Phytochemistry, 1970, Vol. 9, pp. 1277 to 1281. Pergamon Press. Printed in England.

PHENOLIC EXTRACTIVES OF *SALIX PURPUREA* BARK*

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(*Received* 21 *September* 1968, *in revised form* 29 *July* 1969)

Abstract—Fresh June bark of 4-year-old reeds of *Salix purpurea* was processed immediately with ethanol, and the ethanol extract was evaporated to dryness. The hot water-soluble portion of the ethanol extractives was extracted fractionally with ethyl acetate, and the ethyl acetate-soluble fractions were chromatographed on columns of polyamide and eluted successively with water, 20% ethanol and 50% ethanol. Crystalline compounds isolated were (+)-catechin, isoquercitrin, salicin, salicortin, salicyloylsalicin, salicyloylsalicin-2-benzoate, salireposide, naringenin, 5-β-D-glucosides of (+), (±), and (−)-naringenin, isosalipurposide, and an isomer of grandidentatin called purpurein. Discrepancies between the present study and results of previous investigators are discussed.

INTRODUCTION

SEVERAL years ago, Thieme[1] isolated a new glucoside, salicortin, from the bark of *Salix purpurea*. In a subsequent review paper, this author[2] noted that salicortin is a very labile glucoside containing the salicyloylsalicin moiety in its structure, and is probably the precursor of the salicin found in quantity in the barks and leaves of trees of the genera *Salix* and *Populus*. Thieme[3] demonstrated by means of paper chromatography that salicortin was an important component of all the *Populus* and *Salix* barks he had investigated.

The obvious importance of Thieme's findings and hypothesis to our continuing studies on the barks and leaves of the Salicaceae led us to a study of *S. purpurea* bark in an effort to obtain a supply of salicortin for structure determination and for comparison studies with *Populus* bark components. The first studies employed a procedure[4] evolved over the years in our laboratories for the isolation in quantity of components of barks and leaves and comprising allowing the fresh bark to dry, reducing to dust in a Wiley mill, extracting the bark dust with hot water, concentrating the hot water, extracting the concentrated water extract with ethyl acetate, and fractionating the ethyl acetate extractives on a polyamide column by elution with water and ethanol–water mixtures. No salicortin was found, although TLC indicated that a component having the same characteristics was present in several of the early polyamide chromatography fractions. Because our procedure did not yield crystalline salicortin, we modeled a new procedure after the general technique alluded to, but not described by Thieme for processing fresh barks.[2]

RESULTS

In accordance with the new procedure, fresh June bark of *Salix purpurea* was extracted with 95% ethanol by repeated processing in a Waring Blendor. The hot water-soluble

* Part XXI in the series "Studies on the Barks of the Family Salicaceae".

[1] H. THIEME, *Die Pharmazie* **19**, 725 (1964).
[2] H. THIEME, *Planta Medica* **13**, 431 (1965).
[3] H. THIEME, *Planta Medica* **15**, 35 (1967).
[4] I. A. PEARL and S. F. DARLING, *Phytochem.* **7**, 825 (1968).

portion of the ethanol extractives was extracted fractionally with ethyl acetate, and the ethyl acetate extractives were processed by polyamide chromatography with water elution as in the past. In the present investigation, after eluting with water for the collection of ninetyfive fractions, the eluting solvent was changed to 20% ethanol, and after a total of 174 fractions were collected, the eluting solvent was changed to 50% ethanol. 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 two ethyl acetate extracts, A and B, are presented in Table 1.

TABLE 1. CRYSTALLINE COMPONENTS OF ETHYL ACETATE EXTRACTS OF THE HOT WATER EXTRACTIVES OF *Salix purpurea* JUNE BARK

Component	Elution fraction	Extract A yield (g)	Extract B yield (g)	Total yield g	Total yield %*
Crude extract		68·0	85·5	153·5	15·8
Salicin	3	0·01		0·01	
Salicortin	4–5	10·25	P†	10·25	1·060
Salicyl alcohol	12–15	P		P	
Pyrocatechol	16–26	P	P	P	
Purpurein	46–58	1·51		1·51	0·161
Salireposide	56–67	0·57		0·57	0·059
Salicyloylsalicin	59–73		0·62	0·62	0·064
Naringenin glucosides	104–127	6·14	1·08	7·22	0·746
(+)-Catechin	141–151	0·27		0·27	0·028
Salicyloylsalicin-2-benzoate	177	0·01		0·01	
Isoquercitrin	178–179‡	P	P		
Isosalipurposide	180–181	0·87		0·87	0·090
Naringenin	187–190	0·11	2·14	2·25	0·322
Total solids recovered from eluate		67·4§	74·2‖	141·6	14·6

* On the basis of 967 g of oven-dry solids in the 1800 g of fresh bark used in this experiment.

