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STUDIES ON THE BARKS OF THE FAMILY SALICACEAE—XIX.

CONTINUED STUDIES ON THE HOT WATER EXTRACTIVES OF *POPULUS BALSAMIFERA* BARK

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Abstract—The hot water extractives of the smooth green bark of *Populus balsamifera* cut in July were extracted fractionally with ethyl acetate, and the individual ethyl acetate extracts were fractionated by elution chromatography with water on a polyamide column. Crystalline products were salicin, salicyl alcohol, pyrocatechol, 2,6-dimethoxy-*p*-benzoquinone, trichocarpin, salireposide, cinnamic acid, and azelaic acid. This is the first report of azelaic acid in the bark of any tree. The obvious similarity between the components found in this *P. balsamifera* bark with those found previously in a *P. trichocarpa* bark suggests a close relationship between these two species.

RESULTS

THE PRESENT paper communicates the results of the large-scale polyamide chromatography of the ethyl acetate-soluble components of the hot water extractives of the same *Populus balsamifera* bark employed in a small-scale study in the past.[1] The hot water extract from

TABLE 1. IDENTIFIED COMPONENTS OF ETHYL ACETATE-SOLUBLE FRACTIONS OF THE HOT WATER EXTRACTIVES OF *Populus balsamifera* BARK

Component	Fraction A yield (g)	Fraction B yield (g)	Total yield g	Total yield %*
Crude fraction	48	33	81	5·40
Salicin	P†	P	—	—
2,6-Dimethoxy-*p*-benzoquinone	0·002	—	—	0·002
Salicyl alcohol	P	P	—	—
Gentisyl alcohol	P	P	—	—
Pyrocatechol	P	P	—	—
Azelaic acid	0·285	0·044	0·329	0·029
Trichocarpin	6·85	2·91	9·76	0·651
Combined trichocarpin and salireposide	2·76	1·13	3·89	0·259
Salireposide	1·37	0·34	1·71	0·114
Cinnamic acid	0·008	—	0·008	0·001
Unidentified acid	0·020	—	0·020	0·001
Total solids recovered from eluate	28·70‡	17·49§	46·19	3·08

* On the basis of 1500 g of original oven-dry bark solids.
† P = present in quantity, but not isolated and weighed.
‡ Represents 59·8 per cent of material applied to column.
§ Represents 52·8 per cent of material applied to column.

[1] I. A. PEARL and C. R. POTTENGER, *Tappi* **49**, 152 (1966).

1500 g of bark (oven-dry basis) was submitted to fractional ethyl acetate extraction as described previously for triploid *P. tremuloides* bark,[2] and the two ethyl acetate extracts were submitted to column chromatography on polyamide with water elution exactly as described in a previous paper for *P. trichocarpa* bark.[3] The results obtained for the two ethyl acetate extracts, A and B, are presented in Table 1.

DISCUSSION

The elution curves for the two ethyl acetate extractives fractions of this *Populus balsamifera* bark contained three initial peaks essentially identical with those from the analogous chromatograms of similar fractions derived from *P. trichocarpa* bark, and TLC of the corresponding eluate fractions were also identical.[3] The general shape of the elution curves and TLC of most of the fractions of the two species after the first three peaks were similar, but not identical. The obvious similarity between the components and elution curves obtained for fractions of *P. balsamifera* bark and analogous fractions of *P. trichocarpa* bark[3] suggests a close relationship between these two species.

The large-scale polyamide chromatography of the ethyl acetate-soluble portion of the hot water extractives of *P. balsamifera* bark was successful in yielding crystalline components unobtainable from earlier small-scale experiments. Furthermore, TLC of the larger fractions demonstrated that a number of other phenolic compounds are present in substantial quantity, and, in many instances, their proportion in individual fractions is greater than that of the isolated crystalline component. Studies on the further fractionation and isolation of these unknown components are in progress.

The presence in organs of several *Populus* species of the major components isolated as crystalline compounds in this study has become well documented in the past few years. On the other hand, 2,6-dimethoxy-*p*-benzoquinone has been reported only recently in the bark of *P. trichocarpa*,[3] and cinnamic acid in the bark of *P. trichocarpa*[3] and in the leaves of *P. balsamifera*.[4] Azelaic acid has not been found previously in the bark of any tree.

EXPERIMENTAL

Materials

The same smooth green bark dust from authentic *Populus balsamifera* trees cut in Oneida County, Wisconsin, in July which was employed in a previous study,[1] and which had been stored in polyethylene bags, was used in this investigation. Authentic azelaic acid was purchased from Distillation Products Industries Division of Eastman Kodak Co., Rochester, New York.

Bark Fractionation and Processing

The *P. balsamifera* bark dust was processed in the same manner and in the same amount described in detail[3] for *P. trichocarpa* bark dust.

Isolation and Identification of Components

The following crystalline components were isolated from the indicated eluate fractions of the Fraction A chromatogram and identified by mixed m.p. with an identity of i.r. spectra with authentic material: salicin (5–10), 2,6-dimethoxy-*p*-benzoquinone (8), salicyl alcohol (11–15), gentisyl alcohol (12–15), pyrocatechol (16–23), azelaic acid (25–28), trichocarpin (32–39), combined trichocarpin and salireposide (40–49), salireposide (50–64), cinnamic acid (76–80), and unknown acid (83–93). Elution was terminated after 105 fractions had been collected.

[2] I. A. PEARL and S. F. DARLING, *Tappi* **50**, 324 (1967).
[3] I. A. PEARL and S. F. DARLING, *Tappi*, submitted for publication.
[4] I. A. PEARL and S. F. DARLING, *Phytochem.*, submitted for publication.

Isolation of new unsaturated hydroxy acid. Concentration of eluate fractions 83 through 93 yielded small amounts of a crystalline solid. The fractions were combined and concentrated further to yield a total of 17 mg of colorless crystals which were recrystallized from dilute ethanol to give crystals melting at 106–109°. From i.r. and high resolution mass spectra it appears that the material is a long-chain fatty acid of empirical formula $C_{12}H_{20}O_3$ containing one hydroxy group and two double bonds. The exact structure has not been determined as yet.

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