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RESIN ACIDS OF *PINUS RESINOSA* NEEDLES

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Key Word Index—*Pinus resinosa*; *Pinus nigra*; Pinaceae; needles; labdane diterpenes; hybrids.

Abstract—The principal resin acids in the needles of *Pinus resinosa* are the labdane diterpenes, the new 8,13-epoxy-14-labden-19-oic acid (epimanoyl oxide acid), 8,13β-epoxy-14-labden-19-oic acid (manoyl oxide acid), 8(17),*E*-12,14-labdatrien-19-oic acid (communic acid) and 15-oxo-8(17)-labden-19-oic acid (imbricataloic acid). A survey of needles from representative populations of *P. resinosa* showed a limited variability in resin acid composition consistent with the uniformity of other traits. The composition of needle resin acids for putative *P. nigra* × *resinosa* hybrids strongly suggests the improbability of *P. resinosa* as the pollen parent.

INTRODUCTION

American red pine, *Pinus resinosa* Ait., is an important North American pulpwood and lumber conifer in the Great Lakes States and Canada. Historically, recovery of naval stores from the oleoresin has been nearly nonexistent, the only practical use having been as a waterproof sealer for birch bark canoes [1]. Naval stores, however, are recovered from red pine wood as byproducts of kraft pulping.

As part of an evaluation of pine foliage as a potential source for naval stores and fine chemicals, we have investigated the diterpene resin acids of red pine needles. This paper reports on the unique composition of resin acids in red pine needles, on the isolation of two new labdane resin acids, and on specific implications of needle resin acid composition in genetic studies.

RESULTS AND DISCUSSION

Preliminary GLC analysis of *P. resinosa* needle resin acids showed the major components to be different both in kind and quantity from the common tricyclic pimaric, isopimaric and abietadienoic acids found in the xylem of this [2–4] and many other pines. These common resin acids are, however, only about 10% of the total in the needles; four other resin acids, all labdane diterpenes, constitute the major portion.

Identification of resin acids

The resin acid fraction of *P. resinosa* needle ether extract was crystallized from ether–petrol. Recrystallization gave a pure acid which was converted to the methyl ester. Argentative CC of the methylated acids from the acid crystallization-liquors provided a second resin acid methyl ester similar to the above methyl ester in its spectral characteristics. Spectral data for the two esters (M_r 334) suggested that they were C-19 carbomethoxy (chemical shift for C-10 hydrogens at about δ0.6) manoyl oxide/epimanoyl oxide derivatives. Comparison of the ^{1}H NMR patterns of the vinyl ABX hydrogens with those for manoyl oxide and epimanoyl oxide strongly suggested the equatorial vinyl configuration (i.e. the methyl ester, **1b**, of manoyl oxide acid, 8,13β-epoxy-14-labden-19-oic acid, **1a**) for the acid obtained by crystallization, and the axial vinyl configuration for the methyl ester, **2b** (of epimanoyl oxide acid, 8,13-epoxy-14-labden-19-oic acid, **2a**) obtained by argentative CC (Table 1). A stronger silver complexing of **1b** (i.e. greater chromatographic retention) than **2b** further supports these assignments. We have also found **1a** in the needles of *P. nigra* [5, 6] and *P. sylvestris* [5]. Since completion of this work, Bardyshev *et al.* have reported the isolation and identification of **1a** from *P. sylvestris* needles [7]. The enantiomeric, C-18 epimanoyl oxide acid has been isolated from a *Beyeria* species [8] and three double-bond isomers of epimanool-19-oic acid have been isolated from *Agathis robusta* [9].

The two other major labdane resin acids were isolated as the methyl esters by argentative chromatography and identified as methyl communate (methyl 8(17),*E*-12,14-labdatrien-19-oate, **3**) and methyl imbricataloate (methyl 15-oxo-8(17)-labden-19-oate, **4**). Communic acid (communic acid ≡ elliotinoic acid [10]) has been reported to be present in small amounts in the xylem oleoresins of *P. elliottii*, *P. edulis* and *P. strobus* [11] and in the cortex oleoresin of *P. strobus* [12]. Imbricataloic acid has been isolated from the needles of *P. elliottii* [13].

