Phytochemistry, 1968, Vol. 7, pp. 1855 to 1860. Pergamon Press. Printed in England

STUDIES ON THE BARKS OF THE FAMILY SALICACEAE—XX.

VARIATIONS IN THE HOT WATER EXTRACTIVES OF *POPULUS BALSAMIFERA* BARK

IRWIN A. PEARL and STEPHEN F. DARLING

The Institute of Paper Chemistry, Appleton, Wisconsin 54911

(*Received* 17 *April* 1968)

Abstract—The hot water extractives of both the twig and the trunk barks of *Populus balsamifera* cut in May were extracted fractionally with ethyl acetate, and the individual ethyl acetate extracts were fractionated by elution chromatography with water on polyamide columns. The twig bark contained more ethyl acetate-soluble material than did the trunk bark and, in general, the yields of individual crystalline components were higher in the twig bark than in the trunk bark. In addition to previously isolated components of *P. balsamifera* bark, trichoside and gentisyl alcohol were isolated from the trunk bark, and trichocarpigenin was isolated from the twig bark in the present study. This is the first recorded isolation of gentisyl alcohol *per se* from any higher plant material.

INTRODUCTION

IN CONTINUING our studies on the barks of various species of *Populus* we investigated the possible variations in chemical content of hot water extractives of barks taken from different parts of the same tree. Barks were obtained from the twigs, young branches, and trunks of the same vigorous 5-yr-old *P. balsamifera* trees, sacrificed on 31 May, 1967, from which the leaves studied in a previous investigation were obtained.[1] The bark from the twigs and young branches was combined to form one sample, and the trunk bark formed the other sample. Both bark samples were dried, reduced to dust, and processed identically in the same manner reported previously[2] for the trunk bark of a 20-yr-old *P. balsamifera* tree cut in July of 1965.

RESULTS

The hot water extracts of the two bark samples of 1500 g air-dry solids each were extracted fractionally with ethyl acetate to give the fractions noted in Table 1.

TABLE 1. FRACTIONAL ETHYL ACETATE EXTRACTION OF HOT WATER EXTRACTIVES

Ethyl acetate extract	Twig bark		Trunk bark	
	g	%*	g	%
First—A	107·5	7·16	42·5	2·83
Second—B	68·0	4·54	58·0	3·87
Third—C	24·0	1·60	51·0	3·40
Total	199·5	13·30	151·5	10·10

* Basis oven-dry bark.

[1] I. A. PEARL and S. F. DARLING, *Phytochem.*, submitted for publication.
[2] I. A. PEARL and S. F. DARLING, *Phytochem.*, submitted for publication.

Data for the polyamide chromatograms of the three ethyl acetate fractions of each bark are presented in Figs. 1 and 2. The weights noted in these figures are actual weights obtained experimentally from the sample aliquots applied to the polyamide column 50 mm in dia. and 80 cm in length.

