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Studies on the Barks of the Family *Salicaceae*. I. Tremuloidin, a New Glucoside from the Bark of *Populus tremuloides*[1]

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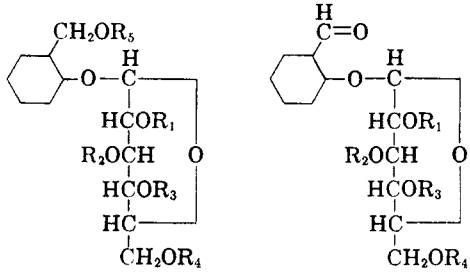
A new glucoside has been isolated from the bark of *Populus tremuloides*. This glucoside, which we have named tremuloidin, is a monobenzoate of salicin and an isomer of populin. Tremuloidin was completely methylated to tetramethyltremuloidin which, in turn, was debenzoylated to a tetramethylsalicin yielding 3,4,6-tri-*O*-methyl-D-glucopyranoside on acid hydrolysis. Thus, tremuloidin was identified as 2-benzoylsalicin. Tremuloidin was oxidized with dilute nitric acid to 2-benzoylhelicin. All products and intermediates were characterized by means of infrared absorption spectra.

In 1830 the glucoside salicin was discovered in the bark of *Salix helix* by Leroux,[2] and in the same year Braconnot[3] isolated salicin from the bark of the European quaking aspen, *Populus tremula*, along with a new substance which he named "populin." Although the structural formulas for these compounds remained unknown for a long time, Piria[4] demonstrated that salicin could be hydrolyzed with dilute acid or enzymatically to yield salicyl alcohol and glucose and that the phenolic hydroxyl in salicyl alcohol was involved in the glucosidic linkage because salicin could be oxidized to helicin, the glucoside of salicylaldehyde. Piria even showed the relationship between salicin and populin by demonstrating that populin yielded salicin and benzoic acid when saponified with barium hydroxide solution. Somewhat later Schiff[5] showed that the benzoyl group in populin must be attached to the glucose and not the salicyl alcohol moiety because populin could be oxidized to benzoylhelicin, a compound he prepared by benzoylating helicin. In addition, Schiff benzoylated salicin by several methods and obtained a synthetic populin which he compared with a sample of natural populin from Piria's laboratory. Identity was established by such properties as taste, solubility, and color with concentrated sulfuric acid, properties now known to be exhibited by similarly related and constituted substances. Dobbin and White[6] improved Schiff's benzoylation technique for preparing synthetic populin from salicin and noted that their synthetic compound had the same melting point (180°) as a purified natural material. The uncertainty of absolute identity was continued by Dobbin and White who wrote: "The purified natural product behaved in every other respect exactly as our synthetic sample did." In 1906, Irvine and Rose[7] completely methylated salicin and hydrolyzed the resulting pentamethylsalicin to 2,3,4,6-tetramethylglucose. This finding together with the fact that salicin is hydrolyzed by emulsin proves salicin to be *o*-hydroxymethylphenyl-*O*-β-D-glucopyranoside (I). Many years later Richtmyer and Yeakel[8] methylated synthetic populin with methyl iodide and silver oxide. The resulting tetramethylpopulin was debenzoylated to a tetramethylsalicin, which on hydrolysis with hydrochloric acid yielded 2,3,4-tri-*O*-methyl-D-glucose. Thus, the structure of synthetic populin was proved to be 6-benzoylsalicin (II), and the intermediate tetramethylpopulin and tetramethylsalicin had the structures III and IV, re-

(1) Presented before the Division of Cellulose Chemistry at the 135th meeting of the American Chemical Society, Boston, Mass., April 5–10, 1959.
(2) Leroux, *Ann. Chim. Phys.*, [2] **43**, 440 (1830).
(3) H. Braconnot, *Ann. Chim. Phys.*, [2] **44**, 296 (1830).
(4) R. Piria, *Ann.*, **56**, 35 (1845); **96**, 375 (1855).
(5) H. Schiff, *Ann.*, **154**, 1 (1870).
(6) L. Dobbin and A. D. White, *Pharm. J.*, [4] **19**, 233 (1904).
(7) J. C. Irvine and R. E. Rose, *J. Chem. Soc.*, **89**, 814 (1906).
(8) N. K. Richtmyer and E. H. Yeakel, *J. Am. Chem. Soc.*, **56**, 2495 (1934).

spectively. Richtmyer and Yeakel reported for synthetic populin $[\alpha]_D$ − 2.0° (c = 5 in pyridine). It is interesting to note that as early as 1852 Biot and Pasteur[9] recorded for natural populin $[\alpha]_D$ − 20.75° (c = 1 in absolute ethanol), while much later Bridel[10] reported $[\alpha]_D$ − 24.73° (in 60% acetone). However, none of the investigators of synthetic populin ever compared rotations of synthetic and natural populins.

