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POPULOSIDE AND GRANDIDENTOSIDE FROM THE BARK OF *POPULUS GRANDIDENTATA**†

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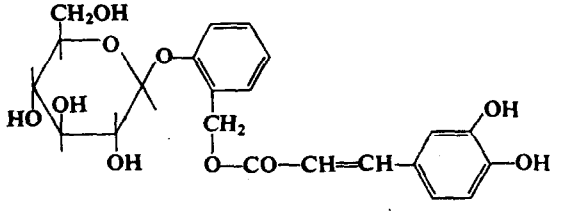
Abstract—Two new glucosides, populoside and grandidentoside, were isolated from the smooth green bark of a *Populus grandidentata* tree felled in May. The structure of populoside was identified by means of mass spectrometry and alkaline and enzymatic hydrolysis as the ω-caffeic acid ester of salicin or *o*-(3,4-dihydroxycinnamoyloxymethyl)phenyl-β-D-glucopyranoside. Alkaline hydrolysis and mass spectrometry demonstrated the structure of grandidentoside to be a caffeic acid ester of the β-glucoside of *cis*-cyclohexanediol and NMR spectrometry suggested that caffeoyl substitution is on the 2-*O*-glucose position.

RESULTS

IN A RECENT study on the hot-water extractives of the green bark of *Populus grandidentata*,[1] we reported the occurrence of two new glucosides, "populoside" and "grandidentoside". Populoside was obtained pure in eluate fractions from the step-gradient aqueous ethanol elution of polyamide chromatographic columns and grandidentoside was obtained in admixture with both grandidentatin and salireposide in eluate fractions from water elution of the same columns.

The structure of populoside has been established as the ω-caffeic acid ester of salicin or *o*-(3,4-dihydroxycinnamoyloxymethyl)phenyl-β-D-glucopyranoside (I). Hydrolysis of populoside with β-glucosidase yielded glucose and alkaline hydrolysis yielded crystalline salicin and caffeic acid. Analysis indicated a monocaffeoyl ester of salicin.



Populoside (I)

Acetylation of I gave a crystalline acetate (II) which was subjected to analysis and mass spectrometry. The mass spectrum of II exhibited a molecular ion with *m/e* 700, corresponding with the structure of the hexaacetate of I. A prominent peak at *m/e* 331, which is characteristic of the acetates of all unsubstituted glucosides,[2] demonstrated that caffeoyl substitution must be on the ω-carbon atom of the salicyl alcohol moiety. Other related glucose ions, such

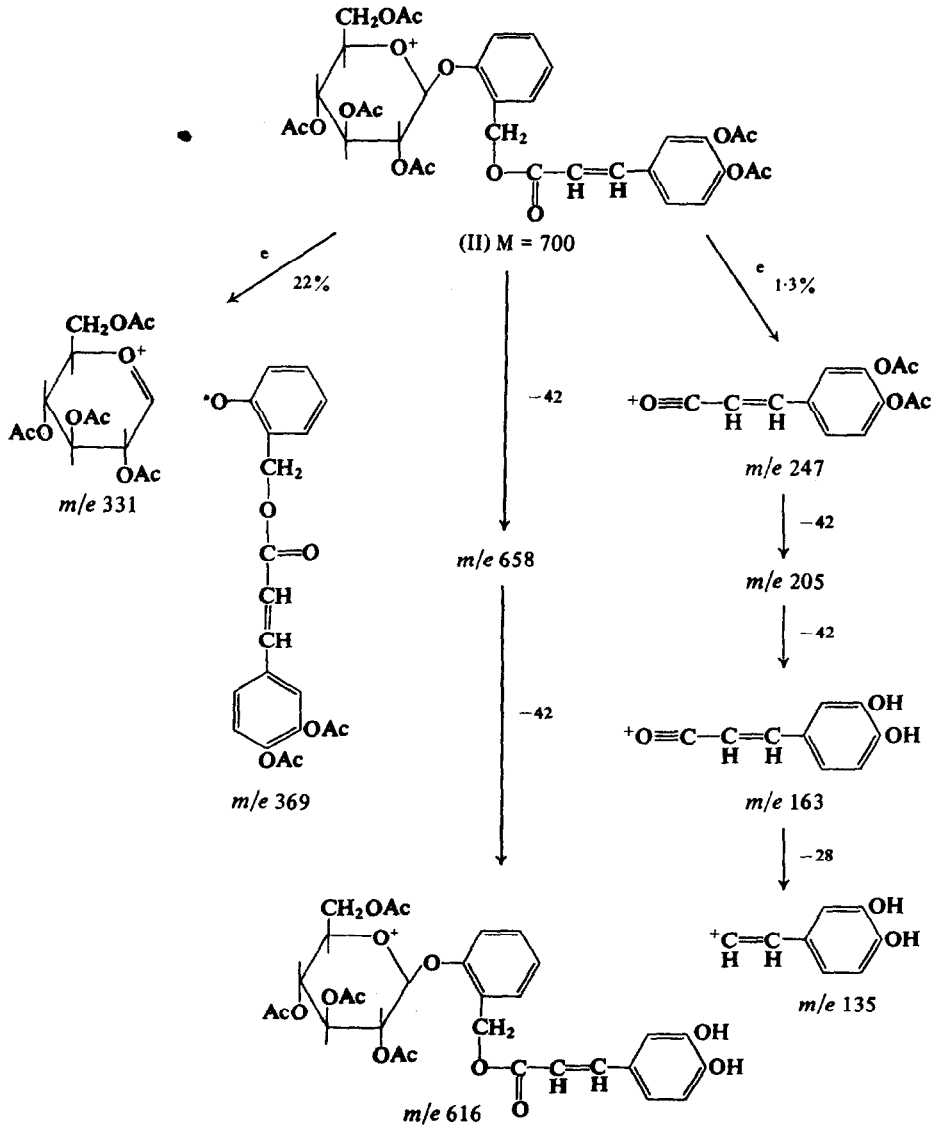
* Part XXV in the series "Studies on the Barks of the Family Salicaceae".