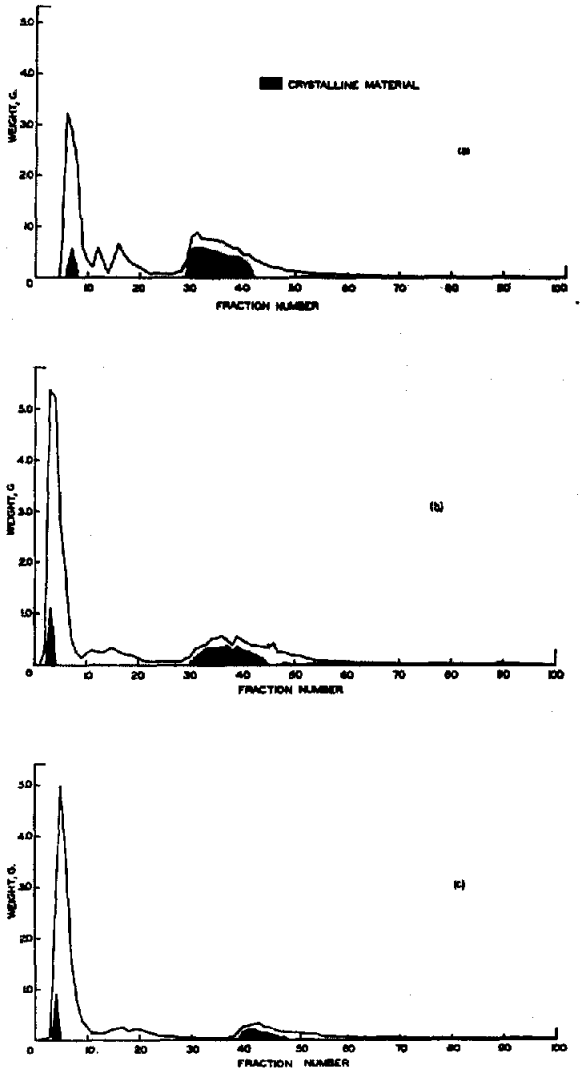

FIG. 1. POLYAMIDE COLUMN CHROMATOGRAPHY OF THE ETHYL ACETATE EXTRACTIVES FRACTIONS FROM *P. balsamifera* TWIG BARK. WEIGHTS OF FRACTIONS APPLIED TO COLUMNS: *a*, 32 g; *b*, 34 g; *c*, 24 g.

Crystalline salicin, salicyl alcohol, pyrocatechol, trichoside, trichocarpin, and salireposide were identified as described in previous papers.[3,4]

[3] I. A. PEARL and C. R. POTTENGER, *Tappi* **49**, 152 (1966).
[4] I. A. PEARL and S. F. DARLING, *Tappi*, submitted for publication.

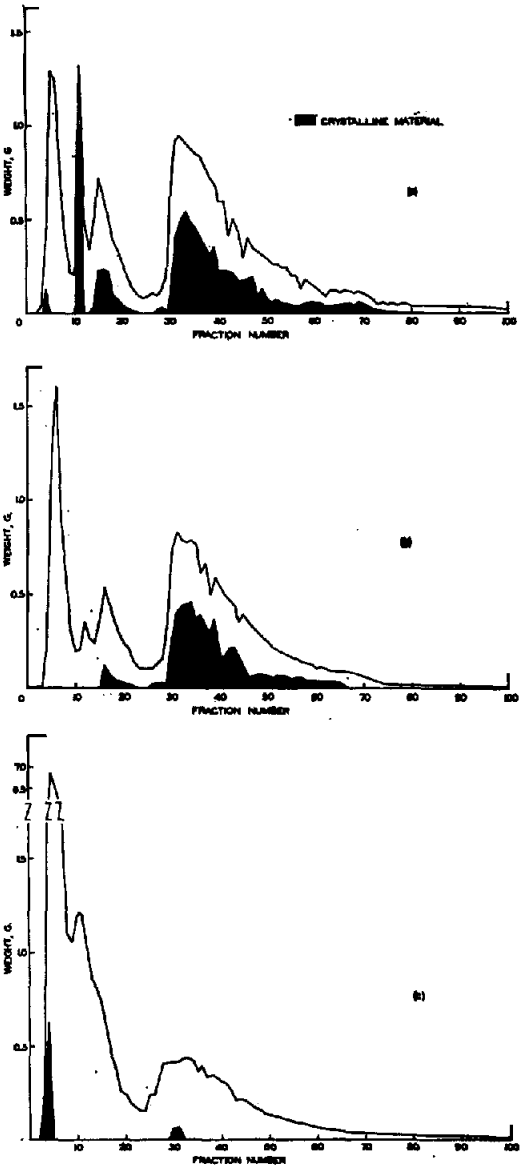

FIG. 2. POLYAMIDE COLUMN CHROMATOGRAPHY OF THE ETHYL ACETATE EXTRACTIVES FRACTIONS FROM *P. balsamifera* TRUNK BARK. WEIGHTS OF FRACTIONS APPLIED TO COLUMNS: *a*, 42·5 g; *b*, 38·6 g; *c*, 31 g.