During the past century the presence of salicin and populin has been reported in the barks of a number of species of *Populus*,[11] but unfortunately, the presence of these glucosides was usually demonstrated by some indirect physical, chemical, or biochemical procedure such as increase in glucose concentration upon hydrolysis, oxidation, and determination of salicylic acid, or calculation of enzymolytic indices. Some of these procedures would give valid results when applied to solutions of glucose and only the two pure glucosides, but results obtained on aqueous extracts of *Populus* barks might be very misleading when interpreted in the light of our present knowledge of possible components of *Populus* bark extracts.



I. $R_1 = R_2 = R_3 = R_4 = R_5 = H$
II. $R_1 = R_2 = R_3 = R_5 = H$; $R_4 = C_6H_5CO$
III. $R_1 = R_2 = R_3 = R_5 = CH_3$; $R_4 = C_6H_5CO$
IV. $R_1 = R_2 = R_3 = R_5 = CH_3$; $R_4 = H$
V. $R_1 = R_2 = R_3 = R_4 = H$; $R_5 = C_6H_5CO$
VI. $R_1 = C_6H_5CO$; $R_2 = R_3 = R_4 = R_5 = H$
VII. $R_1 = R_3 = R_4 = R_5 = H$; $R_2 = C_6H_5CO$
VIII. $R_1 = R_2 = R_4 = R_5 = H$; $R_3 = C_6H_5CO$
IX. $R_1 = H$; $R_2 = R_3 = R_4 = R_5 = CH_3$
XII. $R_1 = C_6H_5CO$; $R_2 = R_3 = R_4 = R_5 = CH_3CO$
XIII. $R_1 = C_6H_5CO$; $R_2 = R_3 = R_4 = R_5 = CH_3$
X. $R_1 = R_2 = R_3 = H$; $R_4 = C_6H_5CO$
XI. $R_1 = C_6H_5CO$; $R_2 = R_3 = R_4 = H$

In the course of our investigations on *Populus tremuloides*, American quaking aspen, some fresh bark obtained in late May was extracted with 95% ethanol, and the ethanol extract was concentrated to approximately 25% solids. The fresh concentrated extract was evaporated to dryness and extracted with water at 25°. The extract was purified by treating with basic lead acetate, filtering, and removing the lead with hydrogen sulfide. Upon partial concentration, the clear filtrate deposited colorless needles melting at 201–202°. The yield amounted to 0.37% based on the original oven-dried *P. tremuloides* bark. Further concentration of the filtrate yielded crystals of salicin melting at 193–194° and very different from the first crystals in water solubility and taste.

Recrystallization of the 201–202° melting material from water and then from methanol raised the melting point to 207–208°. The specific rotation in pyridine $[\alpha]_D^{25}$ + 17.1° (c = 3.1) increased slightly on standing 72 hr. to $[\alpha]_D^{25}$ + 19.5°. The rotation in 80% acetone $[\alpha]_D^{25}$ − 12.3° (c = 1.5) remained unchanged on standing. Hydrolysis with alkali at room temperature yielded benzoic acid and salicin, and analysis of the pure compound and of its acetate indicated it to be a monobenzoate of salicin. Thus, the new compound, which we have named "tremuloidin," is an isomer of synthetic populin.

