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STUDIES ON THE BARKS OF THE FAMILY SALICACEAE—XVII.[1]

TRICHOSIDE, A NEW GLUCOSIDE FROM THE BARK OF *POPULUS TRICHOCARPA*

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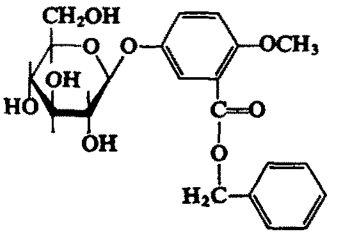
Abstract—The ethyl acetate-soluble portion of the hot-water extractives of the furrowed brown bark of northern black cottonwood (*Populus trichocarpa*) was fractionated by elution chromatography with water on a polyamide column. Trichocarpin and salireposide were the major phenolic glucosides found. In addition, a new glucoside, trichoside, previously found but not identified in the green bark of *P. trichocarpa*, was isolated in substantial quantity and identified by means of mass spectrometry and enzymatic hydrolysis as 3-carbobenzyloxy-4-methoxyphenyl-β-D-glucoside.

IN A recent study on the water extractives of the green bark of *Populus trichocarpa*,[2] we reported the isolation by polyamide chromatography of a new glucoside melting at 163–165°, giving a greenish black spot reminiscent of trichocarpin with sulfuric acid on a silica gel chromatoplate, and having an i.r. spectrum different from any known glucoside. At that time, the minute quantity obtained precluded complete characterization, but hydrolysis with β-glucosidase did demonstrate the presence of glucose as one of the hydrolysis products.

More recently we have investigated the hot-water extractives of the brown furrowed bark from the same *P. trichocarpa* trees used in the earlier study. The ethyl acetate-soluble portion of these extractives was submitted to elution chromatography with water on a polyamide column. In addition to large amounts of trichocarpin (2-carbobenzyloxy-4-hydroxyphenyl-β-D-glucoside) and salireposide, we isolated substantial amounts of the same 163–165° melting compound isolated in the earlier investigation. This new compound has been designated "trichoside", and its structure has been established as 3-carbobenzyloxy-4-methoxyphenyl-β-D-glucoside (I).



(I)

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[1] For paper XVI of this series, see I. A. PEARL and S. F. DARLING, *Phytochem.*, submitted for publication.

[2] T. K. ESTES and I. A. PEARL, *Tappi* 50, 318 (1967).

The structure (I) was established in the following manner. Trichoside was acetylated with acetic anhydride and pyridine, and the resulting acetate (II) was subjected to analysis and mass spectrometry. The mass spectrum of II is remarkably similar to that of trichocarpin acetate (III)[3] (see Fig. 1) suggesting a close relationship between them and that trichoside is also a derivative of a glucoside of the benzyl ether of gentisic acid. The principal difference between the two compounds is in the size of the molecular ion, that for trichoside acetate being 588 and that for trichocarpin acetate being 616. The difference of 28 corresponds to the difference between an acetyl and a methyl group ($CH_3CO—CH_3$) and indicates that one of the hydroxyl groups of trichoside is methylated and, accordingly, cannot be acetylated. The presence of a methoxyl in I was confirmed by analysis.

