Phytochemistry, 1968, Vol. 7, pp. 1845 to 1849. Pergamon Press Printed in England

STUDIES ON THE LEAVES OF THE FAMILY SALICACEAE—XI.

THE HOT WATER EXTRACTIVES OF THE LEAVES OF *POPULUS BALSAMIFERA*

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(*Received* 13 *March* 1968, *revised version received* 16 *May* 1968)

Abstract—The hot water extractives of fresh leaves from *Populus balsamifera* trees cut in May and in September were extracted fractionally with ethyl acetate, and the ethyl acetate extracts were fractionated by elution chromatography with water on a polyamide column. Crystalline compounds isolated were salicin, salicyl alcohol, pyrocatechol, (−)-3-hydroxy-5-phenylvaleric acid, trichocarpin, cinnamic acid, and *p*-coumaric acid. Yields of all identified products were much smaller in the September than in the May leaves. This is the first report of cinnamic, *p*-coumaric, and 3-hydroxy-5-phenylvaleric acids in the leaves of any *Populus* species and the first report of the last acid in any plant source.

INTRODUCTION

RECENT studies in our laboratory on the components of the hot water extractives of *Populus* species barks[1,2] indicated that fractionation of the ethyl acetate-soluble materials could be attained by continuous extraction of the concentrated hot water extract with ethyl acetate with occasional changing of extraction receivers. Other studies in progress demonstrated that the content of *Populus* species bark hot water extractives was a function of the season, and that certain glucosides might be present in quantity in March and be essentially absent in September.

The present communication reports the isolation and characterization of components of the several extracts obtained from the fractional extraction with ethyl acetate of the hot water extractives of the leaves of balsam poplar, *P. balsamifera* collected in May and in September.

RESULTS AND DISCUSSION

The hot water extracts of both leaf samples were extracted fractionally with ethyl acetate, and ethyl acetate fractions were chromatographed on polyamide columns and eluted with water, collecting fractions in the eluate as described previously.[1,3] All eluate fractions were monitored by means of TLC.[3–7] They were then concentrated to small volumes, allowed to stand, filtered if crystals separated, and finally evaporated to dryness. Weights of all fractions

[1] I. A. PEARL and S. F. DARLING, *Tappi* **50**, 324 (1967).
[2] I. A. PEARL and S. F. DARLING, *Tappi*, in press.
[3] I. A. PEARL and C. R. POTTENGER, *Tappi* **49**, 152 (1966).
[4] I. A. PEARL and S. F. DARLING, *Arch. Biochem. Biophys.* **102**, 33 (1963).
[5] I. A. PEARL and S. F. DARLING, *Tappi* **48**, 506 (1965).
[6] I. A. PEARL and S. F. DARLING, *Tappi* **48**, 607 (1965).
[7] I. A. PEARL, S. F. DARLING and S. F. HELLER, *Tappi* **49**, 278 (1966).

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

Component	Fraction A yield (g)	Fraction B yield (g)	Fraction C yield (g)	Fraction D yield (g)	Total yield g	Total yield %*
Crude fraction	52·0	46·5	41·0	30·6	170·1	14·4
Salicin	1·01	1·26	2·73	14·00	19·00	1·60
Salicyl alcohol	P†	P	—	—	P	—
Pyrocatechol	P	P	—	—	P	—
(−)-3-Hydroxy-5-phenylvaleric acid	0·37	0·13	—	—	0·50	0·042
Trichocarpin‡	5·58	3·52	—	—	9·10	0·767
Cinnamic acid	0·91	0·23	—	—	1·14	0·962
p-Coumaric acid	0·08	—	—	—	0·08	0·007

* On the basis of 1185 g of original oven-dry leaf solids.
† P=present in quantity, but not isolated and weighed.
‡ Contains a trace of salireposide.

TABLE 2. IDENTIFIED COMPONENTS OF ETHYL ACETATE-SOLUBLE FRACTIONS OF SEPTEMBER *Populus balsamifera* LEAVES HOT WATER EXTRACTIVES

Component	Fraction A yield (g)	Fraction B yield (g)	Fraction C yield (g)	Total yield g	Total yield %*
Crude fraction	47·0	80·0	20·6	147·6	12·30
Salicin	—	0·90	0·61	1·51	0·126
Salicyl alcohol	P†	P	—	P	—
Pyrocatechol	P	P	T†	P	—
(−)-3-Hydroxy-5-phenylvaleric acid	T	—	—	T	—
Trichocarpin‡	1·64	0·56	—	2·20	0·186
Cinnamic acid	0·25	0·014	—	0·26	0·022
p-Coumaric acid	T	—	—	T	—

* On the basis of 1200 g of original oven-dry leaf solids.
† P=present in quantity, but not isolated and weighed. T=crystals isolated and identified, but present only in trace amounts.
‡ Contains a trace of salireposide.

