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INVESTIGATION OF THE HOT WATER EXTRACTIVES OF *POPULUS BALSAMIFERA* BARK*

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Abstract—A number of components have been found in *Populus balsamifera* bark which were not previously recorded from this important pulpwood species. These are salicortin, trichocarposide, populoside, salicyloylsalicin, and dihydromyricetin. A new procedure for chromatography on polyamide columns gave a greater recovery than previously obtained.

INTRODUCTION

IN TWO recent papers[1,2], methods were described for the examination of the dried trunk and twig bark of *Populus balsamifera* by initial extraction with hot water, followed by fractionation with ethyl acetate. The individual ethyl acetate extracts were further resolved by elution chromatography with water on a polyamide column, and a number of crystalline compounds were isolated and identified. More recently, studies on the hot water extractives of *Salix purpurea* bark[3] demonstrated that considerably more material could be fractionated and isolated from the ethyl acetate extracts if water elution of the polyamide column was followed by a step gradient elution with increasing concentrations of aqueous ethanol. In addition, these studies indicated that better yields of some components were also obtained if fresh bark was processed instead of dried bark, and a large-scale procedure for processing fresh bark with ethanol was described.

RESULTS

Fresh June bark of *Populus balsamifera* was extracted with 95 per cent ethanol in a Waring Blendor, and the hot-water-soluble portion of the ethanol extractives was extracted fractionally with ethyl acetate exactly as described earlier for *Salix purpurea* bark.[3] The individual ethyl acetate extracts were chromatographed on polyamide and submitted to step gradient elution with water, 20 per cent ethanol, and 50 per cent ethanol as detailed in the earlier paper.[3] As in the past, all eluate fractions were monitored by means of TLC, concentrated to small volumes, allowed to stand, filtered if crystals separated, and finally evaporated to dryness. Weights of all fractions and of separated crystals were noted, and elution curves were obtained. The results obtained for the three ethyl acetate extracts, A, B and C, are presented in Table 1.

* Part XXIII of "Studies on the Barks of the Family Salicaceae".

[1] I. A. PEARL and S. F. DARLING, *Phytochem.* 7, 1851 (1968).
[2] I. A. PEARL and S. F. DARLING, *Phytochem.* 7, 1855 (1968).
[3] I. A. PEARL and S. F. DARLING, *Phytochem.*, submitted for publication.

TABLE 1. CRYSTALLINE COMPONENTS OF ETHYL ACETATE EXTRACTS OF THE HOT WATER EXTRACTIVES OF WARING BLENDOR-PROCESSED *Populus balsamifera* BARK

Component	Elution fraction	Ethyl acetate extract*			Total yield†	
		A, g	B, g	C, g	g	%
Crude extract		48·0	69·0	21·0	138·0	14·4
Salicin	3	0·09	0·19	1·41	1·69	0·18
Mixed salicin and salicortin	4–8	+	+	5·70	5·70	0·59
Salicortin	4–11	+	11·50	0·14	11·64	1·21
Pyrocatechol	17–27	0·40	+	+	0·40	0·04
Trichocarpin	34–40	8·55	4·93	–	13·48	1·40
Mixed trichocarpin and salireposide	41–66	5·84	1·33		7·17	0·75
Trichocarpigenin	54–56	+	0·05	–	0·05	0·01
Salireposide	67–95	0·21	0·15	–	0·36	0·04
Salicyloylsalicin	77–80	+	+	0·01	0·01	0·001
Trichocarposide	140–144	0·05	–	–	0·05	0·01
Mixed trichocarposide and populoside	145–154	0·09			0·09	0·01
Populoside	145–154	0·07	0·74	–	0·81	0·08
Dihydromyricetin	165–170	0·77	–	–	0·77	0·08
Total solids recovered from eluate		45·0‡	61·2§	21·0‖	127·2	92·3

* + means detected by TLC but not isolated in a crystalline form. – indicates not found.
† On the basis of 964 g of oven-dry solids in the 1800 g of fresh bark used in this experiment.
‡ Represents 93·8% of material applied to column.
§ Represents 88·6% of material applied to column.
‖ Represents 100% of material applied to column.

DISCUSSION

Relative to the data of Table 1 for ethyl acetate extract A, concentration of fractions 145–154 yielded crystals containing considerable amounts of both trichocarposide and populoside. After the first crystals were removed from these fractions, subsequent concentration deposited crystals of essentially pure populoside. Similarly, for ethyl acetate extract B, after removal of the first mixed crystals of trichocarpin and salireposide from fractions 54–56, further concentration deposited crystals of pure trichocarpigenin, the aglucone of trichocarpin.

The improvement in polyamide column chromatographic technique since the last reports on *Populus balsamifera* bark,[1,2] comprising subsequent elution with aqueous ethanol solutions after water elution, demonstrates dramatically the advantages of this procedure. The new method results not only in much larger overall recovery from the column, but also in the obtainment in relatively pure form of many more crystalline components. Thus, trichocarposide, populoside, salicyloylsalicin, and dihydromyricetin were found in this bark for the first time because water elution alone does not elute these components from a polyamide column. Furthermore, the ethanol dilutions employed for eluting the column prevent diffusion of components normally obtained in high-numbered fractions when using water elution alone.