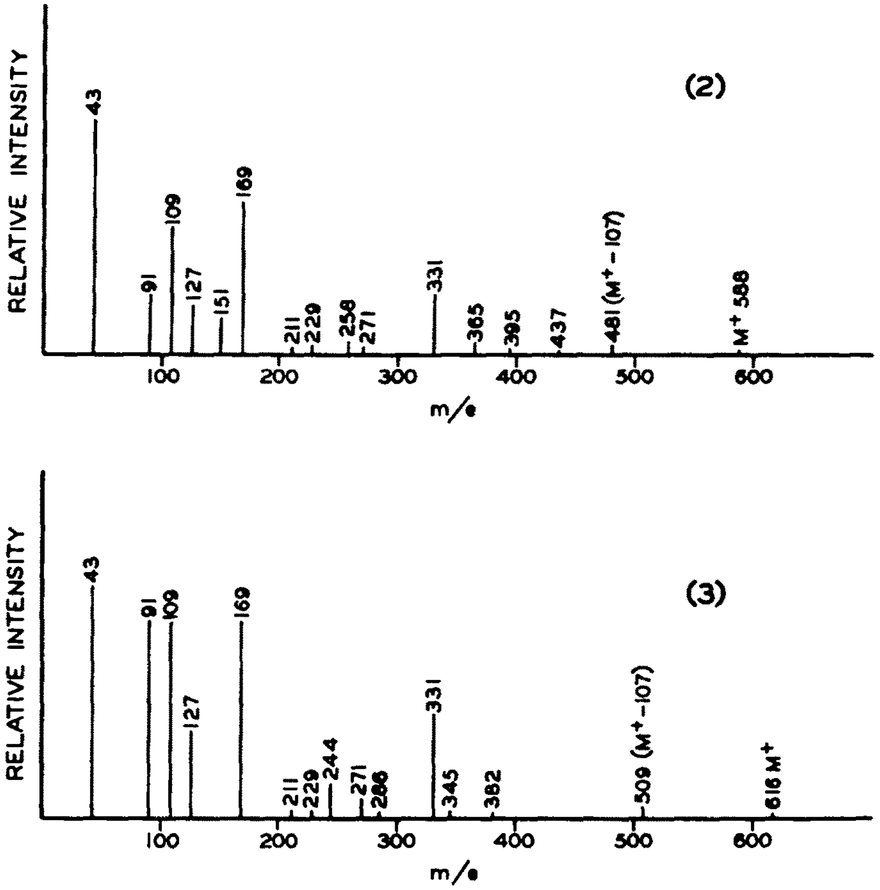

FIG. 1. MASS SPECTRA OF TRICHOSIDE TETRAACETATE (II) AND TRICHOCARPIN PENTAACETATE (III).

Both mass spectra (Fig. 1) show an M-107 peak attributable to the loss of a benzyloxy ($C_6H_5CH_2O$) group. In addition, they both show a prominent peak at *m/e* 331 due to the familiar tetraacetylglucose oxonium ion (IV) reported originally by Biemann and co-workers[4] for the primary fragmentation of glucose pentaacetate and found in our earlier