TABLE 3. POLYAMIDE CHROMATOGRAPHY OF *Populus balsamifera* LEAVES—TOTAL SOLIDS RECOVERED BY WATER ELUTION

Ethyl acetate extract (%)	May leaves X*	May leaves Y†	September leaves X*	September leaves Y†
A	45·9	2·0	42·7	1·7
B	41·5	1·6	29·9	2·0
C	45·3	1·6	48·8	0·8
D	45·6	1·2		

* Basis of individual ethyl acetate extractives fraction.
† Basis of oven-dry weight of original leaves sample.

and of separated crystals were noted, and elution curves were obtained. The results are given in Tables 1 and 2. The total yields of solids recovered from the polyamide chromatograms of the seven fractions of Tables 1 and 2 are reported in Table 3. During the processing of the September (but not the May) leaves considerable ash-containing material separated from the concentrated original hot water extractives. This was removed before fractional extraction with ethyl acetate.

The colorless crystals obtained from eluate fractions 17 through 21 of fraction A were recrystallized from water to give optically active crystals melting at 129–131°. The i.r. spectrum indicated an aromatic hydroxy acid, and carbon and hydrogen analysis suggested a compound $C_{11}H_{14}O_3$ with mol. wt. of 194. A mass spectrum gave a major peak at *m/e* 176 corresponding with M-18 due to loss of water and at *m/e* 91 due to the tropylium ion. An NMR spectrum in deuterated acetone indicated five aromatic and nine side-chain protons including a CH_2—CH—CH_2 configuration. All information suggested an hydroxyphenylvaleric acid. The structure was confirmed as (−)-3-hydroxy-5-phenylvaleric acid by mixed melting point with and identity of i.r. spectrum with that of an authentic sample of inactive 3-hydroxy-5-phenylvaleric acid prepared from 4-hydroxy-6-phenyl-5,6-dihydro-2-pyrone by the method of Reid and Siegel.[9] An attempt to form the acetate of (−)-3-hydroxy-5-phenylvaleric acid by treatment with acetic anhydride in pyridine resulted only in the formation of the well-known 5-phenyl-2-pentenoic acid.

It is obvious from the data of Tables 1 and 2 that although the total ethyl acetate-soluble material present in the hot water extractives of May and September *Populus balsamifera* leaves is present in the same general amount, the distribution of identified crystalline components is completely different. TLC of fractions indicated that analogous eluate fractions contained essentially the same components qualitatively, but in cases where the May leaves contained large quantities of crystalline components, the September leaves contained small amounts, and in instances where the May leaves contained small amounts of crystalline components, the September leaves contained only traces. Thus, the identifiable glucoside and related phenolic compound crystalline components appeared to decrease during the growing season. On the other hand, since no ash-containing organic material separated from the concentrated hot water extractives of the May leaves, it appears that this material increases in concentration during the growing season and reaches a maximum in the fall before the leaves turn.

Salicin, salicyl alcohol, pyrocatechol, and trichocarpin have been isolated in the past from leaves of the *Populus* genus, and their presence in *P. balsamifera* leaves is not surprising. The presence of (−)-3-hydroxy-5-phenylvaleric acid, cinnamic acid, and *p*-coumaric acid in these leaves is noteworthy, however, because none of these compounds have been found in the leaves of any *Populus* species in previous studies. In fact, 3-hydroxy-5-phenylvaleric acid has not been reported previously from any plant source and has been known only in its inactive form as prepared by synthesis. Both samples of *P. balsamifera* leaves contained appreciable amounts of trichocarpigenin, the aglucone of trichocarpin, as noted by TLC. Although never found in the leaves of *Populus* species in the past, its presence would be expected because of the large proportion of trichocarpin present. The similarity of the components isolated from the leaves of *P. balsamifera* in the present study with those obtained in the past from the bark of *P. trichocarpa*[2, 3, 8] suggests the similarity of the two species and that the leaves of *P. trichocarpa* might yield the same crystalline components.