† A portion of a thesis submitted by R. L. E. in partial fulfillment of the requirements of The Institute of Paper Chemistry for the Degree of Doctor of Philosophy from Lawrence University, Appleton, Wisconsin, June 1969.

[1] R. L. ERICKSON, I. A. PEARL and S. F. DARLING, *Tappi*, in press.

[2] I. A. PEARL and S. F. DARLING, *Phytochem.* 7, 831 (1968).

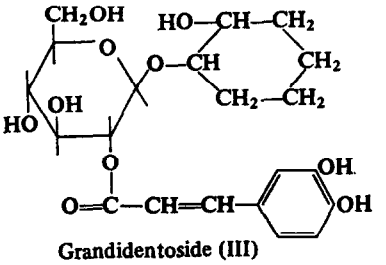
as *m/e* 271, 211, 169, 127, and 109,[2–6] were present in abundance. The peaks at *m/e* 658 and 616, corresponding with the loss of one and two ketone groups (mol. wt. 42) from the molecular ion, indicate the presence of a dihydroxy phenolic moiety since previous studies[2] demonstrated that ketene was usually split from acetylated phenolic hydroxyls before fragmentation of the C-1 linkage of glucosides occurred. The peaks at *m/e* 247, 205, 163, and 135 correspond with the fragmentation of the acetylcaffeoyl moiety. The major fragmentation pattern of II is pictured in Scheme I which accounts for the important peaks in the mass spectrum. The



[3] I. A. PEARL and S. F. DARLING, *Phytochem.* 7, 825 (1968).
[4] K. BIEMANN, D. C. DEJONGH and H. K. SCHNOES, *J. Am. Chem. Soc.* **85**, 1763 (1963).
[5] T. C. SMALE and E. S. WAIGHT, *Chem. Comm.* 680 (1966).
[6] K. HEYNS and S. MUELLER, *Tetrahedron Letters*, 6061 (1966).

largest peak in the mass spectrum was due to the acetyl ion with *m/e* 43. The i.r. spectrum and color reactions correspond with the assigned structure.

Pure grandidentoside was obtained by subsequent column chromatographic fractionation on silica gel of the crude grandidentoside fractions and its structure has been demonstrated to be a caffeic acid ester of *cis*-2-hydroxycyclohexyl-β-D-glucopyranoside. Although not established unequivocally, mass and NMR spectroscopy suggest substitution at the C-2 position on the glucose moiety or *cis*-2-hydroxycyclohexyl 2-*O*-caffeoyl-β-D-glucopyranoside (III).