Composition of P. resinosa *needle resin acids*

A survey of the needle resin acid composition of *P. resinosa* was conducted on samples of nine populations representing the major habitats of the species. The

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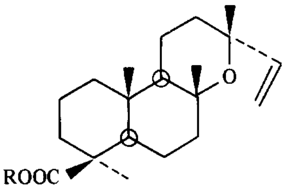
†Maintained by the Forest Service of the U.S. Department of Agriculture, in cooperation with the University of Wisconsin.

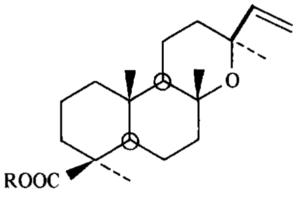
Table 1. ^{13}C chemical shifts of manoyl oxide acid methyl ester **1b**, the dihydro derivative **1c**, and epimanoyl oxide acid methyl ester **2b**

Carbon atom	**1b** δ	**1c** δ	**2b** δ	Carbon atom	**1b** δ	**1c** δ	**2b** δ
1	39.4	39.5	39.8	11	15.5	15.6	16.2
2	19.1	19.2	19.3	12	35.5	35.9	35.1
3	38.2	38.2	38.3	13	73.2	72.8	73.2
4	43.9	43.9	44.0	14	148.0	37.9	147.8
5	55.0*	56.9*	57.0*	15	110.1	7.9	109.6
6	21.5	21.4	21.5	16	28.8	27.1	32.7
7	43.3	43.0	43.2	17	25.0	24.3	23.5
8	74.7	74.1	75.7	18	28.7	28.5	28.6
9	57.0*	57.8*	58.0*	19	177.5	177.6	177.6
10	37.5	37.2	37.4	20	13.0	13.2	13.4
				OMe	51.0	51.0	51.0

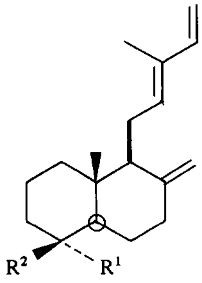
*Chemical shift assignments between C-5 and C-9 and between C-16 and C-17 are interchangeable.



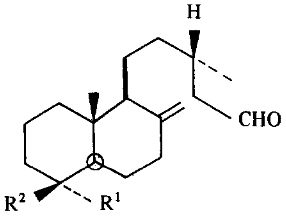
1a R = H
1b R = Me
1c R = Me; vinyl reduced to Et



2a R = H
2b R = Me



	R^1	R^2
3	Me	CO_2Me
5	CO_2Me	Me



	R^1	R^2
4	Me	CO_2Me
6	CO_2Me	Me

methylated resin acids were analysed by packed column GC. The similar retention characteristics for several of the components required analysis on two columns, one containing DEGS and the other SE-30/EGiP [14]; decreasing column temperature from the usual 200 to 186° improved resolution (Table 2).

The needle resin acid composition (Table 3) is relatively consistent among the nine *P. resinosa* populations studied, in agreement with the uniformity in characteristics of the species [16, 17]. The variations that are present correspond fairly well with patterns of latitudinal variation reported for other traits [Lester, D. T., private communication]. Thus, the more southerly provenances 703, 761, and 774 are lower in manoyl oxide/epimanoyl oxide acids (and total oxygenated labdane acids **1a**, **2a**, and **4**) and higher in communic acid than the other more northerly provenances examined.