In order to obviate the remote possibility that natural populin and synthetic populin are not identical and that the populin isolated by investigators more than one hundred years ago without recording the melting point might actually be identical with our tremuloidin, the work of the early investigators was repeated. Natural populin was isolated from the bark of *P. tremula* according to Braconnot[3] and from the leaves of *P. alba* according to Herberger.[12] The isolated populin was compared with synthetic populin[8] by means of optical activity, mixed melting point, and infrared absorption spectra, and the two were found to be identical. Therefore, "tremuloidin" was a new monobenzoate of salicin with benzoyl substitution at some position other than the 6-position on the glucose, which is the known benzoyl substitution of populin.[8]

Controlled periodate oxidation[13] of tremuloidin developed no acidity and consumed one mole of periodate, indicating a compound containing only two adjacent hydroxyl groups. Of the possible monobenzoates of salicin other than 6-glucose substitution (V–VIII) only VI and VIII with substitution at positions 2 and 4, respectively, would satisfy these criteria. The anomalous dextrorotation of the tetraacetate of tremuloidin, $[\alpha]_D^{24}$ + 33.9° (c = 2.5 in chloroform); suggested the structure VI because the data of Pigman[14] indicated that acetates of several glucosides substituted in the 2-glucose position demonstrated this anomalous dextrorotation. For determining the exact location of benzoyl substitution in tremuloidin, the general procedure of Richtmyer and Yeakel[8] was employed.

Tremuloidin was methylated completely with methyl iodide and silver oxide to yield tetramethyltremuloidin as a sirup which failed to crystallize,

(9) J. B. Biot and L. Pasteur, *Compt. rend.*, **34**, 606 (1852).

(10) M. Bridel, *J. Pharm. Chim.*, [7] **20**, 14 (1919).

(11) For a complete bibliography see W. Thies and C. Wehmer, in G. Klein, Handbuch der Pflanzenanalyze, Bd. III, 2 Teil, 845, Vienna, 1932. See also A. Kuhn and G. Schäfer, *Pharm. Ztg.*, **82**, 949 (1937).

(12) J. E. Herberger, *Buchners Report Pharm.*, **51**, 266 (1835).

(13) J. R. Dyer in D. Glick, Methods of Biochemical Analysis, Vol. 3, pp. 123, Interscience, New York, 1946.

(14) W. W. Pigman, *J. Research Natl. Bur. Standards*, **33**, 129, 144 (1944).

but whose purity was demonstrated by paper chromatography and by the fact that paper chromatography of its acid hydrolyzate indicated only one sugar spot, that for a trimethylglucose. The oily tetramethyltremuloidin was debenzoylated by means of sodium methylate in methanol to yield the corresponding tetramethylsalicin as a crystalline compound. The tetramethylsalicin was hydrolyzed by boiling with hydrochloric acid in aqueous methanol to yield 3,4,6-tri-*O*-methyl-D-glucopyranoside which was identified by mixed melting point and by identity of infrared absorption spectra with authentic material.[15] Thus, the structure of the tetramethylsalicin must be ω,3,4,6-tetramethylsalicin (IX) and that of tremuloidin, 2-benzoylsalicin (VI).

The locating of the benzoyl group in populin by Richtmyer and Yeakel[8] establishes the structure of the benzohelicin obtained by Piria[4] and by Schiff[5] by dilute nitric acid oxidation of populin as 6-benzoylhelicin (X). Similar oxidation of tremuloidin gave 2-benzoylhelicin (XI).

Because populin had been reported in the bark of *P. tremuloides* by earlier investigators,[11,16] on the basis of indirect evidence which may have been misleading, it was desired to determine whether this were true or whether tremuloidin had been responsible for earlier reports. Accordingly, the original ethanol extractives left after water extraction at 25° were reextracted with boiling water, and the hot water extract was processed as before. Partial concentration yielded an additional 0.15% tremuloidin as relatively pure material melting at 200–202°. Further concentration yielded 0.26% of crystals melting between 178 and 186° which proved to be a mixture of tremuloidin and populin. Further concentration yielded salicin and other glucosidic material to be described in future papers. Tremuloidin, populin, and salicin were easily recognized separately or in a mixture of all three when paper chromatograms were developed in either 10:3:3 butanol-pyridine-water or 9:2:2 ethyl acetate–acetic acid–water and spots located by means of a modification of Trevelyan's[17] silver spray. In this modification, Trevelyan's procedure for removing unreduced silver oxide with 6*N* ammonium hydroxide is replaced with a concentrated sodium thiosulfate wash resulting in chromatograms with glycoside spots appearing as black spots against a white background instead of brown spots against a light brown background.

