pH 7 with dilute NH_4OH, treated with $Ba(OH)_2$ and centrifuged. The supernatant was adjusted to pH 7.5 with HOAc and passed through a column (6.6 × 75 cm) of Dowex-1 resin (Cl form, 400 mesh, 200 g) which had previously been washed with H_2O.

The adsorbed material was eluted successively with 2 mM (2.5 l., fractions 1–13), 20 mM (1.2 l., fractions 14–19), 0.2 M (0.6 l., fractions 20–22) and 1 M HCl (1.5 l., fractions 23–31) at the rate of 1 ml/min collecting each fraction of *ca* 200 ml, pooling being done on the basis of UV at 280 nm. Each pooled fraction was concd *in vacuo* almost to dryness, dissolved in minimum amount of H_2O (20 ml) and passed over separate columns (2.54 × 40 cm) of activated charcoal (BDH, 18 g) uniformly mixed with Celite (Loba, 2 g). After thorough washing of each column with H_2O, the absorbed material was eluted with EtOH–H_2O (1:1) containing 1% NH_4OH (eluates: fractions 1–13, 300 ml; 14–19, 200 ml; 20–22, 100 ml; and 23–31, 200 ml). Each eluate was adjusted to pH 5 with HOAc and evaporated to dryness *in vacuo*. The fractions were marked as: (A) 1–13: 10 mg, dark brown resinous; (B) 14–19: 70 mg, dark grey amorphous powder; (C) 20–22: 8 mg, dark amorphous powder; and (D) 23–31: 30 mg white crystalline powder containing inorganic material. All samples had an identical UV λ_{max}^{HCl} pH 1, 280 nm. Sample (B) was the purest; UV λ_{max}^{HCl} pH 1, 280 nm $[\alpha]_D^{40}$ −56.64°, (H_2O; *c* 5.3). (A)–(D) had identical UV, NMR and MS and MS of their TMSi derivatives.

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Phytochemistry, Vol. 22, No. 6, pp. 1500–1503, 1983.
Printed in Great Britain.

0031–9422/83/061500–04$03.00/0

ARABINOGALACTAN-PROTEINS FROM DOUGLAS FIR AND LOBLOLLY PINE

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(*Received* 28 *August* 1982)

Key Word Index—*Pseudotsuga menziesii; Pinus taeda*; Pinaceae; Douglas fir; loblolly pine; arabinogalactan-proteins; β-lectins; chemotaxonomy; structural variation during development.

Abstract—Arabinogalactan-proteins, identified as β-lectins by their precipitation with β-glucosyl Yariv antigen, were isolated from the dry seeds, stratified seeds, 2-week-old seedlings, 2-month-old seedlings, 2-year-old saplings, and 1-month-old callus of loblolly pine and Douglas fir. Two-way analyses of variance revealed that certain β-lectin parameters differed significantly from one another depending on the species and developmental state of a sample's origin.

INTRODUCTION

The β-lectins [1] are a class of arabinogalactan-proteins (AGPs) which precipitate with β-glycosyl Yariv antigen [1,3,5-tri-(*p*-β-D-glycosyloxyphenylazo)-2,4,6-trihydroxybenzene]. The function of these AGPs remains unexplained, although their distribution through virtually all phyla of green land plants [1, 2] implies that they may have some fundamental role in plant physiology. It has been suggested that the β-lectins may be cell-surface components involved in the expression of identity of individual plants, tissues, or cell types [3]. This paper describes the first statistically designed study to evaluate compositional variations in the β-lectins in various tissues of *Pseudotsuga menziesii* and *Pinus taeda* (Douglas fir, D, and loblolly pine, L, respectively), with the goal of determining their potential as developmental or chemotaxonomic markers.