The above provenance data for resin acid composition represented mature needles of trees from a 10-year-old plantation. Because early characterization is invaluable in genetic and chemotaxonomic studies, we also examined the consistency of resin acid composition in young needle

Table 2. GC retention data* for the methyl esters of the major resin acids in *P. resinosa* needles

Resin acid (as methyl ester)	DEGS		SE-30/EGiP	
	186°	200°	186°	200°
Manoyl oxide acid, **1a**	1.27	1.19	0.98	0.97
Epimanoyl oxide acid, **2a**	1.26	1.24	1.06	1.04
Communic acid, **3**	1.36	1.28	1.08	1.05
4-Epicommunic acid	1.69	1.53	1.22	1.18
Imbricataloic acid, **4**	3.8	3.63	1.56	1.58

*Retention relative to methyl pimarate = 1.00.

tissue and in young plants. Primary needles of the previous year from 2-year-old seedlings (September sampling) contained only the usual tricyclic resin acids and none of the labdane resin acids, but the secondary needles of current year's growth were high in communic acid (**3**) and still comparatively low in the manoyl oxide/epimanoyl oxide acids (**1a**, **2a**). For 3-year-old seedlings, the manoyl oxide/epimanoyl oxide acid content had increased considerably but the content in the current year's needles was still low (total *ca* 20%). By a tree age of 5 years, however, resin acid content in needles of each of the previous two growing seasons was consistent and in line with values observed for older trees. New-growth needles, however, had a lower content of manoyl oxide/epimanoyl oxide acids similar to observations for manoyl oxide acid in certain *P. nigra* trees [5]. Thus, comparative studies of resin acid composition of *P. resinosa* needles should use only mature needles from plants at least 5 years old.

The resin acids in the cortex oleoresin of several trees were analyzed for comparison with those in the needles. The cortical resin acids of *P. resinosa* are characterized by a high content of levopimaric/palustric, neoabietic and communic acids; manoyl oxide/epimanoyl oxide acids were not observed.

Table 3. Composition of needle resin acids of *Pinus resinosa*

Source*	Percent of total resin acids†						
	Sand	MOA	EMOA	Com	L/P	Neo	Imb
703 Gladwin Co., Michigan	2.2	32.0	7.2	39.0	5.0	4.0	10.3
761 Essex Co., New York	1.8	35.6	11.4	32.5	6.5	3.6	8.3
774 Langlade Co., Wisconsin	1.6	29.6	7.6	45.8	3.2	1.3	10.4
724 Queens Co., New Brunswick	1.1	49.1	14.2	23.4	3.1	3.2	5.6
735 Marquette Co., Michigan	2.4	46.5	11.8	23.7	3.6	2.3	9.2
752 Berthier Co., Quebec	1.9	49.8	14.5	17.7	4.8	2.5	8.6
755 Itaska Co., Minnesota	1.0	50.8	12.0	21.3	2.2	2.0	10.5
777 Douglas Co., Wisconsin	1.6	48.1	12.2	23.2	4.0	4.4	6.3
781 Nipissing Co., Ontario	1.1	50.0	7.5	25.7	2.8	0.9	11.6

*Needles of the previous year were obtained in April 1973 and were a composite from two branches of each of three trees. The trees were from a 10-year-old experimental plot in Central Wisconsin. Provenance (source) designations (numbers) per [15].

†By calculation of GC data from SE-30/EGiP and DEGS packed columns [14] at 186°. Sand = sandaracopimaric, MOA = manoyl oxide acid (**1a**), EMOA = epimanoyl oxide acid (**2a**), Com = communic (**3**), L/P = levopimaric/palustric (unresolved), Neo = neoabietic, Imb = imbricataloic (**4**). Values for Imb are the sum of imbricataloic and an artifact methyl ketone [13] peaks. Small amounts of abietic and dehydroabietic acids were present in all samples.

Table 4. Composition of needle resin acids in *P. nigra* × *resinosa* hybrids

Hybrid*	Percent of total resin acids†						
	Sand	MOA	L/P	Ecom	Ab	DeAb	Neo
11	3.2	—	23.3	31.0	3.3	7.3	21.4
12	5.9	—	14.6	62.8	3.0	4.2	7.1
13	5.0	39.3	14.9	30.7	1.3	2.6	4.4
14	4.4	46.0	18.5	16.6	1.7	6.0	6.7

*Specific hybrid number per Dr W. B. Critchfield. The trees were 15 years old when sampled.

