

RESIN ACIDS OF *PINUS PONDEROSA* NEEDLES

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Abstract — Resin acids of ponderosa pine needle oleoresins are characterized by labdane diterpenes. Imbricataloic and/or acetyl- and succinyl-isocupressic acids predominate. Succinyl derivatives of neutral diterpenes were also found.

INTRODUCTION

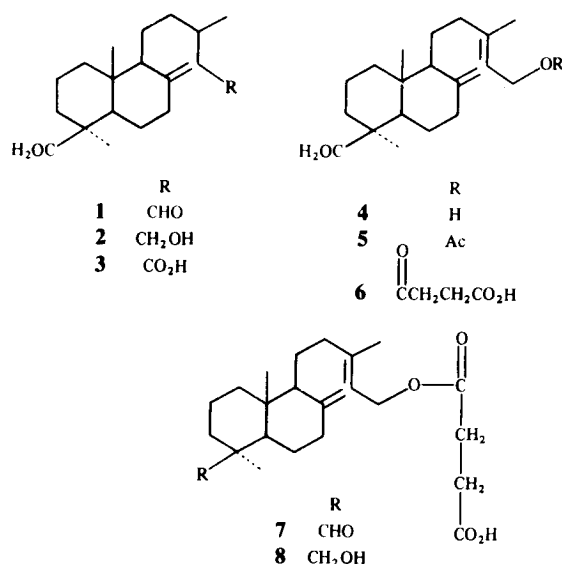
As part of a long-term study, pine foliage is being evaluated as a potential source for naval stores and fine chemicals; the diterpene acid composition is being evaluated as chemotaxonomic indicators. We now report our observations for ponderosa pine, *Pinus ponderosa* Dougl. ex Laws, an important commercial U.S. pine species in the western United States.

Earlier analysis of ponderosa pine needle resin acids from several sources has shown that the needles are characterized by a substantial proportion of imbricataloic acid (**1**) [1, 2], previously reported in the needle resin acids of *Pinus elliotii* [3]. One of these reports also noted the presence of significant amounts of other longer eluting (GC) components in the acid fraction that were loosely ascribed to phenolics [1]. We examined the weak acid, neutral, and strong acid fractions as obtained by DEAE-Sephadex separation of ponderosa pine needle oleoresin and identified several components not defined in the earlier work.

RESULTS AND DISCUSSION

Identification of ponderosa pine needle components

Resin acids. GC inspection of the neutral, weak-acid, and strong-acid fractions from DEAE-Sephadex separation of ponderosa pine needle oleoresin showed the presence of substantial and often dominating amounts of acids other than the usual abietane or pimarane type. Both acetylisocupressic [15-acetoxy-8(17), *E*-13-labdadien-19-oic acid, **5**] and isocupressic [15-hydroxy-8(17), *E*-13-labdadien-19-oic acid, **4**] acids were obtained from the weak-acid fraction and isolated as the methyl esters. Their presence is not surprising as these acids have been found in the xylem oleoresin of ponderosa pine [4]. Two other minor acids, imbricataloic acid [15-hydroxy-8(17)-labden-19-oic acid, **2**] and its dicarboxylic ana-



logue, dihydroagathic acid [8(17)-labden-15,19-dioic acid, **3**], were also found and isolated as the methyl esters. Compound **3** has been reported as a component of *P. elliotii* needle resin acids and of *P. merkusii* xylem oleoresin [5], where it is called mercusic acid (as found in *Juniperus oxycedrus*, **3** is known as junicedric acid [6]).

The primary resin acid found in the strong-acid fraction was also an isocupressic acid derivative. Selective isolation of this acid was accomplished by extraction with aqueous sodium bicarbonate. On purification, the acid was found to be succinylisocupressic acid [15-succinoxy-8(17), *E*-13-labdadien-19-oic acid, **6**], previously identified as a minor component, which constitutes less than 1% of the resin acids in the xylem [7] and needle [8] oleoresins of *P. sibirica*. Lambertianic acid is the primary resin acid in *P. sibirica* needles [9]. The 19-succinyl analogue, succinylagathic acid (interchanged *O*-succinyl and CO₂H functionalities of **6**), has been found in the xylem oleoresin of *P. pumila* [10]. Other succinic acid derivatives of diterpenes were isolated and identified. Because these diterpenes are not resin acids, they are discussed in the following section.