The Waring Blendor method for processing fresh bark enabled us to obtain in quantity suspected labile compounds such as salicortin from *P. balsamifera* bark. The high overall yield of salicortin obtained in the present study was made possible by the discovery that absolutely anhydrous conditions are essential for crystallization of salicortin from ethanol, and that crystallization of sirupy fractions from anhydrous ethanol is expedited by the use of

seed crystals.[3] The fact that all of the pure salicortin was obtained from extracts B and C is somewhat misleading. TLC of individual early eluate fractions from the chromatography of extract A indicated the presence of salicortin in quantity, salicin in small amount, and a variety of other components in trace amounts. Under the conditions employed, salicortin did not crystallize from these sirupy fractions. In the case of the chromatography of extract C, the early eluate fractions contained only salicin and salicortin, and many deposited mixed crystal of the two components. Although the fractions of this study were not resolved, other studies[3] demonstrated that salicin and salicortin could be separated completely by column chromatography on silica gel. The significance of the fractional extraction with ethyl acetate is apparent in this experiment. Under the condition of Thieme's original isolation of salicortin from barks and leaves of *Salix* species[4] it was necessary to rechromatograph all polyamide column effluent samples on cellulose columns to obtain fractions from which salicortin would crystallize. Thieme employed total ethyl acetate-soluble fractions.

The isolation of dihydromyricetin from the bark or leaves of any Salicaceae species had not been reported previously. However, in the process of its identification in the present study, the discovery was made that an unidentified flavonoid compound melting at 239–240° isolated previously from the leaves of *P. tremuloides*[5] was in fact dihydromyricetin, as determined by identity of i.r. absorption spectra.

The mass spectrum of trichocarposide pentaacetate exhibited no peak at *m/e* 331 corresponding with the tetraacetylglucose oxonium ion and characteristic of the mass spectra of the acetates of all glucosides containing an unsubstituted glucose moiety.[6] The presence of a *p*-coumaroyl-substituted glucose moiety was confirmed by the presence of a peak at *m/e* 477 corresponding with the triacetyl-*p*-coumaroylglucose oxonium ion. The strongest peak in the mass spectrum is at *m/e* 189 for the *p*-acetoxycinnamoyl ion, and the next strongest is at *m/e* 147 for the unacetylated *p*-coumaroyl ion corresponding with the loss of ketene from the *m/e* 189 peak. Thus, the mass spectrum of trichocarposide pentaacetate confirms the structure 6-*O*-*p*-coumaroyl-salicin assigned to the parent trichocarposide in the past on the basis of chemical evidence alone.[7]

EXPERIMENTAL

Preliminary Processing of Bark

Fresh bark peeled from the trunk of a *Populus balsamifera* tree cut in Langlade County, Wisconsin, on 17 June 1968 was processed within a few hours after cutting by the Waring Blendor, fractional extraction, and step gradient polyamide chromatography procedures detailed in an earlier paper.[3] A total of 2000 g of fresh bark containing 964 g of oven-dry solids was processed in four equal batches to yield the starting water extractives.

Isolation and Identification of Components

The following crystalline components were isolated from the eluate fractions noted in Table 1 and identified by mixed m.p. with an identity of i.r. spectra with authentic material indicated by reference: salicin,[8] salicortin,[3] pyrocatechol,[1] trichocarpin,[1] trichocarpigenin,[2] salireposide,[9] salicyloylsalicin,[10] trichocarposide,[7] populoside,[11] and dihydromyricetin.[12]

[4] H. THIEME, *Die Pharmazie* **19**, 725 (1964).
[5] H. KINSLEY and I. A. PEARL, *Tappi* **50**, 419 (1967).
[6] I. A. PEARL and S. F. DARLING, *Phytochem.* **7**, 831 (1968).
[7] T. K. ESTES and I. A. PEARL, *Tappi* **50**, 318 (1967).
[8] I. A. PEARL and S. F. DARLING, *J. Org. Chem.* **24**, 731 (1959).
[9] I. A. PEARL and S. F. DARLING, *J. Org. Chem.* **24**, 1616 (1959).
[10] I. A. PEARL and S. F. DARLING, *Archs Biochem. Biophys.* **102**, 33 (1963).
[11] R. L. ERICKSON, I. A. PEARL and S. F. DARLING, to be submitted to *Phytochem.*
[12] Kindly supplied by Dr. H. Hergert, Olympic Research Division, ITT Rayonier Inc., Shelton, Wash., U.S.A.

Trichocarposide acetate. Trichocarposide was acetylated as in the past,[7] and the product was recrystallized from dilute ethanol to give trichocarposide acetate melting at 97–98°. Its mass spectrum contains the following major and important *m/e* peaks: 43 (57·2), 106 (6·0), 107 (5·7), 109 (6·7), 119 (5·7), 146 (13·9), 147 (85·9), 148 (10·2), 189 (100), 190 (13·4), 305 (40·7), 477 (6·7), and M^+ 642.

Spectra

I.r. spectra were obtained with a Perkin-Elmer model 21 recording spectrophotometer using a sodium chloride prism and potassium bromide pellets. Mass spectra were made on a double-focusing Hitachi-Perkin-Elmer RMU-6D instrument by direct introduction of the sample with a probe in the ionizing beam.

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