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STILBENES IN THE BARKS OF FIVE CANADIAN *PICEA* SPECIES

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Abstract—Three stilbenes (astringin, isorhapontin and isorhapontigenin) were present in rhytidome samples from *Picea engelmannii*, *P. glauca*, *P. mariana*, *P. rubens* and *P. sitchensis*. A fourth stilbene (astringenin) was primarily present in *P. sitchensis*, *P. engelmannii* and *P. glauca*, with only small amounts (less than 0·01 per cent) present in *P. rubens* and *P. mariana*. Other compounds present were probably the *cis* forms of the stilbenes, but the yields were too low for full identification. Dihydroquercetin, catechin, and epicatechin were identified in all species.

INTRODUCTION

ISORHAPONTIN (3,4′,5-trihydroxy-3′-methoxystilbene 3-*O*-β-D-glucoside) has been reported in the bark of *P. mariana* (Mill.) B.S.P.,[1] *P. glauca* (Moench) Voss[2] and *P. sitchensis* (Bong.) Carr[3] and represents the only reported occurrence of stilbenes among the Canadian species of *Picea*. Those stilbenes shown to occur in other species of *Picea* include: resveratrol (3,4′,5-trihydroxystilbene) in the leaves of *P. polita* Sieb., *P. koyamai*[4] Shirasawa and *P. bicolor*[5] (Maxim.) Mayr.; piceid (3,4′,5-trihydroxystilbene 3-*O*-β-D-glucoside) in the leaves of *P. glehnii*[6] Fr. Schmidt; astringenin (3,3′,4′,5-tetrahydroxystilbene) in the bark of *P. excelsa*[7] (Lam.) Link = *P. abies* (L.) Karst. Astringin (3,3′,4′,5-tetrahydroxystilbene 3-*O*-β-D-glucoside) and isorhapontin have been recently reported in the needles of *P. abies*.[8]

This investigation represents the first attempt to identify and compare the stilbenes in the five Canadian *Picea* species and astringin, astringenin, isorhapontin, and isorhapontigenin were identified in all five species; astringenin concentration is very low in *P. rubens* and *P. mariana*. Two dimensional chromatograms revealed the eight stilbenes detailed above and minor amounts of eight other compounds with the same fluorescence, colour reactions, and R_fs in organic solvents, but with higher R_fs in aqueous acetic acid. In view of the well known mobility of non-planar molecules (and *vice-versa*) in aqueous solvents, these trace compounds were considered to be the *cis*-forms of the above stilbenes. Table 1 summarizes the spectral and chromatographic data and supplements the extensive data of Hillis and

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TABLE 1

Stilbene	R_f values in						U.v. maxima		
	ULCB	BAW	BzW	BEW	2% HOAc	30% HOAc	EtOH	EtOH—NaOAc—H_3BO_3	EtOH—
Astringin	0·33	0·34	0·08	0·35	0·06	0·25	330	344	34
Astringenin	0·50	0·60	0·27	0·63	0·04	0·27	330	343	35
Isorhapontin	0·52	0·55	0·31	0·47	0·10	0·55	327	327	35
Isorhapontigenin	0·71	0·69	—	0·75	0·06	0·44	327	327	35

Ishikura.[9] The stilbenes were characterized by their R_f values and spectral properties.[9] Isorhapontin was further characterized as the hexaacetate. The differentiation of rhapontin from isorhapontin should be noted.

The taxonomic significance of the stilbenes identified in this investigation is negligible, since all species displayed relatively the same pattern of stilbenes in their barks; furthermore, production is dependent upon the time of year.[8]

EXPERIMENTAL

Sample Collection and Preparation

Bark (rhytidome) from indigenous *Picea* spp. was collected from 10 different sources (see Acknowledgements), with the number of samples ranging from 15 to 41 for each species sample. Bark samples were placed in acetone in the dark for one year. The bark samples were recovered on the filter and percolated with more cold acetone, the combined acetone solutions were evaporated to dryness, taken up in methanol and extracted with petroleum (discarded) to remove any terpenoid material. The methanol extract yield for each species was as follows: *P. engelmannii*, 2·21%; *P. sitchensis*, 6·09%; *P. glauca*, 6·28%; *P. rubens*, 6·47% and *P. mariana* 4·93%.