The mixture of populin and tremuloidin obtained above was submitted to column chromatography by adsorption on a dry packed column of powdered cellulose and elution with the ethyl acetate–acetic acid–water developer noted above. Collection of the

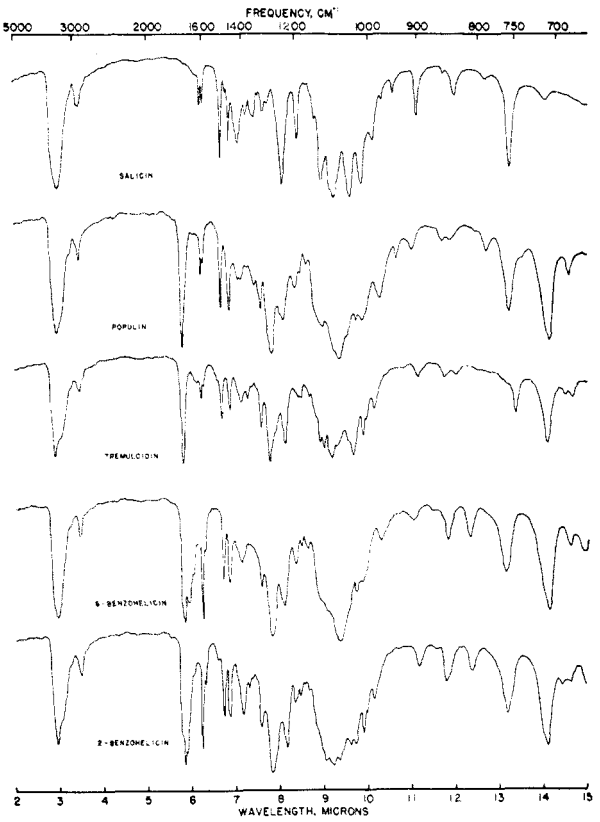


Fig. 1. Infrared absorption curves: salicin, populin, tremuloidin, 6-benzohelicin, 2-benzohelicin

eluate in fractions gave complete separation of populin and tremuloidin, and both glucosides were recrystallized and identified by mixed melting point and by identity of infrared absorption spectra (Fig. 1) and optical activity with authentic samples.

EXPERIMENTAL[18]

Isolation of tremuloidin (VI). An amount of 15 kg. (oven-dry basis) of fresh whole bark peeled from a 35-year-old quaking aspen (*Populus tremuloides*) in late May was broken into strips, placed in three 5-gal. jars, and covered with 95% ethanol. After standing at room temperature one week, the ethanolic extract was decanted, and the bark was covered with fresh ethanol. After another week, the solvent was changed again. The process was repeated once more, and the combined ethanolic extracts were filtered and concentrated under reduced pressure in a circulating evaporator to 8395 g. containing 2240 g. of solids. After a few days, a well mixed sample containing 291 g. of solids was evaporated to dryness below 25° in a rotating evaporator. The residue was stirred with 3 l. of water at 25° and allowed to stand overnight. The aqueous extract was decanted and filtered through a Celite pad. The slightly turbid yellow solution was treated with stirring with an excess of a slurry of basic lead acetate, and the resulting precipitate was filtered. The filtrate was saturated with hydrogen sulfide and filtered to yield a colorless clear solution. This was concentrated under reduced pressure to approximately 1500 ml. volume and cooled. The crystals which separated were fil-

(15) Kindly supplied by Dr. N. K. Richtmyer, National Institutes of Health, Bethesda, Md.

(16) R. L. Hossfeld and F. H. Kaufert, *Forest Products J.*, **7**, 437 (1957).

(17) W. E. Trevelyan, D. P. Proctor and J. S. Harrison, *Nature*, **166**, 444 (1950).

(18) All melting points are uncorrected. Analyses were performed by Huffman Microanalytical Laboratories, Wheatridge, Col.

tered and washed with water to yield 7.0 g. of crude tremuloidin melting at 201–202°. Stepwise concentration of the filtrate and washings yielded crude salicin as bitter crystals melting at 193–194°. The yield in three batches amounted to 12 g., but more precipitated in the filtrate after concentration to a sirup and standing.

The 201–202° crystals were recrystallized from water and then from methanol to give colorless needles melting at 207–208° and with specific rotation in pyridine $[\alpha]_D^{25}$ +17.1° (c = 3.1) which increased slightly on standing 72 hr. to $[\alpha]_D^{25}$ +19.5°. The rotation in 80% acetone $[\alpha]_D^{25}$ −12.3° (c = 1.5) remained unchanged on standing.