†Ecom = epicommunic acid, Ab = abietic acid and DeAb = dehydroabietic acid. Other abbreviations and GC conditions as per Table 1. Hybrid No. 11 contains about 5% each of pinifolic and 4-epiimbricataloic [6] acids; the three other hybrids contained lesser amounts. GC data for hybrid × hybrid and hybrid × *nigra* back crosses are available from the senior author.

Composition of needle resin acids in P. resinosa *hybrids*

Attempts at genetic improvement in red pine through interspecific hybridization have met with only limited success. Critchfield reported [18] a *P. nigra* × *resinosa* cross, albeit only four progeny were selected by foliage characteristics as true hybrids. (A recent publication of Gambles and Dengler [19] provides a detailed description of the morphology of *P. resinosa* needles.) The availability of plant material from these hybrids provided an opportunity to further examine diterpene resin acid composition as a tool for the characterization of hybrids.

The needle resin acid composition for the four *P. nigra* × *resinosa* putative hybrids is given in Table 4. Although the *P. resinosa* parent was no longer living, the limited variability in the species, as seen in Table 1, permits a general needle resin acid characterization of the parent as 30–50% manoyl oxide acid, 7–14% epimanoyl oxide acid, 18–46% communic acid and 6–12% imbricataloic acid. Although the composition of *P. nigra* needle resin acids varies widely [6], the resin acids of the hybrids' *P. nigra* parent consisted primarily of manoyl oxide acid (63%) with a total of 11% of epimbricataloic and pinifolic acids (*P. nigra* N1–N7, [6]); epimanoyl oxide acid and communic acid were not present. Because the needles of both parents are characterized by a high content of manoyl oxide acid **1a**, it was surprising that, while two of the progeny have about 40% **1a** in the needle resin acids, two progeny did not contain any. Equally puzzling was the presence of a resin acid, 4-epicommunic acid, **5**, not found in either of the hybrids' parents. Epicommunic acid is the major needle resin acid of Spanish *P. nigra* [6] and *P. densiflora* [20]. The needle resin acid composition of the four hybrids argues against *P. resinosa* as the pollen parent in the crosses. Rather, a contaminated pollen resulting in some other *P. nigra* hybrid such as *P. nigra* × *densiflora* appears more probable. This is supported by a re-examination of the hybrids based on enzyme-specifying loci [21].

EXPERIMENTAL

GC was done with a Hewlett–Packard model 5750 gas chromatograph equipped with FID. ^{1}H NMR and ^{13}C NMR were recorded at 250 and 62.89 MHz, respectively, in $CDCl_3$ with TMS as int. standard. Mps were determined in evacuated glass capillaries and are corrected. Optical rotations were done using chloroform as solvent.

Isolation and identification of resin acids. Fresh needles of *Pinus resinosa* were shredded and extracted with Et_2O. The volume of Et_2O was reduced and 2 parts petrol added. After refrigeration overnight, the solution was filtered and then extracted with 1 N NaOH. The NaOH solution was washed 2 times with petrol, acidified with HCl and the resulting turbid solution extracted with petrol. The petrol solution was reduced in volume and refrigerated to give a precipitate. Recrystallization of the precipitate from methanol and then from pentane gave pure **1a** (as shown by GC of the methyl ester): mp 147–147.5° (lit. [7] gives an unexplainably low value of 64–66°); $[\alpha]_D^{25}$ −41.5° (*c* 0.9), (lit. [7], −39.2°, EtOH); (Found: C, 74.84; H, 10.10. $C_{20}H_{32}O_3$ requires C, 74.97; H, 10.07%). Methylation with diazomethane gave methyl ester **1b**: mp 84.3–84.5° (lit. [7], 83–85°); $[\alpha]_D^{25}$ −47.6° (*c* 1.2), (lit. [7], −43.2°, EtOH): ^{1}H NMR: δ0.62 (Me-20), 1.17 (Me-18), 1.27 (Me-16), 1.30 (Me-17), 4.91 (1H, *dd*, *J* = 1.5 and 10.7 Hz), 5.14 (1H, *dd*, *J* = 1.5 and 17.5 Hz), and 5.87 (1H, *dd*, *J* = 10.7 and 17.5 Hz); chemical shifts in CCl_4 were 0.02–0.04 further downfield than reported previously [7]. IR and MS were consistent with those reported by Bardyshev *et al.* [7] with the exception of *m/z* at 287, 236, and 189 rather than 288, 237, and 190. ^{13}C NMR is given in Table 1 (cf. [7]).

