Isolation, structure, and synthesis of a stilbene glucoside from the bark of *Picea glauca* (Moench) Voss.

D. H. ANDREWS,[1] J. C. HOFFMAN,[2] AND C. B. PURVES[3]
Division of Industrial and Cellulose Chemistry, McGill University, Montreal, Quebec

AND

H. H. QUON[4] AND E. P. SWAN[5]
Canada Department of Forestry and Rural Development, Forest Products Laboratory, Vancouver 8, British Columbia

Received January 22, 1968

The fractionation of phlobaphenes from white spruce bark by column chromatography gave the non-crystalline stilbene glucoside *trans* 3,4′5-trihydroxy-3′-methoxystilbene-3-*O*-β-D-glucopyranoside (**1**). Purification was achieved through deacetylation of its hexaacetate (**2**). Compound **1** was cleaved with emulsin or dilute acid to the aglycone **4**, and D-glucose. The acetate, benzoate, and dihydro-derivatives of **1**, and the acetate, benzoate, methyl ether, and dihydro-derivatives of **4** were prepared. Oxidation of the benzoate derivative of **1** with chromic acid gave dibenzoyl-α-resorcylic acid. Similar oxidation of **2** gave acetylvanillic acid together with an uncharacterized acid which was hydrolyzed to D-glucose and α-resorcylic acid. The structure of **1** was therefore as shown, and its derivative **2** was synthesized. Vanillin was condensed with 3,5-dihydroxyphenylacetic acid and the product was decarboxylated and deacetylated. This compound was condensed with tetra-*O*-acetyl-α-D-glucopyranosyl bromide to yield a mixture of products which, after acetylation, were separated, using preparative thin-layer chromatography, to give the desired **2**.

Canadian Journal of Chemistry, **46**, 2525 (1968)

Introduction

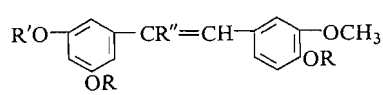
At present, bark is a major waste product in the pulp and paper industry. Not only does it possess little commercial value, but it also provides a difficult disposal problem. One object of this research is to discover whether materials of potential value are present in the bark in sufficient amounts to justify their extraction on a large scale.

In particular, stilbene derivatives have been found in the bark of *Picea* species (1). One of these was piceatannol (2, 3) in *P. excelsa*. This research describes the isolation, characterization, and synthesis of a stilbene glucoside which was not derived from piceatannol. Subsequent to part of this work, this product was isolated by Manson (4).

Discussion of Results

Harwood (5) undertook an extraction, on a pilot-plant scale, of a large quantity of white spruce bark. By using various solvents and precipitants he separated the methanol-soluble extractives into fractions, one of which was material insoluble in water, ether, and ligroin, comprising 1.5% of the original bark. After considerable effort, it was shown that 16% of this material (0.24% of the bark) was compound **1**. The best method of isolation was by Magnesol column chromatography using acetone as the eluant. The removal of the last traces of phlobaphenes was extraordinarily difficult, and since **1** was not obtained crystalline, it was converted to the crystalline acetate derivative **2**, and this in turn was deacetylated. Both **1** and **2** were acid and base labile. The best method of cleaving **1** to the aglycone **4** was by heating with dilute oxalic acid, and the best method of deacetylating **2** was a modified barium methylate-catalyzed methanolysis.

The structure **1** for the product isolated from



1 R = R″ = H, R′ = β-D-glucopyranosyl
2 R = $COCH_3$, R′ = β-D-glucopyranosyl tetraacetate, R″ = H
3 R = R′ = $COCH_3$, R″ = COOH
4 R = R′ = R″ = H

[1]Present address: Central Research Laboratory, Canadian Industries Ltd., McMasterville, Quebec.
[2]Present address: Department of Theology, University of Windsor, Windsor, Ontario.
[3]Deceased.
[4]Present address: Department of Chemistry, Simon Fraser University, Burnaby, British Columbia.
[5]To whom correspondence should be addressed.

the bark was supported by analytical data for it, and its acetyl derivative **2**. Similarly, the aglycone **4** and its triacetate, tribenzoate, and tri-*O*-methyl ether were analytically defensible. The ultraviolet (u.v.) spectra of these products were very similar to those from *trans* stilbenes (6). Compounds **1** and **4** each consumed a mole equivalent of hydrogen to yield the expected products, and the dihydro-derivative of **4** had the expected u.v. spectrum. Treatment of **1** with emulsin yielded D-glucose and **4**, indicating the β-glucoside configuration. Oxidation of the benzoate of **1** as well as **2** with chromic acid gave the appropriate derivatives of α-resorcylic and vanillic acids. Finally, the oxidation of **2** gave an acid which in turn could be cleaved to α-resorcylic acid and D-glucose. This placed the carbohydrate on the resorcinol moiety rather than the guaiacol moiety of **1**, which was therefore *trans* 3,4′,5-trihydroxy-3′-methoxystilbene-3-*O*-β-D-glucopyranoside.

Unfortunately, the structural diagram given for the present stilbene glucoside (**1**) in the previous publication (4) is incorrect and shows rhapontin. The two compounds are not identical, as can be seen by comparison of physical constants found here and given by Kawamura for rhapontin (7). In particular, **2** melted at 162–163 °C and rhapontin hexaacetate melted at 135–136 °C.