Paper Chromatography

Two-dimensional descending paper chromatography on Whatman No. 1 and No. 3 paper was carried out with $CHCl_3$–CH_3COOH–H_2O (8:12:5, upper layer) (3 parts) and butanol (1 part) (ULCB) in the first direction and 2% aq. HOAc in the second direction. Other solvents used included: 30% HOAc, *n*-BuOH–HOAc–H_2O (6:1:2)(BAW); *n*-BuOH–EtOH–H_2O (4:1:5) (BEW); benzene–HOAc–H_2O (125:72:3) (BzW). The stilbenes were highly fluorescent in u.v. light; other phenolics were detected using Barton's reagent (equal vols of 1% aq. $K_3Fe(CN)_6$ and 1% aq. $FeCl_3$,) bis-diazotized benzidine sp. cinnamaldehyde-HCl, diazotized sulfanilic acid, Zn–HCl and phloroglucinol–NaOH spray reagents.

A 1% aq. solution of oxalic acid was used to hydrolyze stilbene glycosides at 50° and the aglycone recovered by $CHCl_3$ or ether extraction.

Thin-layer Chromatography

Stilbene acetates were chromatographed on silica gel G plates in either $CHCl_3$ or benzene–ether (8:2). The acetates were detected with a H_2SO_4–HNO_3 (1:1) spray followed by heating to 150°.

Stilbene acetylation was accomplished using Ac_2O in pyridine (1:1) with subsequent heating to 60° and the removal of residual reagents under vacuum.

Spectral Analyses

The u.v. spectra were determined using ethanol paper 'blanks' and were compared with published data.[9] Most stilbenes produce very similar i.r. spectra, so this technique was mainly for the comparison of the spectra of standard with isolated compounds.

Identification of Stilbenes

Astringin was identified by co-chromatography with a standard sample on paper and of the hexa-acetate on TLC. Astringenin was identified by TLC comparison of its acetate with authentic acetate.

The chromatographic similarity of isorhapontin and piceid (3,4′,5-trihydroxystilbene 3-*O*-β-D glucoside) prevented the identification of the suspect isorhapontin based solely upon chromatographic properties, but comparison of spectral and physical properties was conclusive. Isorhapontin from *P. glauca* bark produced an u.v. max at 327 nm in EtOH, shifting to 351 nm in NaOEt, whereas piceid shows a max of 324 nm in EtOH shifting to 345 nm in NaOEt. The u.v. spectrum of authentic rhapontin was also distinctive (λ_{max} 329 nm in EtOH, 327 nm in NaOEt). Isorhapontin was isolated from a bark fraction on No. 3 paper in 30% HOAc. The isolated material had an i.r. spectrum suggesting a hydroxylated stilbene and a C-H stretching band at 1435 cm^{-1} which is in accord with a methoxyl group in the molecule. It was acetylated and recrystallized from ethanol–water to fine white crystals with a m.p. of 162·5–163·5°, which remained undepressed in a mixed m.p. with a synthetic sample. The i.r. spectra of the acetates were identical.

Chromatographic comparison of the acetylated hydrolysis product of the bark fractions showed the presence of isorhapontigenin triacetate which was compared with an authentic sample.

Identification of Other Polyphenols

Many other polyphenols were present in the extracts in addition to the stilbenes. Dihydroquercetin, catechin, epicatechin, and unidentified leucoanthocyanins were present in all of the *Picea* species investigated

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and their identification was based solely upon chromatographic evidence and use of chromogenic spray reactions.

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