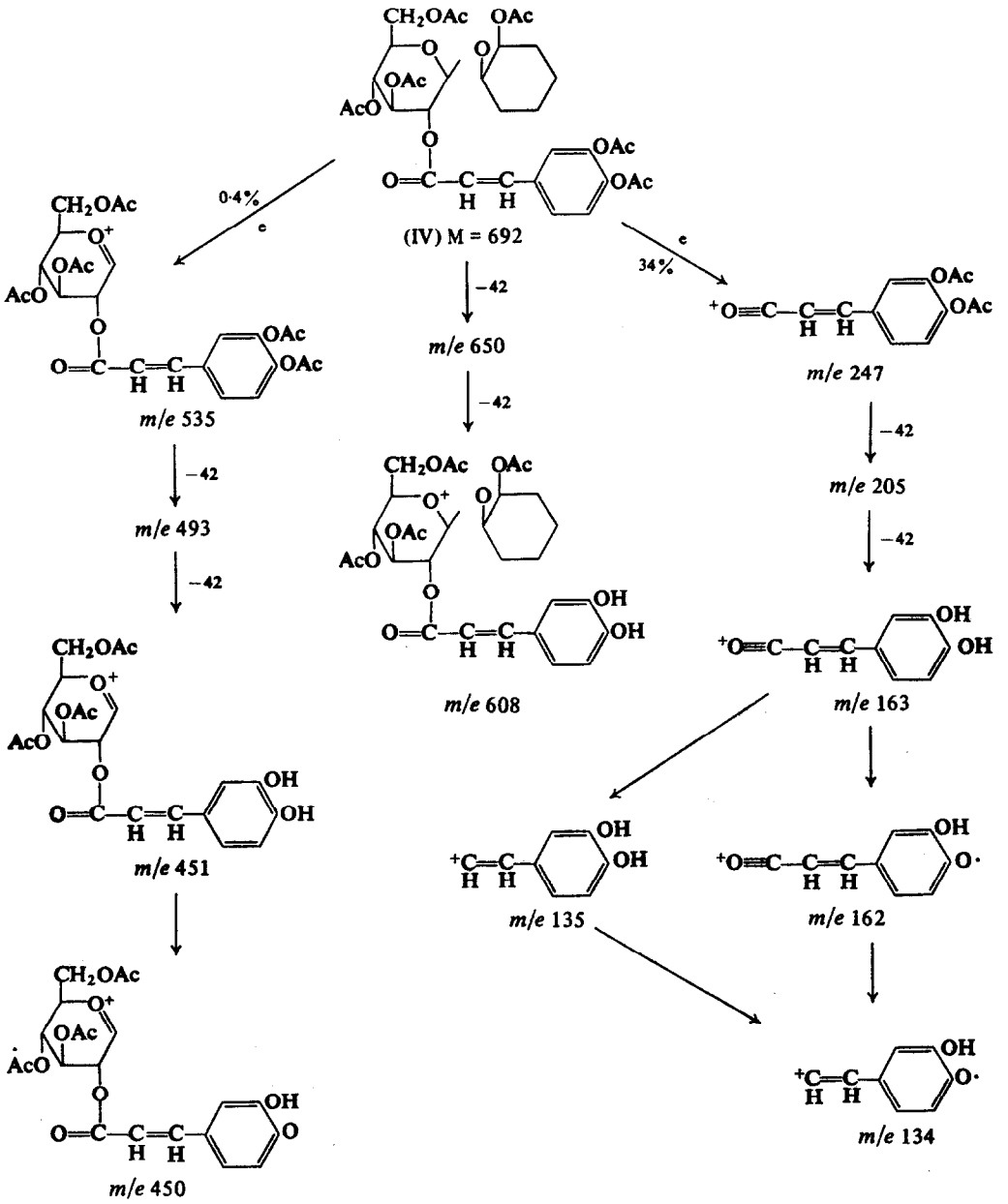
Grandidentoside (III)

Alkaline hydrolysis of grandidentoside yielded grandidentin (*cis*-2-hydroxycyclohexyl-β-D-glucopyranoside) and caffeic acid. Acetylation of grandidentoside yielded grandidentoside hexaacetate (IV) whose mass spectrum indicated a monocaffeoylgrandidentin structure and demonstrated that the caffeoyl substitution was on the glucose moiety.