Table 1. Source means of β-lectin amino acid compositions (mol percent)

Amino acid	Species	Source* DS	SS	CT	TM	SP	CL	$\bar{X}$	s.d.
Glx	D	12.7	17.4	9.9	11.3	13.6	8.2	12.0	3.2
	L	12.8	15.3	9.6	11.7	10.1	8.7	11.3	3.7
Gly	D	9.4	7.5	10.7	12.2	15.4	10.2	11.5	3.3
	L	8.9	9.2	10.4	8.1	15.2	13.9	11.2	3.6
Ser	D	11.7	8.8	9.0	8.2	15.3	12.4	11.7	3.6
	L	11.7	8.1	11.1	8.1	12.5	15.2	11.0	3.8
Asx	D	9.7	9.5	7.0	6.5	12.3	7.3	9.2	2.4
	L	9.5	10.3	10.4	9.1	18.2	10.3	11.9	4.2
Ala	D	9.5	7.0	9.8	16.6	8.9	12.2	10.6	3.2
	L	8.4	9.2	10.5	10.7	9.9	12.7	10.2	2.1
Leu	D	8.2	6.9	8.0	7.5	6.5	6.8	7.2	1.1
	L	8.3	7.9	7.7	8.3	9.7	6.9	8.2	1.4
Arg	D	8.3	11.7	4.5	4.0	9.2	3.5	6.9	4.7
	L	8.9	11.1	4.8	5.1	5.0	3.6	6.4	3.4
Lys	D	4.4	3.4	6.8	6.4	3.6	5.1	4.8	1.4
	L	4.1	4.4	6.5	5.7	6.0	5.5	5.5	1.5
Val	D	6.0	5.2	6.5	6.3	2.4	5.1	4.9	2.0
	L	5.8	5.3	6.6	6.1	0.9	4.6	4.6	2.5
Thr	D	3.0	3.2	7.2	4.6	1.9	6.6	4.2	2.6
	L	2.8	3.4	5.0	6.5	1.6	4.6	3.8	2.5
Ile	D	4.1	3.9	4.6	4.3	3.5	3.4	3.8	0.7
	L	3.9	3.7	4.4	4.3	4.3	3.2	4.0	0.8
Phe	D	3.6	2.8	3.5	3.1	1.1	2.8	2.6	1.2
	L	3.4	2.7	3.0	4.4	0.5	2.8	2.6	1.5
Tyr	D	2.7	2.4	2.6	1.8	1.4	2.0	2.0	1.0
	L	2.5	2.5	2.3	2.8	0.4	2.1	1.9	1.0
Pro	D	2.7	5.6	4.2	1.9	0.0	3.8	2.5	2.2
	L	3.9	2.1	2.6	3.6	0.0	0.6	1.9	2.4
Hyp	D	0.9	0.6	2.3	1.9	0.0	7.9	2.2	2.9
	L	0.7	0.6	1.0	1.7	0.0	1.3	0.8	1.1
Met	D	1.5	1.7	0.7	1.6	1.1	0.8	1.2	0.6
	L	1.8	1.8	1.1	1.5	2.2	1.7	1.7	0.7
Orn	D	0.2	0.1	0.2	0.1	3.3	0.4	1.1	2.2
	L	0.2	0.0	1.2	0.1	3.5	1.1	1.3	2.5
His	D	1.0	0.5	1.6	1.5	0.6	1.2	1.0	0.9
	L	0.8	0.7	1.3	1.9	0.0	0.9	0.9	0.9
1/2Cys	D	0.7	1.8	0.8	0.1	0.0	0.1	0.4	0.6
	L	1.6	1.4	0.7	0.3	0.0	0.0	0.6	1.0

*Sources are abbreviated as DS (dry seed), SS (stratified seed), CT (2-week-old cotyledon stage seedling), TM (2-month-old seedling), SP (2-year-old sapling), and CL (callus). Each value is the mean from three separate isolations of AGP. The mean ($\bar{X}$) and standard deviation (s.d.) for all of the individual Douglas fir (D) and loblolly pine (L) determinations on a parameter are listed in the two right-hand columns.

RESULTS AND DISCUSSION

Two-way analyses of variance indicated that the means of the individual amino acid residues of AGPs isolated from the six developmental states of the two gymnosperms listed in Table 1 differed significantly at the 5.0% level of probability among themselves. Three exceptions were leucine, isoleucine and methionine. However, multiple mean comparisons did not reveal any discernible pattern in the noted variations. In contrast to this were the non-significant differences of the means of the individual residues between the two species. The two exceptions to this interspecies statistical equality of the means were for hydroxyproline and asparagine, two amino acids known to be involved in peptidyl-glycosyl bonds [4, 5].