Anal. Calcd. for $C_{20}H_{22}O_8$: C, 61.53; H, 5.68. Found: C, 61.44; H, 5.66.

Acetylation of tremuloidin with acetic anhydride and pyridine and recrystallization from ethanol yielded crystals of tremuloidin tetraacetate (XII) melting at 114–115°, $[\alpha]_D^{24}$ +33.9° (c = 2.5 in chloroform).

Anal. Calcd. for $C_{28}H_{30}O_{12}$: C, 60.21; H, 5.41. Found: C, 60.25; H, 5.36.

Alkaline hydrolysis of tremuloidin. One g. of tremuloidin was covered with 150 ml. of 1% sodium hydroxide solution and allowed to stand at 25° overnight. The clear yellow solution was exactly neutralized with dilute sulfuric acid and concentrated to half volume in a rotating evaporator. The shiny crystals which separated were filtered, washed with water, and recrystallized from ethanol to give colorless platelets which melted at 119–120° and did not lower a mixed melting point with authentic benzoic acid. The aqueous filtrate was concentrated further, and the crystalline precipitate was filtered and recrystallized from ethanol to give white crystals which melted at 190–191° and did not depress a mixed melting point with authentic salicin.

Populin (II) *from salicin* (I). Salicin was benzoylated with benzoyl chloride and potassium hydroxide solution according to Richtmyer and Yeakel[8] and the product was recrystallized first from water and then from ethanol to give colorless needles of synthetic populin melting at 178–179°, $[\alpha]_D^{24}$ −2.0° (c = 5 in pyridine); $[\alpha]_D^{25}$ −29.7° (c = 5 in 80% acetone).

Populin (II) *from Populus alba leaves.* A batch of fresh leaves from an authentic *P. alba* obtained in June was extracted with hot water according to Herberger,[12] and the hot water extract was purified by means of basic lead acetate. The purified solution was freed from lead by means of hydrogen sulfide and partially concentrated. Some ash-containing crystals which separated were filtered, and the clear filtrate was evaporated further to yield crystals. These were recrystallized from water in the presence of decolorizing carbon to give colorless needles of natural populin which melted at 179–180° and did not depress a mixed melting point with synthetic populin prepared above. Infrared absorption spectra of the two populins were superimposable. The specific rotation of natural populin in both pyridine and 80% acetone was identical with that of synthetic populin.

Natural populin was also isolated from the fresh bark of *P. tremula* in accordance with Braconnot[3] by essentially the same procedure.

Periodate oxidation of tremuloidin. The general procedure outlined by Dyer[13] was modified to some extent. Approximately 50 mg. of tremuloidin was dissolved in 40 ml. of 50% ethanol with warming and then treated with 25 or 50 ml. of 0.01M sodium metaperiodate. The solution was diluted with water to 100 ml. and maintained at 4° in "actinic red" flasks. Aliquots of 5 ml. each were taken at appropriate times for analysis. To each 5-ml. aliquot was added 10 ml. of saturated aqueous sodium bicarbonate, 5 ml. of 0.01M sodium arsenite and 1 ml. of 1% potassium iodide in saturated sodium bicarbonate solution. After 15 min., the remaining arsenite was titrated with iodine to a starch end point. Data for tremuloidin indicated 1 mole periodate consumed per mole of glucoside.

Acidity developed was determined by a modification of the method of Abdel-Akher and Smith[19] in which the aliquot is allowed to stand 60 min. after addition of 10% ethylene glycol to ensure complete destruction of excess periodate before addition of potassium iodide solution. Data indicated no developed acidity with tremuloidin.

Similar oxidations on salicin in water at 25° consumed two moles of oxidant and developed one mole of acid as expected. Oxidations on populin showed overoxidation at 25°, while at 4°, populin was insoluble in water or in the dilute ethanol solutions employed.

