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Diterpene Resin Acids of *Pinus Koraiensis* Needles, Cortex and Xylem*1

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잣나무 Diterpene Resin Acid의 분석*1

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요 약

잣나무의 Diterpene resin acids의 함량을 조사하기 위하여 침엽(needle), 일차조직(cortex), 목질부(sylem)별로 수지를 채취하여 최근 유행하고 있는 capillary column을 사용하는 GC를 이용하여 정량분석을 하였다.

분석결과 7종의 resin acid가 밝혀졌으며, 그 중 lambertianic acid는 needle에서 74-87%, cortex에서 42-57%, xylem에서 18-28%로 분석되었는데, 다른 Pinus류에서 보다 훨씬 많은 양이 함유되어 병리곤충 분야나 농약 분야에서 고찰할 만한 가치가 있다고 생각하며, 그외 *l*-bornyl-trans-p-coumarate, isocupressic acid, pinusolide 등도 분석 되었다.

Keywords: Diterpene, resin acid, lambertianic acid, bornyl coumarate, needles, cortex, xylem, oleoresins

1. Introduction

Korean pine (*Pinus koraiensis* Sieb. et Zucc.) ranges through Korea and eastern Manchuria into southeastern Siberia, with outliers on the Japanese islands of Honshu and Shikoku (1). The composition of gum turpentines (2), neutral diterpenoids (3-10), and mono- and sesquiterpenes (11,12) from the xylem oleoresin are well documented in the literature. However, there is but one report on the composition of the diterpene resin acids, and that report is only for the xylem (13). In the study reported here, a systematic comparison is made of the composition of the resin acids from the three nonconnecting resin systems of the xylem, cortex, and needles. The information in this study will provide a foundation for chemotaxonomic, gentic and insect studies (14).

2. Experimental

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2.1 Collection of oleoresins

Pinus koraiensis oleoresin samples were obtained from the Experimental Forest of Kangweon National University in Korea during August, November, and December, 1986. Oleoresin samples were taken from 16 trees in 2 different areas within the forest. Tree ages were between 40 and 50 years (A-H), and 20 years and younger (I-P).

Xylem oleoresins were taken directly from the tree trunks 30 cm above the ground, and cortical oleoresins were taken from 1 to 2 year old branches; the oleoresins were placed in airtight vials and purged with nitrogen. Mature needles were randomly taken from the designated trees. All the samples were airmailed to the Forest Products Laboratory in Madison and kept refrigerated until the actual chemical analysis. Collection of oleoresin from the needles was done by the Magee-Zinkel method (15). This method involves cutting the tips of the needles and applying slight pressure along the edge between the thumb and forefinger to force oleoresin exudation at the cut. The exudate was then touched to the bottom of a beaker. The ease in obtaining oleoresin varied with time of needle collection. In the needles collected during August, 2 to 5 needles were needed to collect 2 to 5 mg of oleoresin, whereas 5 to 20 needles of the winter samples were needed to obtain the same weight.

2.2 Analysis of oleoresins

The separation of neutrals from acids was accomplished by the semi-micro DEAE-Sephadex method (16). The cortical and xylem oleoresins were diluted 1 to 1 with methyl t-butyl ether; 30μl of the oleoresins solutions were introduced to the semi-micro columns; the entire sample of a needle oleoresin was used.

After separation from the neutrals, the acidic fraction was methylated (CH_2N_2) and analyzed by gas chromatography (Hewlett-Packard model 5840 and 5880 gas chromatographs equipped with a flame ionization detector). Two types of columns were used:

1) Methyl silicone, DB-1, a bonded phase (J. & W. Scientific Inc., Rancho Cordova, Ca) on a fused silica capillary column; 30m x 0.25 mm i.d., with a 0.1 μm film thickness. The column was temperature programmed starting at 170°C for 15 minutes, then increased 10°C/minute to 220°C.
2) Butanediol succinate (BDS), custom coated (Supelco Inc., Bellefonte, Pa) on a fused silica capillary column; 23 m x 0.25 mm i.d., with a 0.25 μm film thickness. The column was kept at an isothermal temperature of 185°C (17). Retention data on capillary columns for the resin acids found in *Pinus koraiensis* have been published (17, 18) or are given in Table 1.

Table 1. Retention characteristics of diterpenoids and bornyl esters from *Pinus koraiensis* oleoresin.

Compounds	Retention time (t_r)* (min)
Methyl isocupressate (IIb)	13.05
ℓ-Bornyl p-methoxycinnamate	15.10
ℓ-Bornyl p-coumarate (I)	17.43
Pinusolide (III)	19.27

*DB-1 column; methyl pimarate=5.54 min, methyl neoabietate 10.75 min. and solvent 0.44 min.

^{1}H NMR specta were obtained at 250 MHz ($CDCl_3$, TMS as internal standard) using a Bruker WM-250 FT Spectrometer. Coupling constant (J) values are in Hz.