† P = present in quantity (0·5–5·0 g), but not isolated and weighed.

‡ In the preliminary studies noted in the introduction, isoquercitrin was isolated as pale-yellow fine needles melting at 221–222° and identified as isoquercitrin by mixed m.p. and identity of i.r. spectra with authentic material.

§ Represents 93·6% of material applied to column in two equal batches.

‖ Represents 86·8% of material applied to column in three equal batches.

DISCUSSION

The data of Table 1 confirm Thieme's contention that salicortin is the major crystalline glucoside obtainable from *Salix purpurea* bark. It should be noted that the yield of 1·06% on the basis of the original solids in the fresh bark represents only a portion of the salicortin present in these fractions, and includes only the weight of the crystalline material separated from two of the many fractions containing salicortin as the only or major component. The fractional ethyl acetate extraction was effective in providing an initial fractionation of the salicortin because polyamide chromatography fractions of Extract A containing salicortin were much cleaner and amenable to crystallization than were corresponding fractions of Extract B. It should be noted further that the brick-red spot at R_f 0·55 characteristic of salicortin on TLC plates was observed in the fractions obtained from the polyamide chromato-

graphy of the extractives of dried *S. purpurea* bark and from polyamide chromatograms of many *Populus* species dried bark extractives in the past. Furthermore, fractions giving this R_f 0·55 brick-red spot, upon drying and seeding with salicortin crystals, deposited crystalline salicortin.

The new glucoside melting at 112–113° isolated from eluate fractions 46–58 was identified as an isomer of grandidentatin.[5] However, no grandidentatin was found. In a recent paper on the glucoside composition of *S. purpurea* bark, Thieme[2] reported the presence of a small amount of grandidentatin, identified only by paper chromatography. Since purpurein is an isomer of grandidentatin and behaves similarly to grandidentatin on paper chromatograms, it is probable that Thieme actually noted purpurein in his studies.

Polyamide chromatography with 20% ethanol yielded naringenin-5-β-D-glucoside in three optically active pure forms identified as the 5-β-D-glucosides of (+)-naringenin, (±)-naringenin, and (−)-naringenin (Table 2). Concentration of eluate fractions 104 and 105 deposited 0·36 g of the (+) form melting at 236–237°; fractions 106–113, 1·54 g of the (±) form melting at 225–227°; and fractions 114–127, 1·17 g of the (−) form melting at 159–160°.

TABLE 2. 5-β-D-GLUCOSIDES OF NARINGENIN

Naringenin form	Trivial name	Fractions	m.p.	Acetate m.p.	Acetate $[\alpha]_D^{25}$ (°)*
(+)		104–105	236–237°	133–134°	−49·2
(±)	Salipurposide[6]	106–113	225–227°	†	−48·8
(−)	Helichrysin A[8]	114–127	159–160°	189–191°	−48·9

* C = 2·0 in $CHCl_3$.

† Because the acetylation of the (±) form does not yield a crystalline acetate comprising equimolar amounts of both forms of the acetate, crystalline products possess m.p.s which are dependent upon the method of crystallization. M.p.s of from 105° to 127° have been obtained.

Acetates of all three forms were prepared, and their mass spectra as well as their i.r. spectra were essentially identical.

Charaux and Rabaté[6] and Zemplén, Bognár and Székely[7] isolated the (±) form, but neither of the others from *S. purpurea* bark. Hänsel and Heise[8] found both the (±) and (−) forms in fresh *S. purpurea* bark extract, but obtained the (+) form only by selective absorption on neutral aluminum oxide or by preparative paper chromatography of a (±) sample obtained from the blossoms of *Helichrysum arenarium*. Charaux and Rabaté[9] found isosalipurposide, the chalcone isomer of the (±) form in the old, but not in the young bark of *S. purpurea*, while Jarrett and Williams[10] reported isosalipurposide as the principal component of the young bark and found that, in old bark, this was accompanied by stereoisomeric forms of salipurposide as demonstrated by paper chromatography.