The major fragmentation pattern of IV is pictured in Scheme II which accounts for the important peaks in the mass spectrum. The mass spectrum of IV is remarkably similar to that of the pentaacetate of grandidentatin (the 2-*O*-*p*-coumaroyl ester of grandidentin),[2] but reflecting only the difference in phenolic hydroxyl substitution. The molecular ion at 692 corresponds with the proposed structure of IV. The loss of one and two ketene molecules (42) to give peaks at *m/e* 650 and 608 confirm the caffeoyl group as already noted for populoside. From the relative abundance of ions, the primary fragmentation route appears to be through the splitting of the caffeoyl group (*m/e* 247) with the subsequent loss of one and two ketenes to give peaks at *m/e* 205 and 163. It is interesting to note that the unacetylated caffeoyl ion (*m/e* 163) readily lost a proton to form an ion with *m/e* 162 and both of these ions lost carbon monoxide to give important ions with *m/e* 135 and 134, respectively. Another important peak in the mass spectrum at *m/e* 535 corresponds with the acetylated caffeic acid ester of the triacetyl glucose oxonium ion and this ion loses one and two ketenes to give peaks at *m/e* 493 and 451. The latter ion also loses a proton to give a peak at *m/e* 450. The presence of the peak at *m/e* 535 and the absence of one at *m/e* 331 demonstrates conclusively that caffeoyl substitution must be on the glucose moiety and not on the cyclohexanediol portion of the grandidentin molecule.

The small amount of material available precluded the absolute location of caffeoyl substitution on the glucose moiety by the methylation procedures employed in the past for tremuloidin,[7] grandidentatin[8] and salireposide.[9] Recourse was made to NMR spectrometry (see below) which suggested caffeoyl substitution at the C-2 position.

[7] I. A. PEARL and S. F. DARLING, *J. Org. Chem.* **24**, 731 (1959).
[8] I. A. PEARL and S. F. DARLING, *J. Org. Chem.* **27**, 1806 (1962).
[9] I. A. PEARL and S. F. DARLING, *Phytochem.* **7**, 821 (1968).

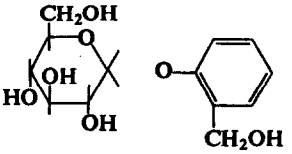


Nuclear Magnetic Resonance Spectrometry

The application of NMR spectrometry to carbohydrate chemistry has been reviewed recently,[10] and its use for location of substitution location on glucosides appeared feasible. As a first step in the location of caffeoyl substitution in grandidentoside, a number of phenolic glucosides with established structures were examined. These included salicin (V), tremuloidin (2-*O*-benzoylsalicin), populin (6-*O*-benzoylsalicin), ω-salicyloylsalicin, grandidentin and

[10] L. D. HALL, in WOLFROM'S *Advances in Carbohydrate Chemistry*, Vol. 19, p. 51, Academic Press, New York (1964).

grandidentatin. Chemical shifts are reported in parts per million [ppm τ] and the primary region of interest is 4·0 to 6·0 ppm.



Salicin (V)

In the NMR spectrum of V, three protons were observed between 5·0 and 5·7 ppm. The anomeric proton (H-1) of glucose appeared as a masked doublet at 5·10 ppm with a coupling constant of 7 c/s. For the remaining two protons, corresponding with the ω-methylene protons of the benzyl group, an AB quartet was observed. The two protons were located at 5·32 and 5·42 ppm with a coupling constant of 15 c/s. Although free benzyl alcohol[11] and salicyl alcohol exhibited a strong singlet for the methylene protons at 5·45 ppm, their non-equivalency in the case of V would be expected from their diastereomeric environments and a hindered rotation as a result of a locked conformation from intramolecular hydrogen bonding between the benzylic hydroxyl proton and the glycosidic oxygen, a type of hydrogen bond which has been proposed previously.[12] Intramolecular hydrogen bonding can exist in methyl sulfoxide solution even though most of the glucose hydroxyl protons are hydrogen bonded strongly to the solvent.[13]

Substitution at the ω-position, as in ω-salicyloylsalicin, eliminated the AB pattern producing a singlet at 4·45 ppm, which corresponded closely with the singlet observed in benzyl benzoate.[11] Collapsing of the quartet to a singlet by substitution provided additional evidence that the benzylic proton could be involved in intramolecular hydrogen bonding.