3. Results and Discussion

3.1 Resin Acids

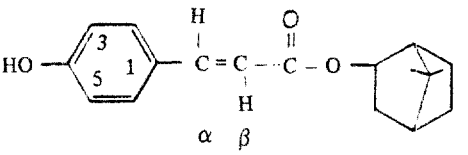
The determination of the composition of resin

acids in oleoresins and rosins is usually accomplished by gas chromatographic analysis with a single column, most often using a polyester liquid phase such as BDS (16). However, in some situations, incomplete resolution of components on both polar and nonpolar columns leads to a need for the combination of data from the analysis on two columns. In this work, the lack of resolution of methyl neoabietate/lambertianate (α= 1.008) on the polar BDS column and insufficient resolution of methyl levopimarate/palustrate on the nonpolar methyl silicone, DB-1 (18), necessitates such a two column analysis.

The predominant resin acids found in three resin systems (Table 2) are: lambertianic (81.2%) and neoabietic acid (14.7%) in the needles; lambertianic (48.9%), neoabietic (18.0%) and abietic acid (16.9%) in the cortex; abietic (30.0%), isopimaric (25.2%) and lambertianic acid (22.0%) in the xylem. The relative amount of lambertianic acid increases from xylem to cortex and it is the predominating component in the needles. These observations are in accord with the enhanced biosynthesis of labdane in needle oleoresin (14) whereas tricyclic common resin acids predominate in xylem. Lambertianic acid is the principal resin acid in the needles of *P. lambertiana*, *P. armandii*, *P. griffithii*, and *P. parviflora* (14).

3.2 *l*-Bornyl *trans-p*-coumarate

The chromatograms of the acidic fractions of the needle oleoresins contained a peak (5 to 10% of te needle acids, 0.5 to 1% of the cortex acids, and trace in the xylem acids) which was not a resin acid. The compound forms a sharp yellow band on the DEAE-Sephadex column in the same manner as does bornyl ferulate (19). Isolation of the compound was achieved in the same manner as the isolation of bornyl ferulate from *P. ponderosa* (19). The proton NMR spectrum [δ 0.88, 0.88, 0.94 (3 Me,*s*,bornyl group), 6.33 (βH,*d*,J= 16), 6.84 (2H at C-2 and C-6,*d*,J=8), 7.44 (2H at C-3 and C-5,*d*,J=8) and 7.61 (αH,*d*,J=16)], the MS (M^+ m/z 300 and base peak at m/z 147, *cf.* with bornyl ferulate, ref, 19), GLC retention, and optical rotation are consistent with the data for the *l*-bornyl *trans-p*-coumarate (I) isolated from *P. bungeanna* (17). The *trans* assignment is made by comparison of the α and β hydrogen coupling constant with those of authetnic *cis*- (δ 5.83 and 6.88, 2H, pair of *d*,J=12) and *trans*- (δ 6.30 and 7.64, 2H, pair of *d*,J=16) methyl coumarate. The report that the isolate from *P. bungeanna* was the *d*-isomer was in error; the pure compound as isolated from *P. bungeanna* had a rotation $[\alpha]_D^{22} = -30°$ (*c* 0.7, $CHCl_3$) (D.F. Zinkel, personal communication). The compound was isolated previously from *Vergesina rupetris* (20).



I. *l*-bornyl *trams-p*-coumarate

During the diazomethane methylation of the acidic fraction of the needles, an artifact of the bornyl coumarate was found to an extent of 10 to 40% of I, depending upon the degree of excess diazomethane. The artifact was separated from the bornyl coumarate (the artifact was a "neutral" compond) by DEAE-Sephadex. Its identity as the methyl ether of I was confirmed by the NMR spectrum (identical to I except for an additional *OMe* at δ 3.84) and a M^+ of m/z 314.

3.3 Isocupressic Acid

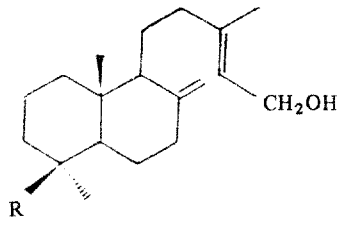
A peak corresponding to methyl isocupresate (IIb) was seen in the gas chromatograms of the (methylated) needle acids (1 to 10%) and cortex resin acids (1 to 3%) only after the DEAE-Sephadex separation but was not detected after direct methylation of oleoresin. There was no evidence in the chromatograms of a DEAE-Se-

Table 2. Composition of the resin acids of needle, cortex and xylem oleoresins from *Pinus koraiensis*.