Isolation and Identification of Gentisyl Alcohol

Thin-layer chromatography indicated that fractions 11–13 of Fig. 2*a* contained salicyl and gentisyl alcohols. The combined residues were chromatographed on a column of silica gel and eluted with methanol–chloroform (4:1). The crude gentisyl alcohol was recrystallized from chloroform containing 2 per cent methanol to give colorless crystals melting at 101–102° and not depressing a mixed melting point with authentic gentisyl alcohol.[5] The i.r. spectra of the crystals and authentic gentisyl alcohol were identical.

[5] J. H. BIRKINSHAW, A. BRACKEN and H. RAISTRICK, *Biochem. J.* 37, 726 (1943).

Isolation and Identification of Trichocarpigenin (Benzyl Gentisate)

The crystals obtained upon concentration of fractions 48 and 49 of Fig. 1*b* were recrystallized from methanol–water (3:2) to give colorless needles melting at 102–104° and not depressing the melting point of a mixture with authentic trichocarpigenin.[6] The i.r. spectrum of the needles and authentic trichocarpigenin were identical and contained bands at 2·95, 5·95, 6·13, 6·23, 6·72, 7·12, 7·20, 7·32, 7·67, 7·88, 8·26, 8·60, 8·85, 9·26, 9·73, 9·96, 10·41, 10·56, 11·02, 11·45, 11·61, 11·72, 12·06, 12·59, 12·73, 12·80, 13·40 and 14·44 μ.

Qualitative Determination of Pyrocatechol and Salicyl Alcohol

Pyrocatechol and salicyl alcohol were determined quantitatively by isothermal gas chromatography at 220° on a column of 10 per cent Apiezon N on Fluoropak 80 ¼ in. in dia. and 6 ft in length. Retention times were 4·2 min for pyrocatechol and 6·9 min for salicyl alcohol.

Isolation of Individual Components

Crystalline salicin was always obtained under the first major peak of all chromatogram elution curves, salicyl alcohol under the second, and pyrocatechol under the third. The crystals isolated from fractions under the fourth major peak (approximately 30–60) are trichocarpin and/or salireposide. First, fractions deposit pure trichocarpin upon concentration. Then a number of fractions yield a crystalline mixture of both glucosides and, finally, the fractions give crystals of pure salireposide. In the case of twig bark fractions B and C (Figs. 1*b* and 1*c*) the crystalline product appearing in fractions 48–49 and 51, respectively, is trichocarpigenin. Trichoside, when present, was isolated in fractions just preceding those which yielded trichocarpin. When gentisyl alcohol was present in fractions under the second major peak which also contained salicyl alcohol, it was separated from the latter by silica gel chromatography as already noted.

The yields of identified components of the three ethyl acetate fractions of the two bark samples are given in Tables 2 and 3. The data of Tables 2 and 3 have been recalculated to the basis of the original 1500 g of oven-dry bark solids employed in both experiments. In some

TABLE 2. COMPONENTS IDENTIFIED AFTER POLYAMIDE CHROMATOGRAPHY OF *P. balsamifera* TWIG BARK*

	Fraction A (g)	Fraction B (g)	Fraction C (g)	Total	
				g	%
Salicin	1·84	2·22	0·92	4·98	0·33
Salicyl alcohol	+	+	+	+	
Gentisyl alcohol	+	+	+	+	
Pyrocatechol	+	+	+	+	
Trichoside	–	–	–	–	
Trichocarpin	18·40	7·69	1·188	27·28	1·82
Salireposide	0·23	0·17	–	0·40	0·03
Trichocarpigenin	+	0·12	0·003	0·12	0·01
Total	20·47	10·20	2·11	32·78	2·19

* Basis 1500 g oven-dry bark.

[6] Kindly supplied by Dr. V. LOESCHCKE, Institut für Biochemie, Hann.-Münden, Germany.