[8] I. A. Pearl and S. F. Darling, *Phytochem.*, submitted for publication.
[9] E. B. Reid and J. R. Siegel, *J. Am. Chem. Soc.* **76**, 938 (1954).

Chromatography on a polyamide column was successful in fractionating the ethyl acetate-soluble portion of the hot water extractives of these leaves to the point where a relatively large number of components separated as crystalline solids. The monitoring of these fractions by TLC indicated that substantial numbers of nonisolatable components still existed in most of the fractions obtained. In many instances, the crystals which separated from concentrated fractions were only minor components of these fractions.

Weights of ethyl acetate fractions in Tables 1 and 2 and yields based on these fractions in Table 3 were obtained from the actual weights of these fractions after evaporation under reduced pressure. During the interval of time between the processing of the May and September leaves, it was found that considerable weight loss was obtained when these samples were absorbed on polyamide preparatory to column chromatography. Because no absolute data was obtainable for the May leaf fractions, data for both leaf samples were reported on the crude weight basis for the individual ethyl acetate extractives fractions.

As in all studies on the separation of components from extractives of biological materials, the question arises as to whether the compounds isolated in the present investigation are true components of the *P. balsamifera* leaves or are artifacts of the processing involved. Because our leaves were extracted with boiling water within a few hours after sacrificing the tree, very little if any enzymatic degradation could have taken place. Only water and neutral solvents were employed in processing and, therefore, even though there is a possibility of hydrolysis, we believe that most of the material isolated by our processing scheme is present *per se* in the leaves.

EXPERIMENTAL

Materials

Fresh leaves were obtained from *Populus balsamifera* of approximately 5 yr of age cut near Clintonville, Wisconsin, on 31 May 1967 and 5 September 1967. Leaves were processed within a few hours of the tree cutting.

Preliminary Processing of Leaves

The hot water extract of fresh leaves containing approximately 1200 g of oven-dry solids was concentrated under reduced pressure in a circulating evaporator to a volume of 1000 ml and extracted fractionally with ethyl acetate in a liquid–liquid extractor. Quantitative data for these extractions are given in Tables 1 and 2.

Polyamide Chromatography of Ethyl Acetate-Soluble Fractions

The entire fraction or aliquot containing approximately 40 g of solids was dissolved in tetrahydrofuran, absorbed on polyamide powder,* placed on a column of polyamide (50 mm × 80 cm), and developed with water, collecting 210-ml fractions in the eluate.[1] Eluate fractions were concentrated under reduced pressure to 5 ml and allowed to stand 24 hr. If crystals appeared, they were filtered, washed, dried, weighed, and identified. If crystals did not appear, the fractions were evaporated to dryness under reduced pressure and weighed. The total weight and weight of crystals in each fraction was noted. The composition of each fraction was monitored by TLC on silica gel plates, developed in 4:1 chloroform–methanol, sprayed with 50% sulfuric acid, and heated at 105°.[4]

Representative Polyamide Chromatogram

In the elution chromatogram of the first ethyl acetate extract of the May leaves (fraction A), salicin crystals were obtained when eluate fractions 2–4 were concentrated, and TLC indicated that salicin was the major component of fractions 2–7. No crystals separated on concentration of fractions 8–15, but TLC indicated these fractions to be predominantly salicyl alcohol and pyrocatechol with several phenolic impurities. Upon concentration to small volumes under reduced pressure, eluate fractions 17 through 21 deposited colorless crystals. These were filtered and recrystallized from water to give optically active crystals melting at 129–131° which were identified as (−)-3-hydroxy-5-phenylvaleric acid (see below). Crystals of pure trichocarpin separated from eluate fractions 26 to 35, and crystals of almost pure trichocarpin containing a trace of salirepo-

* Polyamide Woelm, manufactured by M. Woelm, Eschwege, Germany.

side separated from fractions 37 to 41. Mixtures containing a variety of phenolic compounds were obtained from fractions 42 through 62, but no major crystalline component was obtained. Fractions 63 through 84, upon concentration, deposited colourless crystals melting at 132–134° which proved to be cinnamic acid. Fractions 85 through 103 contained phenolic mixtures which yielded no crystals, but fractions 104 through 109 did deposit colourless crystals of *p*-coumaric acid upon concentration. The elution was stopped after 110 fractions.