Methylation of tremuloidin. In a small flask fitted with a reflux condenser and silicone-sealed stirrer was placed a mixture of 1.0 g. of tremuloidin, 10 ml. of methyl iodide, and 15 ml. absolute methanol. With stirring and boiling under reflux, 6.0 g. of freshly prepared silver oxide was added over a period of 3 hr. in 1-g. lots. After the second addition, 5 ml. of acetone were added to completely dissolve all tremuloidin. The mixture was allowed to stand overnight at room temperature and filtered. The silver oxide was washed thoroughly with acetone, and the combined filtrate and washings were evaporated to dryness in a rotating evaporator. The colorless sirup was dissolved in 10 ml. of methyl iodide and a few drops of methanol and methylated as before. The process was repeated three times making a total of four methylations. After the third methylation, the product was completely soluble in methyl iodide without the addition of methanol. The final product tetramethyltremuloidin (XIII), was obtained as 1.05 g. of clear colorless viscous oil having a specific rotation $[\alpha]_D^{25}$ +6.56° (c = 4.2 in chloroform). All attempts at crystallization failed. Complete methylation was demonstrated by the fact that, upon hydrolysis with hydrochloric acid, paper chromatograms of the hydrolyzate showed only one sugar spot, that for a trimethylglucose. Paper chromatograms were developed with 9:2:2 ethyl acetate–acetic acid–water, and spots were located by means of the *p*-anisidine spray.[20]

Debenzoylation of tetramethyltremuloidin to ω,3,4,6-tetramethylsalicin (IX). A solution of 1.05 g. of tetramethyltremuloidin in 20 ml. of anhydrous methanol was treated with a solution of 0.1 g. of metallic sodium in 10 ml. of anhydrous methanol, and the mixture was boiled under reflux for 10 min., diluted with 30 ml. of water, and partially evaporated in a rotating evaporator to remove all methanol. The turbid aqueous solution was extracted with ether, and the ether was washed with water, dried with sodium sulfate, and evaporated in a rotating evaporator to yield a colorless oil which solidified upon standing. The crystals were dissolved in a little anhydrous ether and filtered. The clear filtrate was diluted with petroleum ether (b.r. 30–60°) and placed in the freezer. The colorless needles which separated were filtered and recrystallized again in the same manner to yield 0.31 g. of IX melting at 85–86°, $[\alpha]_D^{25}$ −39.1° (c = 1.2 in chloroform).

Anal. Calcd. for $C_{24}H_{26}O_7$: C, 59.63; H, 7.65; CH_3O, 36.3. Found: C, 59.64; H, 7.68; CH_3O, 36.1.

The aqueous layer remaining after the ether extraction was acidified with dilute hydrochloric acid and allowed to stand. The crystals which separated were filtered and recrystallized from water to give shiny platelets of benzoic acid which melted at 119–120° and did not depress the melting point of a mixture with authentic benzoic acid.

Hydrolysis of ω,3,4,6-tetramethylsalicin. A mixture of 0.35 g. of ω,3,4,6-tetramethylsalicin, 4 ml. of methanol, and 6 ml. of 2N hydrochloric acid was heated on the steam bath under reflux for 2 hr. The mixture became turbid after 1 hr. and had deposited a reddish gum after 2 hr. Methanol was removed under reduced pressure, and the residual aqueous mixture was filtered with the aid of a little Celite. The clear

(19) M. A. Abdel-Akher and F. Smith, *J. Am. Chem. Soc.*, **73**, 996 (1951).

(20) L. Hough, J. K. N. Jones, and W. H. Wadman, *J. Chem. Soc.*, **1950**, 1702.

filtrate was treated with excess IR-4B ion-exchange resin in the acetate form and filtered. The resin was washed thoroughly with water, and the combined filtrate and washings were evaporated to dryness under reduced pressure in the rotating evaporator to leave a slightly yellow syrup. Paper chromatography in the ethyl acetate–acetic acid–water developer and spraying with *p*-anisidine indicated only a trimethylglucose and some phenolic aglucone material with good separation. The entire yellow sirup was dissolved in 2.5 ml. of methanol and streaked on four eight-inch wide Whatman 3M papers, previously washed with methanol. The papers were developed in the ethyl acetate–acetic acid–water developer. One-fourth inch strips were cut from each paper for monitoring, and the located bands of trimethylglucose were cut from the papers. These bands were eluted with methanol in a Soxhlet apparatus, and the methanol eluate was evaporated in a rotating evaporator to yield a colorless sirup. After standing for two weeks, the partially crystalline sirup was covered with a few milliliters of diisopropyl ether and filtered. The crystals were recrystallized from 2 ml. of diisopropyl ether to give colorless crystals which melted at 97–98° and did not depress a mixed melting point with authentic 3,4,6-tri-*O*-methyl-D-glucopyranoside.[15] The infrared curves of the authentic 3,4,6-tri-*O*-methyl-D-glucopyranoside and the trimethylglucose obtained by hydrolysis of IX were identical.