[5] I. A. PEARL and S. F. DARLING, in preparation.
[6] C. CHARAUX and J. RABATÉ, *Compt. rend.* **192**, 1478 (1931).
[7] G. ZEMPLÉN, R. BOGNÁR and I. SZÉKELY, *Ber.* **76**, 386 (1943).
[8] R. HÄNSEL and D. HEISE, *Archiv. Pharm.* **292**, 398 (1959).
[9] C. CHARAUX and J. RABATÉ, *Compt. rend.* **196**, 816 (1933).
[10] J. M. JARRETT and A. H. WILLIAMS, *Phytochem.* **6**, 1585 (1967).

Zemplén and co-workers[7] were the only previous investigators who prepared acetates of any of these naringenin glucoside isomers. These workers acetylated the (±) form to obtain an acetate of salipurposide melting at 183–184°, more than 50° higher than the m.p. of our acetate. They assigned the empirical formula $C_{38}H_{37}O_{16}$ and a molecular weight of 666·58 to their hexaacetyl salipurposide, but reported no analyses. These values are erroneous and the formula should be $C_{33}H_{34}O_{16}$ with a molecular weight of 686, values which agree with our analysis and mass spectral data. Although Zemplén and co-workers were unaware of the existence of the several stereoisomers of naringenin-5-β-D-glucoside, it is possible that they prepared their acetate from a crude sample that was largely the (–) form rather than the (±) form, and thus obtained an acetate which was predominantly the (–) form which melts at 189–191°. Hänsel and Heise[8] demonstrated that crystalline salipurposide was an equimolar compound of (–)-naringenin-β-D-glucoside and (+)-naringenin-β-D-glucoside. We have found that when crystalline salipurposide is acetylated with acetic anhydride in pyridine solution, the resulting acetate does not crystallize as a single compound, but as a mixture of two acetates which can be separated by fractional crystallization. Thus, it is also possible that Zemplén and co-workers actually recrystallized their product to yield a relatively pure (–)-naringenin-5-β-D-glucoside.

Although naringenin glucosides have been found in Salicaceae bark extracts in the past, no previous investigator has reported the finding of naringenin *per se*. Isoquercitrin and (+)-catechin, too, have been found in a great many species, but have never been reported heretofore in the Salicaceae.

The overall yield for Extract A of identified crystalline components in Table 1 amounted to about 30% of the eluate recovered from the polyamide column, and much less for Extract B. TLC of filtrates from the crystals noted in Table 1 in all instances indicated more of the specific compound together with one or more components, some in substantial amount. Further fractionations will be necessary to separate these components in order to obtain crystalline compounds for identification.

EXPERIMENTAL

Preliminary Processing of Bark

Four-year-old reeds of authentic *Salix purpurea* were cut in Outagamie County, Wisconsin, on 10 June 1968. The reeds were peeled and processed immediately. The thin bark was cut into small pieces, and 500-g batches were covered with 2·5 l. of 95% ethanol in a gallon-size stainless-steel Waring Blendor. The mixture was blended for 5 min, and the hot mixture was filtered with suction. The residual bark fiber was reprocessed twice more in the same manner with 2·5 l. each of 95% ethanol, and the total ethanol extract combined. In this manner 1800 g of fresh bark was processed, and the fresh bark contained 967 g of oven-dry solids. The combined ethanol extracts were concentrated under reduced pressure to dryness. The residue was heated with 3 l. of water, allowed to cool, and filtered through Celite. The residue was re-extracted with 3 l. of hot water in the same manner, and the combined clear aqueous filtrate was concentrated to approx 1l. The concentrated aqueous solution was extracted fractionally with ethyl acetate and processed as described previously for *Populus trichocarpa* bark.[4] One-half of the first ethyl acetate extract (Extract A) and one-third of the second ethyl acetate extract (Extract B) were applied to the polyamide column for elution chromatography. After collecting ninety fractions of aqueous eluate, the eluting solvent was changed to 20% ethanol, and after fraction 165, the eluting solvent was changed to 50% ethanol. A total of 216 fractions was collected.

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 and identity of i.r. spectra with authentic material indicated by reference: salicin,[11]

[11] I. A. PEARL and S. F. DARLING, *J. Org. Chem*, **24**, 731 (1959).

purpurein,[5] salireposide,[12] salicyloylsalicin,[13] (+)-catechin,* salicyoylsalicin-2-benzoate,[13] isoquercitrin,† and naringenin.‡

Isolation of salicortin. Evaporation of eluate fractions 4 through 10 yielded colorless syrups totalling 13·90 g with fractions 4 and 5 containing almost 10 g of this total. TLC[13] indicated that the major component of all these fractions was a compound giving a brick-red spot at R_f 0·55 along with traces of several other components. Authentic salicortin§ gave an identical spot.