Isolation and Identification of Known Components

Salicin, salicyl alcohol, pyrocatechol, trichocarpin, and cinnamic acid were isolated as crystals and identified as in the similar study of the components of *P. trichocarpa* bark. Crystalline *p*-coumaric acid was identified by mixed m.p. and identity of i.r. spectra with authentic material.

Isolation and Identification of (−)-3-Hydroxy-5-phenylvaleric Acid

Upon concentration to small volumes under reduced pressure, eluate fractions 17 through 21 of fraction A from May leaves deposited colorless crystals. These were filtered to give a combined yield of 0·37 g of crystals which were recrystallized from water. M.p. 129–131°, $[\alpha]_{546}^{25}$ −10·4° (c=2·55 in ethanol). (Found: C, 68·22; H, 7·19. Calc. for $C_{11}H_{14}O_3$: C, 68·04; H, 7·26 per cent.) Its i.r. spectrum contained bands at 3·15, 3·43, 3·80, 3·94, 5·93, 6·22, 6·68, 6·86, 6·94, 7·06, 7·38, 7·67, 7·86, 8·34, 8·41, 8·68, 9·20, 9·38, 9·70, 9·85, 10·70, 11·43, 13·30, and 14·20 μ. The mass spectrum contained the following major and important *m/e* peaks with relative *m/e* intensity for each peak noted in parentheses: 176 (7), 158 (2), 130 (9), 117 (16), 91 (100), 78 (10), 77 (9), 65 (19), 51 (12), 43 (9), 39 (13), 27 (6), and 18 (7). In the NMR spectrum absorption at 4·59 ppm δ which disappeared when deuterated with D_2O indicated OH and COOH groups. A methine group between two methylene groups (CH_2—CH—CH_2) was indicated by one proton at 4.02δ. Integration of NMR spectrum indicated five aromatic protons (7·22δ) and nine side-chain protons for a total of fourteen protons.

An attempt to form the acetate of (−)-3-hydroxy-5-phenylvaleric acid by treatment with acetic anhydride in pyridine resulted only in the formation of known 5-phenyl-2-pentenoic acid.[10] M.p. 99–101°. (Found: C, 74·30; H, 6·90. Calc. for $C_{11}H_{12}O_2$: C, 74·96; H, 6·75.)

Preparation of authentic 3-hydroxy-5-phenylvaleric acid. Authentic 4-ethoxy-6-phenyl-5,6-dihydro-2-pyrone was deethylated by hydriodic acid by the method of Reid and Ruby[11] to yield 4-hydroxy-6-phenyl-5,6-dihydro-2-pyrone which in turn was catalytically reduced with hydrogen and Raney nickel to yield the desired 3-hydroxy-5-phenylvaleric acid according to Reid and Siegel.[9] M.p. 129–131°. The i.r. spectrum was identical with that of the product isolated from the *P. balsamifera* leaves. A mixed m.p. of the two was not depressed.

Spectra

I.r. spectra were obtained with a Perkin-Elmer model 21 recording spectrophotometer using a NaCl prism and KBr 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. NMR spectra were made on a Varian-Aerograph Model A60A Analytical NMR Spectrometer with spin decoupler. Deuterated acetone was used as the solvent and TMS (tetramethylsilane) was employed as an internal standard. Spectra were obtained before and after addition of deuterium oxide which deuterized the hydroxyl and carboxyl groups.

Acknowledgements—All melting points are uncorrected. Analyses were performed by Micro-Tech Laboratories, Skokie, Illinois. I.r. and NMR spectra were determined by Mr. Lowell Sell of The Institute of Paper Chemistry Analytical Department. Mass spectra were determined by Morgan-Schaffer Corp., Montreal, Quebec, Canada. The authors wish to thank Mrs. Charlotte Robbins for her help in the processing and analytical monitoring required in this investigation. The authors are grateful to Dr. E. B. Reid of Colby College, Waterville, Maine, for a generous supply of authentic 4-ethoxy-6-phenyl-5,6-dihydro-2-pyrone.

[10] R. Fittig and T. Hoffmann, *Ann.* **283**, 317 (1894).
[11] E. B. Reid and W. R. Ruby, *J. Am. Chem. Soc.* **73**, 1054 (1951).