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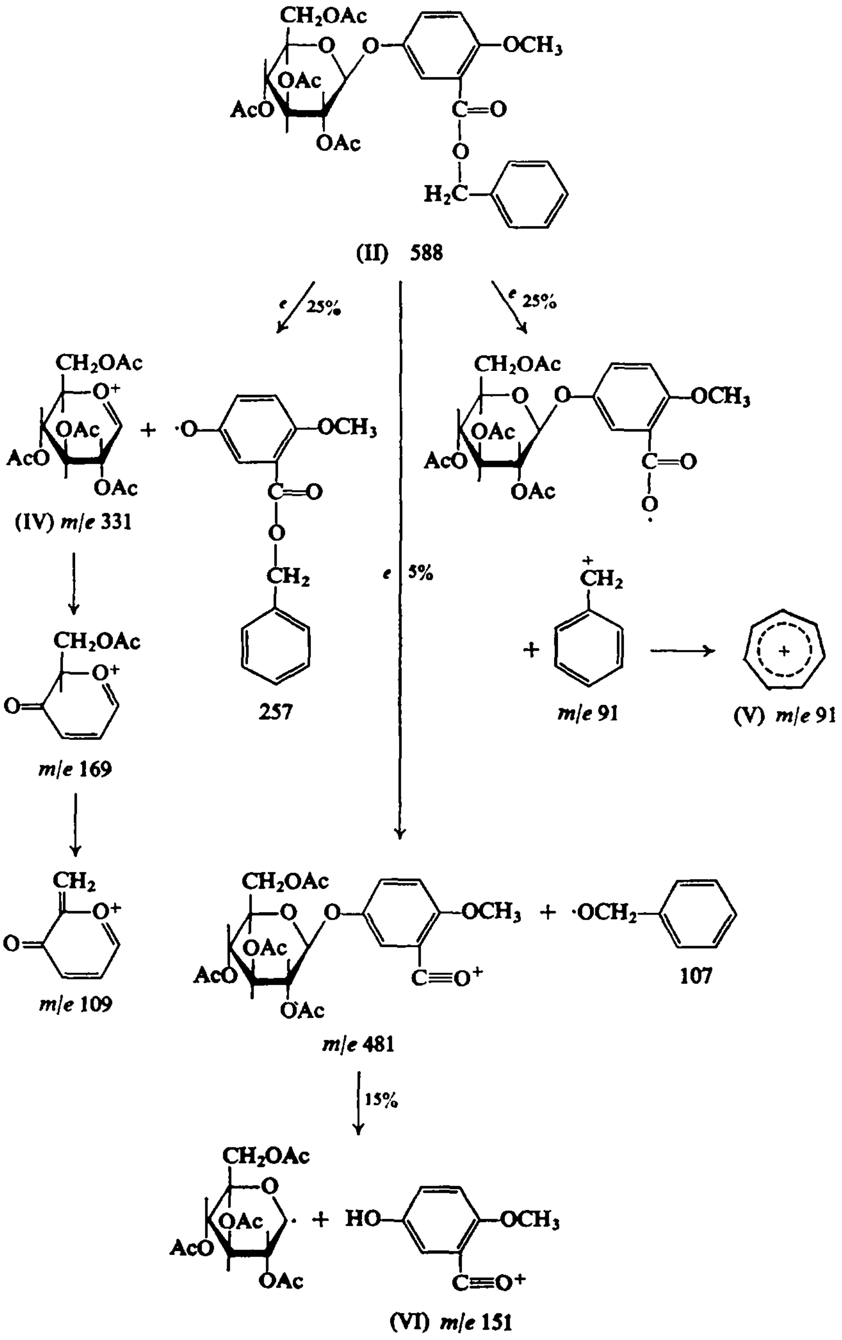
[3] I. A. PEARL and S. F. DARLING, *Phytochem.*, submitted for publication.

[4] K. BIEMANN, D. C. DEJONGH and H. K. SCHNOES, *J. Am. Chem. Soc.* **85**, 1763 (1963).

studies to be present in the fragmentation pattern of the acetates of all glucosides without substitution in the glucose moiety.[3] Since none of the glucose hydroxyls of trichoside is substituted, the free hydroxyl of the gentisic acid moiety must be methylated. This conclusion



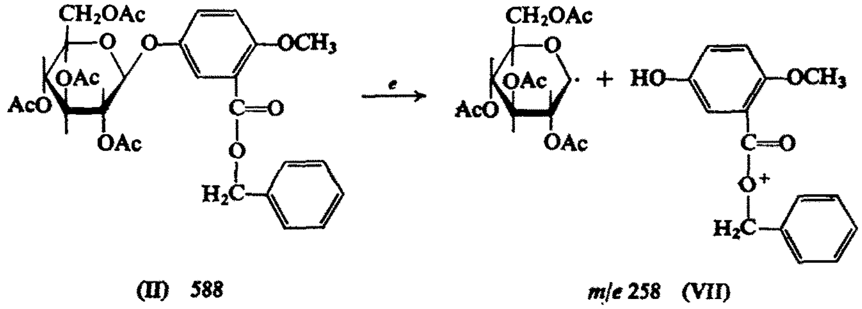
SCHEME I.

is confirmed by the fact that trichocarpin is readily soluble in aqueous alkali and forms a colored derivative with diazotized *p*-nitroaniline whereas trichoside is insoluble in aqueous alkali and gives no color with the diazo reagent.