The molecular parameters listed in Table 2 were more easily described in terms of the results of the two-way analyses of variance. The group means listed for the sedimentation coefficient, the weight percent protein, the mol percent galactose, and the mol ratio of non-galactose sugars to galactose were all shown to differ significantly from one another depending on the developmental state and species from which the samples originated. Both sedimentation coefficient and percent protein increased in magnitude with time from the convergent dry seed state up to the cotyledon seedling (2-week-old seedling) state and then decreased: for both parameters within a given developmental state, those of Douglas fir were consistently larger than those of loblolly pine. During the

Table 2. Source means of β-lectin molecular parameters

Parameter	Species	Source* DS	SS	CT	SP	CL	$\bar{X}$	s.d.
$S_{25,W}$	D	5.35	6.35	6.79	6.19	7.59	6.39	0.81
	L	4.98	5.80	5.93	5.24	6.51	5.56	0.66
% protein	D	8.8	23.5	23.7	3.0	3.7	11.3	9.8
(wt %)	L	6.1	10.1	11.1	1.6	2.8	6.8	5.0
Galactose	D	63.4	50.5	46.9	41.2	47.6	48.9	9.2
(mol %)	L	61.7	57.8	51.2	49.3	51.9	53.8	6.1
Arabinose	D	24.2	20.8	31.3	31.6	35.6	29.0	6.1
(mol %)	L	24.9	26.8	32.1	27.9	28.4	28.0	3.2
Glucose	D	4.4	15.1	18.4	21.4	13.8	15.4	9.3
(mol %)	L	6.3	6.7	10.3	14.8	12.2	10.6	5.2
Rhamnose	D	6.9	6.6	0.0	2.2	0.0	3.0	4.0
(mol %)	L	6.0	6.5	2.4	4.5	4.2	4.7	2.3
Mannose	D	0.4	3.8	3.3	2.0	2.5	2.4	1.9
(mol %)	L	0.6	1.5	2.8	2.4	2.2	1.9	1.4
Xylose	D	0.8	3.2	0.0	0.9	0.5	1.0	1.4
(mol %)	L	0.5	0.7	0.5	0.9	1.1	0.7	0.4
G/A (mol	D	2.64	2.47	1.50	1.31	1.34	1.79	0.63
ratio)	L	2.54	2.15	1.60	1.78	1.83	1.95	0.42
T/G (mol	D	0.58	1.01	1.15	1.48	1.10	1.11	0.40
ratio)	L	0.63	0.73	0.97	1.04	0.94	0.88	0.21

*The footnote following Table 1 applies here also. G/A is the ratio galactose–arabinose, and T/G is the ratio of the sum of the monosaccharides shown (excluding galactose)–galactose. $S_{25,W}$ is the principal sedimentation coefficient in Svedberg units taken at 25° in aqueous buffer. In a few samples, a poorly resolved shoulder could be observed at higher values in accord with the report of Yariv *et al.* [7].

development of both species from dry seed to sapling, the mol percent galactose of the AGP consistently declined as the proportions of the other constituent sugars increased. Douglas fir β-lectins were, on a relative basis, more abundant in non-galactose sugars than loblolly pine β-lectins.

Anderson and Dea have discussed the use of carbohydrate fine structural features in the *Acacia* arabinogalactans as a chemotaxonomic marker [6]. The present investigation tends to support a model of the β-lectins as molecules with an evolutionarily conserved (compositionally relatively invariable) protein moiety and a variable carbohydrate moiety. Furthermore, the data reported in Tables 1 and 2 have shown that AGP parameters within development stages of a single species exhibit as great a range of variation as those between species; e.g. weight percent protein [2], galactose–arabinose ratio [2] and sedimentation coefficient [7, 8]. In view of the dependence of β-lectin structure on the subject plant's developmental state, caution ought to be exercised in looking for general chemotaxonomic differences in AGPs isolated from a given state, such as that of suspension-cultured cells [8].

EXPERIMENTAL

Plant materials. Northern Washington Douglas fir seeds were obtained from Brown Seed Company and Alabama loblolly pine seeds from Herbst Brothers Seedsmen, Inc. Seeds were stored at −15° until used. Stratification was achieved by refrigerating water-soaked (48 hr), drained seeds at 1–2° for 60 days. Seeds were sown in redwood flats and grown in a greenhouse. Douglas fir bud tip callus was initiated from a 32-month-old seedling and grown on a modified Murashige and Skoog medium with seven subcultures. Loblolly pine stem callus was initiated from a 3-month-old seedling and grown on another modified Murashige and Skoog medium with six subcultures. The calluses were grown in incubators set for a diurnal cycle of 8 hr of light (125–175 footcandles).

