed to assess the amounts of each of these compounds by difference.

IR data. Peaks are listed in order of their decreasing intensity, as before [17]. S = shoulder.

α-Amorphene (neat—600–4000 cm^{-1}): 2820–3020, (1430, 1470), 1375, 814, 1155, 872, 1160*s*, 1068, 1200, 768, 1100, 940, 828, 3050, 1354, 1306, 1261, 1325, 2730, 1030, 1315*s*, 1040, 1340, 1020, 922, 961, 1670, 694, 658, 2675, 1125, 1288, 1280*s*, 605, 856, 2608, 780*s*, 2635, 1630, 1656*s*.

γ-Amorphene (neat—600–4000 cm^{-1}): (2920, 2870*s*, 2960*s*, 2840*s*), (1430, 1426*s*, 1460*s*, 1470*s*), 880, 1380, 1366*s*, 1638, 3080, 793, 867, 950, 826, 1170, 1066, 1271, 1150, 1145*s*, 1353, 3042, 1092, 2724, 600, 1286, 1252, 928, 1668, 1320, 988, 1302, 920*s*, 1332*s*, 1199*s*, 970*s*, 2700*s*, 1013, 1052, 1085*s*, 2640, 690, 1210, 1777, 1032, 2570*s*, 630*s*, 705.

Unknown ester R_t = 0·39: (in CCl_4—600–700; 850–4000 cm^{-1}); 1738, 2960, 2930*s*, 1170, 1240, 2875, 1205*s* (CCl_4), 1110, 1370, 1468, 1040, 1550 (CCl_4), 1388, 1020*s* (CCl_4), 1300, 630*s*, 1450*s*, 1350, 980, 1420.

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