Compound **1b** was hydrogenated at ambient conditions in benzene using triphenylphosphine rhodium chloride catalyst. Recrystallization from MeOH–H_2O gave the hydrogenated derivative, methyl 8,13β-epoxy-19-labdanoate **1c**: mp 51–52°; $[\alpha]_D^{25}$ −34.3° (*c* 0.7); (Found: C, 74.86; H, 10.71. $C_{21}H_{36}O_3$ requires C, 74.95; H, 10.78%). ^{1}H NMR: δ0.60 (Me-20), 0.85 (*t*, Me-15), 1.17 (Me-18), 1.19 (Me-16), 1.29 (Me-17), and 3.63 (OMe). EIMS *m/z* (rel. int.): 336 (M^+, 1), 321 (22), 307 (100), 289 (50), 275 (21), 261 (15), 247 (51), 236 (12), 229 (43), 189 (12), 139 (28), 121 (73), 109 (32), and 107 (39) (cf. MS for dihydromanoyl oxide [22]).

The acids in selected mother liquors from the crystallization of **1a** were methylated with CH_2N_2 and chromatographed on 20% $AgNO_3$–SiO_2 with Et_2O–petrol. The first major compound to elute (10% Et_2O–petrol) was recrystallized twice from pentane to give pure **2b**: mp 88–88.5°, $[\alpha]_D^{25}$ −61° (*c* 0.7); (Found: C, 75.18; H, 10.07. $C_{21}H_{34}O_3$ requires C, 75.33; H, 10.23%). ^{1}H NMR: δ0.56 (Me-20), 1.17 (Me-18), 1.23 (Me-16), 1.14 (Me-17), 3.62 (OMe), 4.91 (1H, *dd*, *J* = 0.5 and 10.8 Hz), 4.96 (1H, *dd*, *J* = 0.5 and 18 Hz) and 6.01 (1H, *dd*, *J* = 10.8 and 18 Hz). The ^{13}C NMR is given in Table 1. The MS of **2b** is indistinguishable from that of **1b**. The IR spectrum (in CCl_4 for 4000–1350 cm^{-1} and CS_2 for 1350–430 cm^{-1} per the requirements of the Coblentz Society for Class II spectra [23]) of **2b** is nearly identical to that of **1b** with the exception of vinyl CH_2 out-of-plane deformation at 910 cm^{-1} for **2b** and 920 cm^{-1} for **1b** (a similar difference between axial and equatorial vinyl CH_2 deformation is seen for methyl pimarate and sandaracopimarate [24]) and 'fingerprint' differences between 1250 and 1050 cm^{-1}.

Manoyl oxide acid methyl ester **1b** eluted from $AgNO_3$–SiO_2 after **2b**, and was closely followed by methyl imbricataloate **4** (identified by GC retention characteristics and MS, NMR, and IR spectra [13, 24]). Increasing the Et_2O content of the eluant to 20% gave methyl communate [3], identified by spectral (NMR, UV, IR and MS) and physical data (GC retention and optical rotation) [24, 25].

GC determination of resin acid composition. Fresh needles were cut into *ca* 1 cm pieces and extracted 5 times with Et_2O. The acidic fraction of the extract, consisting almost entirely of diterpene resin acids, was quantitatively separated using DEAE-Sephadex [26, 27]. The acids (in 9:1 Et_2O–MeOH) were converted to the methyl esters with ethereal diazomethane. Composition of the resin acids was determined by GC with DEGS and SE-30/EGiP packed columns [14] at 186° (Table 2).

Retention data with capillary columns has been reported by Foster and Zinkel [28].

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