Trichoside could not be the methyl ether of trichocarpin because hydrolysis with dilute hydrochloric acid yielded a substance which gave a bright blue spot on a paper chromatogram with diazotized *p*-nitroaniline, whereas hydrolysis of the methyl ether of trichocarpin would yield 2-hydroxy-5-methoxybenzoic acid, a compound which gives a tan-colored spot with diazotized *p*-nitroaniline. The structure of trichoside was determined by hydrolysis with β-glucosidase followed by saponification of the resulting benzyl ester of the methylated gentisic acid. Saponification yielded 5-hydroxy-2-methoxybenzoic acid, proving that trichoside is an isomer of the methyl ether of trichocarpin and has structure I.

The major fragmentation pattern of trichoside tetraacetate (II) is pictured in Scheme I which accounts for the important peaks in the mass spectrum. The several minor peaks (Fig. 1) are associated with the fragmentation of the glucose acetate moiety and are discussed in an earlier paper.[3]

The peak in the trichoside tetraacetate spectrum at *m/e* 258 corresponding with the 3-carbobenzyloxy-4-methoxyphenol ion VII indicates that primary fragmentation also takes place to a lesser extent in accordance with Scheme II in a manner previously noted for the two nonacetylated glycosides, vimalin (1-*O*-*p*-methoxycinnamyl-β-D-glucose) and picein (1-*O*-*p*-acetylphenyl-β-D-glucose).[3] The ion VII can also fragment further to yield ion V with *m/e* 91 and ion VI with *m/e* 151 noted in Scheme I. It is interesting to note that the spectrum in Fig. 1 for trichocarpin acetate also shows analogous peaks not discussed in the earlier communication.[3] The peak at *m/e* 286 corresponds with the 3-carbobenzyloxy-4-acetoxyphenol ion, and that at *m/e* 244 agrees with the latter ion less ketene (42).



SCHEME II.

EXPERIMENTAL[5]

Preparation of Trichoside

The mature, furrowed, brown bark of the same *Populus trichocarpa* tree employed in the earlier green-bark study[2] was dried when received, reduced to dust in a Wiley mill, air-dried, and stored in polyethylene bags until used. A sample of air-dried bark dust containing 1500 g of oven-dry solids was extracted with hot water and concentrated under reduced pressure using our modified procedure.[6,7] The concentrated solution (1 l.)

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[5] All m.p.s are uncorrected. Analyses were performed by Micro-Tech Laboratories, Skokie, Illinois. I. 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.

[6] I. A. PEARL, S. F. DARLING, H. DEHAAS, B. A. LOVING, D. A. SCOTT, R. H. TURLEY and R. E. WERTH, *Tappi* **44**, 475 (1961).

[7] I. A. PEARL and S. F. DARLING, *Tappi* **50**, 324 (1967).

was extracted for 12 hr with ethyl acetate in a nonagitated liquid–liquid extractor. Evaporation of the ethyl acetate under reduced pressure yielded 40 g of amber-colored syrup.

The entire extract was dissolved in tetrahydrofuran, absorbed on polyamide powder,[8] placed on a column (50 mm × 80 cm) of polyamide and developed with water, collecting 210-ml fractions.[7] 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. The composition of each fraction was monitored by thin-layer chromatography on silica gel plates, developed in 4:1 chloroform–methanol, sprayed with 50 per cent H_2SO_4 and heated at 105°.[9]

Salicin, salicyl alcohol, and pyrocatechol were found in early fractions along with other unidentified materials in small quantity. Fractions 22–29 deposited 75 mg of colorless crystals which were recrystallized from dil. EtOH and dried to yield crystals of trichoside containing ½ molecule of water and melting at 163–165°. Its i.r. spectrum was identical with that of the compound isolated earlier from the green bark of *P. trichocarpa*[2] and contained bands at 3·00, 3·48, 5·81, 6·21, 6·31, 6·68, 6·88, 7·05, 7·30, 7·67, 7·83, 8·06, 8·30, 8·50, 9·35, 9·57, 9·80, 10·89, 11·23, 12·23, 12·77, 13·16, 13·35, 13·60, and 14·40 microns. (Found: C, 58·67, 58·65; H, 5·90, 5·90; CH_3O, 7·30. $C_{21}H_{24}O_9.\frac{1}{2}H_2O$ required: C, 58·74; H, 5·87; CH_3O, 7·23.)