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Neutrals

The DEAE-Sephadex separation of *P. ponderosa* needle extracts yielded a neutrals fraction that was chromatographed on silica gel using gradient elution with ether-hexane. The first material to elute was 10-non-cosanol, an alcohol previously found in several conifers, including pines [11]. Rechromatography on silica gel of the material eluting with 20% Et₂O provided a component whose ¹H NMR spectrum showed it to be the hydroxyaldehyde, 15-hydroxy-8(17), *E*-13-labdadien-19-al. {This compound was first named contortolal by Rowe and Scroggins [12, 13] and later, isoagatholal [14]. Others have designated the same compound agatholal. Establishing priority of trivial names that could be based on agath-, isocupress-, copal-/anticopal-, or imbricat- (but not isoagath-, because it is tricyclic [15]) stems is a fruitless exercise in that rigorous nomenclature [16] is unambiguous and prevents confusion and error. }

As noted above, succinic acid derivatives of diterpenes other than resin acids (resin acids being defined as having CO₂H functionality on the parent hydrocarbon) were isolated from the strong acids fraction. These materials behave as strong acids with DEAE-Sephadex, but they were isolated from the ether phase after sodium bicarbonate extraction of the needle extract. This ether phase was essentially free of succinylisocupressic acid. Methylation (CH₃N₂) of the ether phase material and chromatography on silica gel gave the methyl ester of 15-succinoxy-8(17), *E*-13-labdadien-19-al (7) followed by the diol derivative, 15-succinoxy-8(17), *E*-13-labdadien-19-ol (8), both characterized from their ¹H NMR spectra. The corresponding, succinyl-free, labdadienal and labdadiendiol found in this study may be natural products and/or products of hydrolysis during isolation.

Resin acid composition of ponderosa pine needle oleoresin

In a limited survey of *P. ponderosa*, several samples of needles were obtained from provenance studies to pro-

vide some representation of the range of the species. The three varieties of *P. ponderosa* [17] were represented by var. *scopulorum* (702, 720, 813, 816, 820, 840, 848, 860), var. *ponderosa* (865, 866, 867), and var. *arizonica* (766), although only one example of the latter variety was obtained. The needle oleoresins were separated by our micro-DEAE-Sephadex method [18], but the succinoyl acid, 6, was eluted with 1% acetic acid in ether-methanol. To avoid isomerization during further work-up, the whole eluate was methylated immediately with diazomethane.

The original observations that ponderosa pine needle oleoresin resin acids are characterized by substantial amounts of imbricatolal acid [1, 2] do not appear to be generally true in this study, three samples from two varieties consisted of only ca 5% of this acid (Table 1). Succinylisocupressic acid (6) was the major resin acid in all three samples of var. *ponderosa* but there was no pattern in the other two varieties; succinylisocupressic acid content was in the range 0–38%. No pattern could be discerned for isocupressic acid and acetylisocupressic acid, although both tended to be low for samples with a high content of 6. Because both the acetyl and succinyl isocupressic acids are susceptible to hydrolysis during DEAE-Sephadex separation and subsequent work-up, the isocupressic acid may be a combination of naturally occurring material and a hydrolysis artifact.

Other resin acids are typical of pine in general, and the elevation in neoabietic acid content in respect to xylem oleoresins is consistent with our experience with pine needles from various species [2]. Not included in Table 1 are peaks for minor components (0–2%), including sandaracopimaric, dihydroagathic, imbricatolal, and acetyl-imbricatolal acids (identified by GC comparison with authentic material from slash pine xylem oleoresin [19]) and two unidentified components as well as the succinyl derivatives (7, 8) of neutral diterpenes. Because of the similarity in retention behaviour of methyl dihydroagathate, imbricatolal, and isocupressate on the DB-1 col-

Table 1. Needle resin acid composition of selected ponderosa pine*

Provenance and origin	% of total resin acids†									
	Iso	Lev	Pal	Ab	DeA	Neo	Ial	IC	AcIC	SIC
702 West N. Dakota	5.7	5.4	1.5	1.0	1.9	22.1	38.4	2.2	7.5	11.0
720 Nebraska	1.7	1.4	18.1	2.8	1.6	26.2	30.8	0.5	8.7	—
766 South New Mexico	4.2	3.7	11.5	1.4	1.0	11.7	44.1	6.1	13.1	—
813 Central Montana	5.2	0.3	7.4	0.8	4.6	6.3	12.6	13.4	41.6	20.6
816 Montana Transition	7.6	3.2	4.9	1.7	2.5	10.2	11.4	7.9	11.6	36.0
820 West Montana	11.8	4.1	5.8	1.3	1.7	11.8	13.9	3.4	0.4	38.4
840 South S. Dakota	4.4	2.7	4.9	0.8	1.4	16.2	30.2	3.5	25.4	—
848 East Wyoming	10.3	12.5	3.0	0.5	1.4	7.8	3.0	15.4	18.7	26.0
860 Central Colorado	9.6	2.8	18.1	0.9	1.5	22.4	21.2	9.3	4.6	—
865 Central Oregon	13.0	3.3	5.5	1.7	0.9	15.2	4.5	1.2	1.2	49.2
866 North Washington	8.8	5.0	4.0	2.2	3.0	24.6	5.3	—	0.5	43.4
867 West Idaho	4.1	1.3	4.3	0.7	3.4	3.5	25.2	2.1	1.3	49.7

*Needle samples were provided through the courtesy of Dr Glenn Petersen (USDA Forest Service Forestry Sciences Laboratory, University of Nebraska, Lincoln). The trees were part of a provenance study; seed sources as indicated [17]. The data shown are representative for the three trees of each provenance.