Tremuloidin exhibited four protons in the region of interest. Again, an AB quartet was observed for the ω-methylene protons. However, the difference in chemical shifts increased from 0·10 to 0·24 ppm (protons at 5·55 and 5·79 ppm), while the coupling constant remained at 15 c/s. These data indicate that substitution at C-2 forced the ω-methylene protons into environments even different than in salicin. The remaining two protons are attributed to the anomeric (H-1) proton and the H-2 proton. Esterification of a ring hydroxyl causes the corresponding ring proton to shift downfield by 1·0 to 1·2 ppm.[10] Benzoylation at C-2 shifted H-2 into the region of 4·8 ppm. Substitution at C-2 also shifted the anomeric proton slightly downfield.

In the NMR spectrum of populin, an AB quartet similar to that for salicin and the anomeric proton at 5·1 ppm were observed. Since integration yielded exactly five protons between 4·0 and 6·0 ppm, the remaining two protons can be attributed to the C-6 methylene protons brought downfield by esterification.

Thus, NMR spectrometry data for salicin and its derivatives demonstrated that substitution at C-2 or C-6 could be distinguished by the number and nature of the protons observed in the region of 4·0 to 6·0 ppm. Substitution at C-2 only shifted one proton downfield by esterification, whereas substitution at C-6 shifted two protons downfield. Although derivatives substituted at C-3 or C-4 were not investigated, other studies on acetylated methyl

[11] N. S. Bhacca, L. F. Johnsol and J. N. Shoolery, *NMR Spectra Catalog*, Vols. 1 and 2, Varian Associates, Palo Alto, Calif. (1962).
[12] B. Capon, *Tetrahedron Letters*, 911 (1963).
[13] B. Casu, M. Reggiani, G. G. Galle and A. Vigenvani, *Tetrahedron* 22, 3061 (1966).

β-D-glucopyranosides indicated that if either of these positions were esterified, the corresponding ring protons would be shifted downfield slightly more than those of C-2 or C-6.[14] Since glucosides of natural products are substituted predominantly in the latter two positions, NMR spectrometry should prove extremely valuable for differentiating the nature of substitution on the glucose moiety of such compounds. Mass spectrometry proves unequivocally whether or not the glucose is substituted.[2]

In the case of grandidentin and related derivatives, the region of interest was narrower than that observed for salicin derivatives and extended from 5·0 to 6·0 ppm. A doublet corresponding with the anomeric proton of grandidentin appeared at 5·67 ppm, with a coupling constant of 7 c/s. This value compared with 5·91 and 5·72 ppm for methyl β-D-glucopyranoside and cyclohexyl β-D-glucopyranoside, respectively. Grandidentatin exhibited two protons between 5·0 and 6·0 ppm. Esterification at C-2 shifted the H-2 proton downfield by 1·0 to 1·2 ppm and shifted the anomeric proton downfield slightly. Since there is only a slight difference between the *p*-coumaroyl and caffeoyl moieties, if substitutions were in identical positions, similar NMR spectra would be expected. This was shown to be the case since identical peaks and splitting patterns were observed for grandidentatin and grandidentoside between 5·0 and 6·0 ppm.

EXPERIMENTAL*

Preparation of Populoside

In the polyamide chromatogram of *Populus grandidentata* whole bark ethyl acetate extractives described in a previous paper,[1] after water elution of the column, the column was extracted with hot ethanol and the extractives were rechromatographed on polyamide and subjected to step gradient elution with aqueous ethanol. During elution with 25% ethanol, eluate fractions 72 through 87, upon concentration, yielded colorless crystals which were recrystallized from water to give crystals of populoside containing 2·5 moles of water, melting at 186–188° and having a specific rotation in methanol of $[\alpha]^{27}_{Hg-546}$ −46·4° and $[\alpha]^{26}_{D}$ −38·0° (*c.* 2·23). Its i.r. spectrum contained bands at 2·95, 3·47, 5·98, 6·12, 6·23, 6·59, 6·68, 6·90, 7·28, 7·86, 8·03, 8·38, 8·65, 9·00, 9·17, 9·30, 9·60, 10·22, 11·17, 11·62, 11·77, 12·40, 13·20 μ. (Found: loss in wt. on drying, 9·7. Calc. for $C_{22}H_{24}O_{10}.2{\cdot}5H_2O$: 9·2%. Found after drying:C, 58·60, 58·54; H, 5·56, 5·61. Calc. for $C_{22}H_{24}O_{10}$:C, 58·92; H, 5·36%.)