Yariv antigen. The Yariv glucoside, [1,3,5-tri-(*p*-β-D-glucosyloxyphenylazo)-2,4,6-trihydroxybenzene] was synthesized by coupling diazotized *p*-aminophenyl-β-D-glucopyranoside to phloroglucinol according to the method of Yariv *et al.* [9].

Isolation and purification of the AGPs. The β-lectins were isolated and purified according to the method of Clarke *et al.* [2].

Amino acid analyses. Acid hydrolysates were prepared according to the method of Spitz [10] and run on a Beckman Amino Acid Analyzer Model 119CL.

Weight percent protein. The weight percent protein of each sample was calculated from the data for its corresponding amino acid analysis.

Sedimentation coefficients. Sedimentation velocity determinations were carried out with a Beckman Model E ultracentrifuge at 56 000 rpm (*r* 6.5 cm) at 25°. Samples (5 mg/ml) were run in a 0.02 M KPi buffer (pH 7.0) containing 0.17 M NaCl. A single sector, synthetic boundary cell with quartz windows and Schlieren optics was used.

Carbohydrate analyses. Alditol acetates were prepared according to the method of Borchardt and Piper [11], multiple injections of each sample being made into a Packard Model 417 gas chromatograph connected to a Hewlett–Packard 3385A data system. The integrated peak areas were reported on a percent basis of the total neutral sugars present.

Computation. The two-way analyses of variance were conducted using a multiple regression program from Utah State University's Statpac on a Burroughs System 6900 computer. The sedimentation coefficients were calculated from a program run on an IBM 360 computer.

Acknowledgements—We thank John A. Carlson, Russ Feirer, Leroy Borchardt and Tom Rehfeldt for their assistance in procuring the analytical data reported herein. John Teed's advice on the statistical analysis of the data is gratefully acknowledged.

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Phytochemistry, Vol. 22, No. 6, pp. 1503–1505, 1983.
Printed in Great Britain.

0031-9422/83/061503-02$03.00/0

VOLATILE COMPONENTS OF CALIFORNIA LIVE OAK, *QUERCUS AGRIFOLIA*

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(*Revised received* 12 *November* 1982)

Key Word Index—*Quercus agrifolia*; Fagaceae; California live oak; volatiles.

Abstract—The essential oil isolated from *Quercus agrifolia* leaves (*ca* 0.02% wt) was investigated using GC, GC/MS and IR. The most abundant components were *E*-hex-2-enal, *Z*-hex-3-en-1-ol, *Z*-hex-3-en-1-yl acetate and nonanal, respectively, which together comprise 51% of the oil. Of the 15 compounds identified 10 contained a C_6 straight-chain carbon skeleton as part of their structures. Only traces of terpenes were present with linalool and α-terpineol the only terpenes identified with certainty.

INTRODUCTION

The California live oak, *Quercus agrifolia* is a perennial evergreen tree of the black oak group typical of coastal and central California. This investigation of the volatile components present in the leaves of live oak was part of a long-range study in the food-chain relationships of browsing ruminants with respect to the damage they do to industrially important browse species. Although the many different oak species growing in California exhibit a wide range of palatability for browsing deer, the members of the black oak group are generally highly palatable species [1]. Critical observation of deer, both in the field and under penned conditions at the Hopland Field Station of the University of California, has shown that they use olfaction to make their initial selection of forage. In this report we present the results of the first investigation of the volatile components present in the foliage of an oak species.

RESULTS AND DISCUSSION

The aroma of live oak leaves is quite mild. *Ca* 0.2 g of essential oil per kg of fresh leaves can be isolated from the leaves by conventional steam distillation and extraction techniques. Analysis of the essential oil by high resolution glass capillary GC indicated the presence of *ca* 105 volatile components, the majority of which were present in amounts too small to permit identification.

Table 1 lists the volatile components identified in live oak essential oil, the percent composition of the components in the oil and the basis for identification of each component. Identifications of components were based primarily on spectral data with further confirmation by GC retention indices. The mass and IR spectra for the compounds identified in the oil compared favorably with lit. spectra [2, 3] or with the spectra of known compounds measured under the same experimental conditions. The Kovats' indices, listed in Table 1, were measured in