1 g of polyamide fraction 4 was dissolved in $CHCl_3$–MeOH (4:1) and placed on top of a column of silica gel‖ (72 cm × 2·5 cm). The column was eluted with $CHCl_3$–MeOH (7:1) containing 0·5% acetic acid, and 25-ml fractions were collected. Fractions 7 through 17 contained pure salicortin as indicated by TLC. These were combined and evaporated to dryness to leave 0·60 g of colorless syrup which crystallized on standing. The crystals were covered with absolute ethanol, filtered, and washed with absolute ethanol to yield 0·39 g of salicortin melting at 135–137° undepressed on admixture with authentic material. Its i.r. spectrum was identical with that of the authentic compound. Polyamide fraction 5, containing 4·96 g of syrup, was dried by evaporation under reduced pressure, covered with absolute ethanol, and seeded. After standing a few days, the mixture was thick with crystalline precipitate. The precipitate was filtered and washed with absolute ethanol to yield 3·30 g of salicortin melting at 135–137°. Crystalline salicortin could regularly be obtained from syrupy fractions provided they were absolutely anhydrous and they were seeded.

Naringenin-5-β-D-glucosides. Concentration of fractions 104 through 127 gave crystalline materials as noted in Table 2. TLC on cellulose¶ with 15% acetic acid and detection with alcoholic KOH and u.v. light gave the following R_fs: (+), 0·61; (−), 0·55; and (I), 0·61 and 0·55. Similarly, paper chromatography on Whatman No. 1 with 30% methanol and location with alcoholic KOH gave R_f 0·62 for (+), 0·55 for (−), and both for (±). Acetates were prepared and recrystallized. (Found (+): C, 57·50, 57·25; H, 4·96, 5·04. (±): C, 57·42, 57·63; H, 5·16, 4·95. (−): C, 57·62, 57·57; H, 5·27, 5·16. Calc. for $C_{33}H_{34}O_{16}$: C, 57·72; H, 4·99.) The i.r. absorption spectra of all acetates are essentially similar, and that of (±)-naringenin-5-β-D-glucoside acetate contains bands at 2·92, 3·41, 5·68, 5·88, 6·20, 6·31, 6·61, 6·96, 7·30, 7·52, 8·25, 8·38, 8·58, 8·85, 9·36, 9·65, 10·20, 11·03, and 11·88 μ. Similarly, the mass spectra are practically identical and may be represented by that of the (±) acetate which contains the following major and important *m/e* peaks: 43 (100), 69 (3·6), metastable 70·5, 81 (6·1), 97 (7·1), 103 (4·3), 109 (56·5), 115 (3·2), 120 (5·7), 127 (16·8), 139 (5·7), 145 (5·7), 153 (8·9), 169 (76·1), 170 (7·5), 211 (2·5), metastable 222, 229 (2·9), 271 (7·1), 272 (3·9), 313 (1·4), 314 (3·2), 331 (21·4), 332 (3·2), 356 (1·4), and M+ 686. All high intensity and metastable peaks represent ions derived from the acetylated glucose moiety.[14]

Acknowledgements—All m.p.s are uncorrected. Analyses were performed by Micro-Tech Laboratories, Skokie, Illinois, U.S.A. Infrared absorption spectra were determined by Mr. Lowell Sell of The Institute of Paper Chemistry Analytical Group. Mass spectra were determined by Morgan-Schaffer Corp., Montreal, Quebec, Canada. The authors wish to think Mrs. Charlotte Robbins for her help in the processing and analytical monitoring required in this investigation.

* Obtained from S. B. Penick and Company, Jersey City, New Jersey, U.S.A.
† Kindly supplied by Dr. S. H. Wender, University of Oklahoma, Norman, Oklahoma, U.S.A.
‡ Obtained from K. and K. Laboratories, Jamaica, New York, U.S.A.
§ Kindly supplied by Dr. H. Thieme, Karl-Marx University, Leipzig, Germany.
‖ Silica Gel-Grade 950 (60–200 mesh) from Fisher Scientific Company, Fair Lawn, New Jersey, U.S.A.
¶ MN-POLYGRAM CEL-300 UV_{254} manufactured by Machery-Nagel & Co., Düren, Germany.

[12] I. A. PEARL and S. F. DARLING, *J. Org. Chem.* **24**, 1616 (1959).
[13] I. A. PEARL and S. F. DARLING, *Arch. Biochem. Biophys.* **102**, 33 (1963).
[14] I. A. PEARL and S. F. DARLING, *Phytochem.* **7**, 831 (1968).