Trichoside Tetraacetate

Trichoside (63 mg) was acetylated with 2 ml of acetic anhydride and 3 ml of pyridine to give a colorless syrup, which crystallized from EtOH as colorless glistening platelets of the *tetraacetate*, m.p. 116–118°. The i.r. spectrum contained bands at 2·96, 3·41, 5·71, 6·21, 6·27, 6·66, 6·84, 7·04, 7·27, 7·66, 7·87, 8·07, 8·21, 8·72, 8·98, 9·30, 9·59, 10·00, 10·16, 10·43, 10·80, 11·03, 11·21, 11·65, 12·11, 12·74, 13·13, 13·40, 14·31, and 15·03 microns. (Found: C, 59·24, 59·24; H, 5·51, 5·66; mol.wt. by mass spectrometry, 588. $C_{29}H_{32}O_{13}$ required: C, 59·15; H, 5·44; mol.wt., 588.)

Enzymatic Hydrolysis of Trichoside and Isolation of 5-Hydroxy-2-methoxybenzoic Acid

A suspension of 51 mg of trichoside in 25 ml of 0·01 M sodium acetate solution brought to pH 5·0 with acetic acid was heated to effect a clear solution and allowed to cool to 40°. β-Glucosidase (25 mg) was dissolved in the clear solution which was then allowed to stand 1 hr at 40°. During this time, the solution became progressively turbid, and a curdy precipitate separated. The mixture was extracted twice with ether, and the ether was washed twice with bicarbonate and dried. The ether was removed to leave 28 mg (93 per cent) of colorless syrup (benzyl 5-hydroxy-2-methoxybenzoate). The syrup was dissolved in 2 ml EtOH, treated with several drops of 4 per cent NaOH, and heated on the steam bath for 15 min. The reaction mixture was diluted with water, and the clear solution was distilled to remove ethanol. The resulting aqueous solution was acidified with dil. HCl and extracted with ether. Evaporation of the ether yielded 5-hydroxy-2-methoxybenzoic acid, melting at 152–153° and not depressing the m.p. when mixed with authentic material.[10] The i.r. spectra of natural and authentic 5-hydroxy-2-methoxybenzoic acid were identical and contained bands at 2·94, 3·45, 4·95, 5·98, 6·15, 6·30, 6·68, 6·92, 6·97, 7·14, 7·49, 7·63, 7·76, 7·94, 8·20, 8·29, 8·65, 9·27, 9·85, 10·54, 11·05, 11·24, 12·20, 12·65, 12·93, 13·31, 14·77, and 15·03 microns.

Isolation of Trichocarpin and Salireposide

Fractions 30–35 gave no crystals upon concentration and thin-layer chromatography indicated a mixture of components. Fractions 36–40, upon concentration, yielded 45 mg of trichocarpin, m.p. 135–136°, which was identified by mixed m.p. and identity of i.r. spectra with authentic trichocarpin.[11] Fractions 41–60 gave crystalline precipitates upon concentration, but thin-layer chromatography indicated that the crystals were mixtures of trichocarpin and salireposide, with gradually increasing proportions of salireposide.

Mass Spectra

Mass spectra were made on a double-focusing Hitachi RMU-6D instrument by direct introduction of the sample with a probe in the ionizing beam.

Infrared Spectra

I.r. spectra were obtained with a Perkin-Elmer model 21 recording spectrophotometer using a sodium chloride prism and potassium bromide pellets prepared by hand-grinding with sample before pressing.

Acknowledgement—The authors wish to thank Mrs. Charlotte Robbins for her help in the processing and analytical monitoring required in this study.

[8] Polyamide Woelm, manufactured by M. Woelm, Eschwege, Germany.

[9] I. A. Pearl and S. F. Darling, *Arch. Biochem. Biophys.* **102**, 33 (1963).

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

[11] I. A. Pearl and C. R. Pottenger, *Tappi* **49**, 152 (1966).