†Iso = isopimaric, Lev = levopimaric, Pal = palustric, Ab = abietic, DeA = dehydroabietic, Neo = neoabietic, Ial = imbricatolal, IC = isocupressic, AcIC = acetylisocupressic and SIC = succinylisocupressic acid. The value for imbricatolal acid is the sum of the peaks for the imbricatolal and the artifact methyl ketone derivative that inevitably forms during diazomethane esterification [3].

umn at 170°, supplementary GC was performed at 190° and with butanediol succinate (BDS) columns [20].

In summary, the resin acids of ponderosa pine needles are characterized by labdane-type acids of which imbricatolalic, acetylisocupressic, and succinylisocupressic acids are the predominant components. Because the plantation source for our sampling had a widespread *Dothistroma* infection, the limited availability of apparently healthy trees hampered the selection of a full complement of varieties. Thus, our data on resin acid composition can only be viewed as an indication of variability. Further work will be necessary to establish the specifics of ponderosa pine needle resin acid composition as chemotaxonomic and genetic indicators. Our data on ponderosa pine needle resin acid composition may have important implications for a toxicological problem. Ingestion of needle resin acids has been implicated in reproductive failure of cattle [21]; none of the labdane resin acids was recognized or found in that study. Perhaps the labdane acids, particularly the succinylisocupressic acid, are the abortifacients.

EXPERIMENTAL

Mature needles from a Nebraska provenance study conducted near Lincoln, Nebraska were obtained in July 1984. Provenances were selected that were relatively free of a prevalent *Dothistroma* infection. Three trees from each selected provenance were sampled. The needle oleoresin was obtained by gently squeezing freshly sliced needle segments and collecting sufficient resin canal exudate on a glass surface. The collected oleoresin was separated into neutral and acidic fractions by our micro-DEAE-Sephadex method [18], but the (resin) acid fraction containing the succinyl derivatives was recovered by elution with 15 ml 1% HOAc in the Et₂O-MeOH solvent. The HOAc eluate was immediately methylated with ethereal CH₃N to minimize acid-catalysed hydrolysis of the acetyl and succinyl components.

GC analysis was performed in a Hewlett-Packard model 5880 GC equipped with FID using a 14 m × 0.25mm (0.1 μm film) FSOT methyl silicone column (DB-1, J & W Scientific, Folsom, Calif.), usually temp. programmed isothermal 170° for 15 min followed by a gradient of 8° min⁻¹ to 230° [22]. The program was varied as necessary to achieve specific separations. The GC retention data for the methyl esters of resin acids including those found in ponderosa pine needles have been summarized [19]. ¹H NMR data were recorded at 250 MHz (CDCl₃ soln, unless noted otherwise, and TMS as int. standard). EIMS: 70eV and reported as *m/z* (rel. int.).

Needle resin acids. The oleoresin from the needles for resin acid isolation and characterization was obtained by extraction. Mature ponderosa pine needles were washed with pentane, cut into segments and extracted with Et₂O. The resin acids were separated from the Et₂O extract by several analytical DEAE-Sephadex columns [23]. These resin acids were then fractionated on silica gel with Et₂O-hexane. Fractions were methylated (CH₃N₂) for further chromatography.

The fr. eluted with 10–15% Et₂O-hexane was further chromatographed as the methyl esters on neutral alumina activity III. Elution with 4% Et₂O gave methyl acetylisocupressate (methyl 15-acetoxy-8(17), *E*-13-labdadien-19-oate, methyl **5**) having spectral and GC retention characteristics identical to that for the material isolated from ponderosa pine xylem oleoresin and oleoresins [4]. Chromatography of another methylated fr. (similar to the 10–15% Et₂O-hexane eluate) on silica gel gave, on elution with 6% Et₂O-hexane, the dimethyl dihydrogathate [dimethyl 8(17)-labdene-15,19-dioate, dimethyl **3**] with GC and

spectral characteristics identical to those of authentic material and with the spectral characteristics for this compound named as mercusic acid [5].