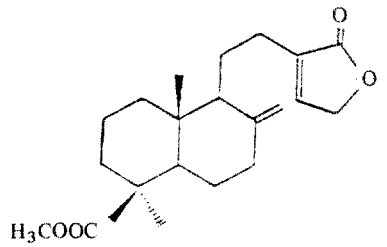
		Percent of total resin acids*						
Source	Tree	Isop	Levo	Palu	Lamb	Deab	Abie	Neo
Needles	A	.0	.9	.0	83.0	.5	.0	14.8
	B	.2	1.6	.2	82.0	.8	.3	12.7
	C	.0	2.2	.0	81.3	.6	.4	13.8
	D	.2	1.7	.0	81.8	1.0	.4	13.0
	E	.0	.4	.0	87.7	1.2	.0	8.7
	F	.1	1.5	.0	78.1	.7	.0	18.2
	G	.1	.6	.2	87.5	.8	.2	9.3
	H	.1	2.0	.0	81.2	.8	.0	14.9
	I	.0	1.9	.0	79.6	.0	.0	17.8
	J	.0	.8	.0	85.6	.0	.0	12.5
	K	.1	1.2	.3	77.8	.7	.3	18.0
	L	.1	2.0	.2	75.0	.7	.3	20.4
	M	.1	2.7	.2	74.1	.7	.3	20.9
	N	.1	.6	.2	85.8	.7	.3	11.5
	O	.0	1.4	.0	82.0	.4	.0	15.2
	P	.1	1.4	.2	76.4	.4	.3	14.2
	mean	.1	1.4	.1	81.2	.6	.2	14.7
Cortex	A	6.5	5.8	.6	43.2	.2	21.6	21.5
	B	9.2	5.7	.8	51.8	.3	17.8	13.5
	C	1.3	5.1	.9	45.4	.4	17.3	18.5
	D	7.4	7.0	1.1	49.3	.4	17.5	16.0
	E	8.2	6.5	.6	47.7	.2	16.0	19.7
	F	10.7	4.3	.7	48.3	.2	20.2	14.8
	G	12.0	3.4	.5	54.8	.2	11.0	17.3
	H	9.6	5.6	1.2	50.1	.3	13.9	18.3
	I	4.3	5.3	1.2	51.1	.3	19.6	17.5
	J	8.8	4.1	.6	50.4	.3	17.0	18.1
	K	5.7	4.9	1.5	57.4	.4	14.5	15.1
	L	9.8	8.4	.8	42.4	.3	19.3	18.1
	M	8.8	8.4	.5	48.7	.3	14.1	18.5
	N	6.1	5.0	.6	45.9	.3	19.1	22.0
	O	9.2	4.8	.6	52.1	.3	14.3	18.0
	P	9.1	7.9	.6	43.1	.3	17.5	20.4
	mean	7.9	5.8	.8	48.9	.3	16.9	18.0

Source	Tree	Percent of total resin acids*						
		Isop	Levo	Palu	Lamb	Deab	Abie	Neo
Xylem	A	28.5	9.5	1.2	24.4	.8	29.4	5.1
	B	25.1	9.3	2.1	28.4	1.2	27.0	4.8
	C	26.7	9.6	1.6	26.9	1.2	27.0	5.4
	D	27.1	9.0	2.0	25.4	1.0	29.2	4.9
	E	29.4	9.4	1.7	18.5	.5	31.2	7.6
	F	25.8	10.5	2.5	23.3	1.0	29.0	5.7
	G	25.0	12.3	1.7	16.2	1.1	33.0	8.2
	H	6.4	9.1	1.8	20.6	1.0	30.8	8.3
	I	28.7	9.6	1.8	21.0	.8	30.8	5.8
	J	28.7	8.6	1.6	21.1	.9	30.0	7.0
	K	25.0	10.7	2.0	27.3	.8	28.0	5.3
	L	27.9	12.6	2.0	17.8	.8	30.0	6.4
	M	25.5	11.0	.6	19.2	1.2	33.5	6.9
	N	25.4	10.7	.9	19.1	1.1	32.5	7.5
	O	22.9	8.6	1.9	19.0	.8	30.7	8.7
	P	25.3	9.6	1.1	23.4	1.3	27.3	6.5
	mean	25.2	10.0	1.7	22.0	1.0	30.0	6.5

*Isop=isopimaric, Levo=levopimaric, Palu=palustric, Lamb=lambertianic, Deab=dehydroabietic, Abie=abietic, and Neo=neoabietic acid. Trace amounts of Δ^8 pimaric, pimaric, sandaracopimaric and communic acids were found in the needle oleoresins; trace amounts of Δ^8 pimaric and sandaracopimaric acids were found in the cortex and xylem oleoresins.



II. a. R=COOH Isocupressic acid
b. R=COOMe Methyl isocupressate



III. Pinusolide

phadex-hydrolyzable precursor of IIa such as acetyl isocupressic acid [found in the xylem oleoresins of *P. ponderosa* (19) *P. elliottii*, and *P. palustris* (21)] or succinyl isocupressic acid [found in the xylem oleoresin of *P. sibirica* (22) and the needle oleoresin of *P. ponderosa*, D.F. Zinkel, personal communication] nor was there any evidence on GLC at 240°C of any other potential precursor. No further attempt was made to identify this apparent 15-O-acyl derivative of IIa.

3.4 Pinusolide

Pinusolide (III) was found by Raldugin *et al* (9) in the xylem oleoresins of *P. sibirica* and *P. koraiensis* and was isolated as the naturally occurring methyl ester. Pinusolide was present at about 0.5% of the needle, cortex, and xylem

oleoresins that we examined. There was no indication of the free acid of III.

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