TABLE 3. COMPONENTS IDENTIFIED AFTER POLYAMIDE CHROMATOGRAPHY OF *P. balsamifera* TRUNK BARK*

	Fraction A (g)	Fraction B (g)	Fraction C (g)	Total g	Total %
Salicin	0·13	+	0·627	0·76	0·05
Salicyl alcohol	1·14	+	–	1·14	0·08
Gentisyl alcohol	0·10	–	–	0·10	0·01
Pyrocatechol	0·91	0·29	–	1·20	0·08
Trichoside	0·60	0·74	–	1·34	0·09
Trichocarpin	2·49	2·71	0·12	5·32	0·35
Mixed trichocarpin and salireposide	2·84	4·41		7·25	0·48
Salireposide	0·79	0·80	+	1·59	0·11
Trichocarpigenin	+	–	–	+	
Total	9·00	8·95	0·75	18·70	1·25

* Basis 1500 g oven-dry bark.

instances individual components were identified in fractions by thin-layer chromatography, but could not be obtained in crystalline form. These cases are noted by plus signs in the tables. Where components were suspected, but not found, minus signs appear in the tables.

The total yields of solids recovered from the polyamide chromatograms of the six fractions by water elution are reported in Table 4.

TABLE 4. POLYAMIDE CHROMATOGRAPHY OF *P. balsamifera* BARKS

Ethyl acetate extract, %	Twig bark X*	Twig bark Y†	Trunk bark X	Trunk bark Y
A	78·4	5·6	65·5	1·9
B	86·8	3·9	63·1	2·4
C	90·5	1·4	70·4	2·4

* Basis of individual ethyl acetate extractives fraction.
† Basis 1500 g oven-dry bark.

DISCUSSION

The results of Table 1 demonstrate that the content of ethyl acetate-soluble water extractives of the twig bark is much higher than that of the trunk bark of the same *Populus balsamifera* tree. Furthermore, these data suggest that the nature of individual components of the several ethyl acetate-soluble-fractions vary much more in the twig bark fractions than in those of the trunk bark.

The combined results of Tables 2 and 3 show that all identified components except trichoside were present to some extent in both barks. In general, the twig bark was much richer in isolatable crystalline material than was the trunk bark. Especially noteworthy are the cases of the dominant glucoside, trichocarpin, which was present in twice the concentration in the twig bark than in the trunk bark, and salicin, the concentration of which was seven times as much in the twig bark.

The isolation of gentisyl alcohol from the trunk bark is the first record of this compound *per se* from any higher plant material. It was obtained originally by J. Rabaté in 1935 by enzymatic hydrolysis of debenzoylated salireposide.[7] Subsequently, it was isolated from the substrate of *Penicillium patulum, P. urticae*, and *P. divergens*.[5,8–10]

Trichoside had been isolated previously from the brown bark of *Populus trichocarpa*,[4] and its isolation from *P. balsamifera* bark is still another instance of the close relationship between these two species in the *Populus* genus.[2]

A comparison of the data of Table 4 with those of Tables 2 and 3 together with the data of Figs. 1 and 2 demonstrate that the recovery of crystalline products represented a relatively small proportion of the total products recovered from the water-eluted chromatograms. In most instances, the crystals which separated from concentrated individual fractions were only minor components of these fractions. Further monitoring of these fractions by thin-layer chromatography indicated that substantial numbers of nonisolated components still existed in most of the fractions obtained. Furthermore, the 10–35 per cent loss noted in Table 4 for the water-eluted polyamide chromatograms suggest that considerable material of some fractions cannot be eluted from the polyamide columns with water, and that other solvent systems must be employed for their elution and fractionation. The results of investigations along these lines will be the subject of future communications.

EXPERIMENTAL

Materials

The barks samples were obtained from a tree cut in Waupaca County, Wisconsin on 31 May 1967.

Bark Fractionation and Processing

The samples of *Populus balsamifera* bark dust were processed in the same manner and in the same amount described in detail for *P. trichocarpa* bark dust in an earlier study.[4]

Acknowledgement—The authors wish to thank Mrs. Charlotte Robbins for her help in the processing and analytical monitoring required in this investigation and Mr. Lowell Sell for his i.r. spectrographic analyses.

[7] J. RABATÉ, *Bull. Soc. Chim. Biol.* **17**, 328 (1935).
[8] B. G. ENGEL and W. BREZESKI, *Helv. Chim. Acta* **30**, 1472 (1947).
[9] A. BRACK, *Helv. Chim. Acta* **30**, 1 (1947).
[10] J. BARTA and R. MEČIR, *Experientia* **4**, 227 (1948).