TLC on silica gel in $CHCl_3$–MeOH (4:1, + trace of HCO_2H) and spraying with H_2SO_4 and heating gave a lavender spot at R_f 0·32. Diazotized *p*-nitroaniline gave a tan-yellow spot at R_f 0·75 on paper chromatograms developed in EtOAc–pyridine–H_2O (8:2:1). Populoside fluoresced in u.v. light.

Populoside Hexaacetate

Populoside (100 mg) was acetylated with 1 ml Ac_2O and 2 ml of pyridine to yield 165 mg of colorless sirup which crystallized from ethanol as the hexaacetate, m.p. 93–95° and having a specific rotation $[\alpha]^{27}_{Hg-546}$ −9·33° and $[\alpha]^{26}_{D}$ −8·04° (*c.* 2·40 in $CHCl_3$). The i.r. spectrum contained bands at 2·95, 3·42, 5·70, 5·82, 6·08, 6·20, 6·27, 6·65, 6·86, 7·00, 7·30, 7·55, 8·20 (broad), 8·51, 9·00, 9·37, 9·61, 9·88, 10·17, 11·05, 11·40, 11·98, 13·22, and 14·38 μ. Its mass spectrum contains the following major and important *m/e* peaks: 43 (100), 81 (6·4), 97 (6·1), 107 (12·5), 109 (55·5), 110 (5·1), 127 (17·5), 135 (4·0), 139 (6·4), 145 (6·7), 163 (12·8), 169 (86·9), 170 (8·7), 205 (5·0), 211 (3·7), 247 (1·3), 271 (5·0), 328 (5·0), 331 (21·5) and M^+ 700. (Found after drying: C, 58·22, 57·93, 57·94; H, 5·24, 5·18, 5·17; mol. wt. by mass spectrometry, 700. $C_{34}H_{36}O_{14}$ required: C, 58·29; H, 5·14; mol. wt., 700.)

Alkaline Hydrolysis of Populoside

100 mg of populoside, 200 ml of sat. $Ba(OH)_2$ and 5 ml of water were heated to boiling 4 min and filtered to give a dark reddish-brown solution. The clear solution was neutralized with H_2SO_4 and extracted with ether. The ether was evaporated to yield 23 mg of caffeic acid melting at 212–213° which was identified by mixed m.p., identity of i.r. spectrum and gas chromatographic retention time of trimethylsilyl derivative with

* All m.p.s are uncorrected. Analyses were performed by Micro-Tech Laboratories, Skokie, Illinois. I.r. spectra were determined by Mr. Lowell Sell of The Institute of Paper Chemistry. The mass spectra were determined by Morgan-Schaffer Corp., Montreal, Quebec, Canada.

[14] A. P. TULLOCH and A. HILL, *Can. J. Chem.* **46**, 2485 (1968).

those of authentic caffeic acid. The slightly acidic aqueous raffinate, after ether extraction, was treated with excess $BaCO_3$ and filtered. The clear filtrate was evaporated to dryness and the residue was taken up in ethanol, clarified by centrifugation and evaporated to dryness to give 45 mg of pure salicin melting at 198–199° which was identified by mixed m.p. and identity of i.r. spectrum with that of authentic salicin.

Enzymatic Hydrolysis of Populoside

β-Glucosidase (50 mg) was added to a solution of 96 mg of populoside in 50 ml of 0·1 N NaOAc buffered to pH 5·0 and the clear solution maintained at 40° for 3 hr. The solution was extracted with ether and the aqueous raffinate was passed through a column of Amberlite IR-120 cation-exchange resin and concentrated. Glucose was identified in the concentrated solution by TLC and by paper chromatography.