Paper chromatography of glucosides. The silver spray procedure of Trevelyan and co-workers[17] indicated the glucosides under study as dark brown spots on a light brown background. As such, the procedure was unsatisfactory for the location of small amounts of glycosidic materials. In the Trevelyan procedure the chromatogram is sprayed with an acetone solution of silver nitrate, allowed to dry, sprayed with an alcoholic solution of sodium hydroxide, and allowed to stand at room temperature for 5 to 10 min. to develop the spots. The paper is then washed with 6*N* ammonium hydroxide to remove unreduced silver oxide, then washed with water and dried. The procedure was modified as follows. The paper, after standing at room temperature to develop the spots, is bathed a few times in a concentrated (350 g. per liter) solution of sodium thiosulfate, washed thoroughly with water and dried. In this modified procedure, glycoside spots appear as almost black spots against a white background, and much smaller amounts of glycosidic materials can be detected. It is important in this modified procedure that all excess sodium thiosulfate be removed from the paper by washing if it is desired to store the chromatograms. Otherwise, the spots will gradually fade over a period of several weeks. Very recently, Hathaway and Seakins[21] published a modification of the Trevelyan procedure in which a 4% sodium thiosulfate wash was employed.

The three glucosides, salicin, populin, and tremuloidin, were easily recognized separately or in admixture when paper chromatograms were developed in either 10:3:3 butanol-pyridine-water (BPW) or 9:2:2 ethyl acetate–acetic acid–water (EAW) and sprayed with the modified silver spray. R_f values at 25° for BPW are: salicin, 0.52; populin, 0.69; and tremuloidin, 0.77. R_f values for EAW are: salicin, 0.60; populin, 0.84; and tremuloidin, 0.85.

Isolation of populin from P. tremuloides. The residue remaining after water extraction at 25° noted above under the isolation of tremuloidin was covered with 1 l. of hot water, and the mixture boiled under reflux for 1 hr. The mixture was allowed to cool, and the turbid yellow aqueous layer was decanted from the residual heavy oil. The boiling water extraction was repeated on the heavy oil, and the combined turbid aqueous extracts were filtered through Celite and purified by means of basic lead acetate precipitation followed by hydrogen sulfide treatment. The resulting clear solution was concentrated to one-half volume and allowed to stand overnight. The crystals which separated were filtered, washed with water, and dried to yield 2.8 g. of almost pure tremuloidin melting at 200–202°. The filtrate and washings were concentrated again to a smaller volume and allowed to stand. The crystals which separated at this point weighed 0.8 g. and melted at 178–181°. Paper chromatography indicated about 75% tremuloidin and 25% populin. Another concentration and crystallization yielded 4.1 g. of crystals melting at 185–186° comprising about 50% each of tremuloidin and populin. Crystalline products obtained on further concentration contained substantial amounts of salicin and other glucosidic materials.

A 0.1-g. sample of the 50% mixture of populin and tremuloidin melting at 185–186° was dissolved in EAW developer and absorbed on a dry-packed column of powdered cellulose (Whatman Standard Grade) 2 cm. in diameter and 15 cm. in height. The column was eluted with EAW, and the eluate was collected in 5-ml. fractions. The fractions were monitored by means of paper chromatography as follows: 1, pure tremuloidin; 2, tremuloidin with trace of populin; 3, pure populin; 4, pure populin; 5, pure populin; 6, trace of populin; 7 ff., nothing. Fractions 3, 4, and 5 were combined and evaporated to dryness. The residue was covered with a little water and filtered to give crystals of populin which melted at 179–180° and did not depress a mixed melting point with synthetic populin.

It is interesting to note that Fraction 1 deposited crystals of tremuloidin melting at 209–210°, slightly higher than that of any sample purified by recrystallization alone.

Infrared spectra. Infrared absorption 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.

Acknowledgment. The authors wish to thank Dr. John W. Green for the controlled periodate oxidation data and Mr. Lowell Sell for the infrared spectra reported in this paper.

APPLETON, WIS.

(21) D. E. Hathaway and J. W. T. Seakins, *Biochem. J.* **70**, 158 (1958).