Argentation resin chromatography [24] of the methylated silica gel-acids fr. (eluted with 20% Et₂O) gave, on elution with 50% Me₂CO-Et₂O, methyl imbricatololate [15-hydroxy-8(17)-labden-19-oate, methyl **2**] whose GC retention and spectral characteristics were identical to authentic material as isolated from *P. elliotii* [3]. Elution with 100% Me₂CO gave methyl isocupressate [15-hydroxy-8(17), *E*-13-labdadien-19-oate, methyl **4**], identical with authentic material from *P. ponderosa* xylem oleoresin [4].

Another preparation of oleoresin was extracted from provenance 867 material (Table 1) with Et₂O as described above. The Et₂O soln was filtered to remove wax materials and then extracted with 2% aq. NaHCO₃. The bicarbonate extract was acidified with dil H₂SO₄ and extracted with Et₂O. This Et₂O extract was methylated with CH₃N₂ and chromatographed on silica gel with increasing gradients of Et₂O in hexane. A fr. eluting with 10% Et₂O provided 99% (GC) dimethyl succinylisocupressate [dimethyl 15-succinoxy-8(17), *E*-13-labdadien-19-oate, the dimethyl ester of **6**], [α]_D²⁰ 43.9° (CHCl₃; *c* 0.21, lit. [7] 38.9°, CHCl₃; *c* 4.6). ¹H NMR: δ 5.33 (*t*, *J* = 7 Hz, H-14 = CH), 4.60 (*d*, *J* = 7 Hz, CH₂, H-15), 4.51 and 4.85 (2s, exocyclic = CH₂, H-17), 3.69 (*s*, CO₂Me-19), 3.62 (*s*, succinyl OMe), 2.64 (*s*, 4 methylene H of succinyl), 1.69 (*s*, Me-16), 1.19 (*s*, Me-18), and 0.51 (*s*, Me-20); (CCl₄, 5.23, 4.52, 4.48 and 4.80, 3.64, 3.57, 1.69, 1.15, and 0.47 (cf. [8]); MS *m/z*: 448 (tr) [M], 316 (17) [M-succinyl], 301 [M-succinyl-Me], 241 (9), 189 (22), 180 (17), 133 (12), 121 (100), 115 (30), 107 (25), 93 (21), 81 (27).

Neutrals. The Et₂O phase from the bicarbonate extraction of succinylisocupressic acid was fractionated by DEAE-Sephadex. The neutral portion was chromatographed on silica gel using a gradient of hexane-Et₂O. First to elute (10% Et₂O) was 10-noncosanol, identical in all respects with authentic material [11]. Elution with 20% Et₂O then gave the hydroxyaldehyde, 15-hydroxy-8(17), *E*-13-labdadien-19-al having an ¹H NMR spectrum in CCl₄ essentially identical to that reported in ref. [6]. However, our spectrum in CHCl₃, 9.74 (*s*) 5.38 (*t*), 4.89 and 4.56 (2s), 4.15 (*d*), 1.69, 1.02 and 0.57 (3s), differed somewhat from that of ref. [25]. Further elution of this same silica gel column with 40% Et₂O gave agathadiol having GC retention and spectral characteristics identical to those for authentic material.

Methylation (CH₃N₂) of the HOAc fr. from the DEAE-Sephadex column described in the preceding paragraph and chromatography on silica gel gave as the primary component: 15-succinoxy-8(17), *E*-13-labdadien-19-al (7) as the methyl ester, characterized by ¹H NMR: δ 9.74 (*d*, *J* = 1.3 Hz, CHO), 5.30 (*t*, *J* = 6 Hz, =CH), 4.89 and 4.55 (2s, =CH₂), 4.61 (*d*, *J* = 7 Hz, C H₂O), 3.69 (*s*, OMe), 2.62 (*s*, succinyl CH₂), 1.69 (*s*, Me C=), 1.02 and 0.57 (2s, C-19 and C-20 Me). Elution with 40% Et₂O then gave 15-succinoxy-8(17), *E*-13-labdadien-19-ol (**8**) as the methyl ester characterized by ¹H NMR: δ 5.32 (*t*, *J* = 7 Hz, =CH), 4.82 and 4.51 (2s, =CH₂), 4.56 (*d*, *J* = 7 Hz, C-15 CH₂O), 3.74 and 3.39 (*dd*, *J* = 10.8 Hz, C-19 CH₂O), 3.69 (OMe), 2.63 (*s*, succinyl CH₂), 1.69 (*s*, MeC=), 0.98 and 0.66 (2s, C-19 and C-20 Me). On GC (DB-1), the elution order of the methyl esters was the succinoxy-al (7), succinylisocupressate (**6**), followed by the succinoxy-ol (**8**).

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