Preparation of Grandidentoside

The crystalline mixture of grandidentatin and grandidentoside isolated from eluate fractions 60 through 80 of the water elution polyamide chromatography of the *P. grandidentata* whole bark ethyl acetate extractives described in a previous paper[1] (53 mg) was dissolved in a little $CHCl_3$–MeOH–HCO_2H (93:5:2) and the solution was pipetted on top of a column of silica gel* 1 cm in diameter and 57 cm in length packed as a slurry from the same solvent mixture. Elution was initiated and 20-ml fractions were collected in the eluate. After 10 fractions were collected, the methanol concentration was increased to 10%; after 14 fractions, to 12%; and after 23 fractions, to 20%. Grandidentatin was eluted between fractions 15 and 22 and grandidentoside was eluted between fraction 25 and 30. 20 mg of the sirup obtained upon evaporation of the latter fractions were dissolved in 1 ml of water containing a little methanol and formic acid. Most of the methanol was removed under reduced pressure, and the solution was stored in the refrigerator. After 2 days, the mixture was filtered to yield 3·5 mg of grandidentoside as colorless needles melting at 196–197° and containing the following bands in its i.r. spectrum: 2·97, 3·16, 3·45, 5·85, 6·14, 6·21, 6·61, 6·92, 7·28, 7·48, 7·76, 8·00, 8·46, 8·65, 8·97, 9·30, 9·40, 9·68, 10·01, 10·94, 11·22, 11·75, 12·26, 12·73, and 13·03 μ.

TLC on silica gel in $CHCl_3$–MeOH (4:1, + trace of HCO_2H) and spraying with H_2SO_4 and heating gave a lavender spot at R_f 0·35. Diazotized *p*-nitroaniline followed by Na_2CO_3 gave a yellow-tan spot at R_f 0·81 on paper chromatograms developed in *n*-BuOH–EtOH–H_2O (1:1:1).

Grandidentoside Hexaacetate

Grandidentoside was acetylated with Ac_2O and pyridine and the product was recrystallized from 90% ethanol to give grandidentoside hexaacetate as fine colorless needles melting at 175–177° and having the following bands in its i.r. spectrum: 2·93, 3·40, 3·50, 5·72 (broad), 6·11, 6·67, 7·01, 7·30, 8·06, 8·29, 8·49, 9·00, 9·47, 9·67, 10·16, 10·45, 11·11, 12·00, and 16·68 μ. Its mass spectrum contained the following major and important *m/e* peaks: 28 (16·4), 42 (11·8), 43 (100), 81 (31·7), 97 (11·4), 98 (10·8), 99 (27·2), 109 (11·2), 115 (11·9), 127 (5·9), 134 (12·6), 135 (6·1), 139 (8·0), 141 (22·9), 157 (5·1), 162 (63·7), 163 (89·4), 169 (15·9), metastable 170 [247 → 205 + 42], 189 (10·6), 204 (5·7), 205 (92·0), 206 (13·9), 219 (5·1), 228 (8·4), 247 (33·6), 248 (5·7), metastable 412 [492 → 450 + 42], 450 (10·2), 451 (3·0), 492 (5·2), 493 (1·7), 535 (0·4), metastable 569 [650 → 608 + 42], 608 (5·0), 650 (0·5), and M^+ 692. (Found by mass spectrometry: mol. wt., 692. $C_{33}H_{40}O_{16}$ required: mol. wt. 692.)

Alkaline Hydrolysis of Grandidentoside

Grandidentoside was hydrolyzed with $Ba(OH)_2$ as described for populoside. The ether extract of the acidified hydrolysis mixture yielded caffeic acid, identified as before. The aqueous raffinate was passed through a column of Amberlite MB-3 mixed-bed ion-exchange resin and evaporated to dryness under reduced pressure to yield a sirup giving only one spot on TLC identical with that of grandidentin.[8] This chromatographically pure sirup was acetylated and the product was recrystallized from 30% ethanol to yield colorless crystals of grandidentin pentaacetate melting at 120–121°, identified by mixed m.p. and identity of i.r. spectrum with authentic material.[8]

Mass Spectra

The 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 measured using a NaCl prism and KBr pellets.

Nuclear Magnetic Resonance Spectra

NMR spectra were determined on a Varian Model A-60-A analytical spectrometer using methyl sulfoxide -d_6† as the solvent. Exchange of hydroxyl protons was accomplished with deuterium oxide. Tetramethylsilane was used as a reference.

* Silica gel—60 to 200 mesh, Grade 950, Fischer Scientific Co., Fair Lawn, New Jersey.

† Stohler Isotope Chemicals, Rutherford, New Jersey.