The synthesis of **2** was similar to the synthesis of 3,3′,4′,5-tetrahydroxystilbene by Cunningham, Haslam, and Haworth (2). 3,5-Dihydroxyphenylacetic acid was condensed with vanillin under Perkin reaction conditions to give **3** which was deacetylated and decarboxylated to the aglycone **4**. This latter reaction gave low yields, probably because of concurrent demethylation. The isolation of the product was facilitated by the use of polyamide columns (8) rather than silica gel. The aglycone (**4**) was condensed with tetra-*O*-acetyl-α-D-glucopyranosyl bromide in a Koenigs–Knorr reaction. The reaction was monitored by thin-layer chromatography (t.l.c.) and the desired product, from reaction of the glucosyl moiety with the resorcinol ring, was identified and separated by preparative t.l.c., from the other major product which resulted from similar substitution on the guaiacol ring. It was easy to differentiate the former from the latter because, for mono-substitution, there was much more of the former produced. The desired product was then acetlyated to give **2**, mixture m.p. undepressed, and with u.v. and infrared spectra, together with t.l.c. behavior, all identical with the original **2**.

Experimental

Melting points are uncorrected. Elemental analyses in the synthetic work only, were performed by Clark Microanalytical Laboratory, Urbana, Illinois. Paper chromatography was performed on Whatman No. 1 paper using the organic phase of butanol – acetic acid – water (4:1:5) as a developing solvent.

Isolation of the Stilbene Glucoside (1)

The phlobaphene obtained from Harwood (5) was dried in a vacuum desiccator over phosphorus pentoxide for several days, and then extracted exhaustively with ether. One hundred grams of the product were extracted with acetone. The extract was not concentrated, but was applied as obtained to a short column (95 × 70 mm), consisting of a sintered glass funnel of Magnesol and operated under suction. Elution with 4500 ml of acetone required approximately 1 h. The acetone extracts yielded 15.6 g of slightly colored material which showed as **1** on a paper chromatogram.

Reactions of the Stilbene Glucoside (1)

Stilbene glucoside (**1**) was a yellow-white amorphous powder. It produced a characteristic fluorescent spot on a paper chromatogram, R_f 0.40; $[\alpha]_D^{21}$ −54.1° (*c*, 2 in acetone). Found: OCH_3, 7.33, 7.34. Ultraviolet (u.v.) spectrum (methanol): λ_{max} 325, shoulder at 300 mμ (log ε 4.22 and 4.06).

Compound **1** (1.0 g) in pyridine (10 ml) was reacted with acetic anhydride (4 ml) for three days at room temperature. The reactants were poured into water to yield (crystallized from hot methanol) compound **2**, 1.35 g of white needles, m.p. 162–163 °C, $[\alpha]_D^{20}$ −22.6° (*c*, 2 in acetone). Ultraviolet spectrum (methanol): λ_{max} 300 and 315 mμ (log ε of both 4.37).

Anal. Calcd. for $C_{20}H_{15}O_8$ $(COCH_3)_6(OCH_3)$, (mol. wt., 672): C, 58.9; H, 5.36; OCH_3, 4.61; acetyl, 38.4. Found (mol. wt., 635, 654; Rast): C, 58.8, 59.1; H, 5.50, 5.54; OCH_3, 4.54, 4.60; acetyl, 38.1, 39.4.

Hydrogenation of **2** was carried out in acetic acid (3 ml) with palladium–charcoal in the Siggia (9) apparatus. Duplicate estimations showed the uptake of 1.08 and 0.97 moles of hydrogen per mole of **2**. The product was recrystallized from ethanol. The white needles, 0.105 g had m.p. 94–95.5 °C.

Anal. Calcd. for $C_{20}H_{17}O_8$ $(CH_3CO)_6(OCH_3)$: acetyl, 38.3; OCH_3, 4.60. Found: acetyl, 37.9, 37.5; OCH_3, 4.3, 4.5.

Compound **1** was methylated repeatedly with ethereal diazomethane. The product had R_f 0.66. Attempts to crystallize the product were not successful.

Hydrolyses

A sample of **2** (0.9 g) dissolved in anhydrous methanol (50 ml) was added to barium methylate in methanol (10 ml of 0.69 *N*). A yellow precipitate formed immediately. The reaction mixture was kept at about 5 °C with occasional shaking for 5 h. The precipitate was separated from

the supernatant liquid by filtration, and was washed with anhydrous methanol. The precipitate was then added to a mixture of dry ice and water, and the barium carbonate formed was separated by filtration. The filtrate was evaporated under reduced pressure to dryness, and an acetone extract of the dry residue (made at room temperature) was again evaporated to dryness. The residue was taken up in *n*-butanol and the product recovered in the usual way. The material was nearly white in color. Chromatographic analysis showed the presence of **1** with very little impurity; yield, 0.40 g. Found: acetyl, 0.7, 0.9. The product (34 mg) was re-acetylated. This product had m.p. 162–163 °C and a mixture m.p. with **2** was not depressed.

Compound **1** (2.0 g) was added to oxalic acid solution (100 ml of 1%) and the mixture was warmed. After 1 h the material was still not completely dissolved. After a further hour of heating on the steam cone, a chromatogram showed two spots of R_f 0.47 and 0.78. The hydrolysate was extracted overnight with chloroform, and chromatography of the chloroform extract showed the spot, R_f 0.79, of **4** while that of the aqueous residue retained a strong spot for compound **1** plus a faint spot for the aglycone. A brown solid which had again formed in the still for the chloroform was recovered and dried. This process was repeated five more times, the length of the heating period being increased gradually. A total of 0.563 g of crude aglycone (**4**) was obtained. The above procedure was repeated four times until 2.65 g of crude **4** had been accumulated.

The aqueous residue was treated with a slight excess of calcium hydroxide, and the calcium oxalate which precipitated was removed by filtration. The excess calcium hydroxide was removed by shaking the filtrate with IR 120. The filtrate was clarified and then it was mixed with phenylhydrazine hydrochloride and sodium acetate, in excess, and heated on a water bath. Yellow crystals of glucosazone were separated, m.p. 204–205 °C (decomposed). The product (0.2 g) was mixed with water (18 ml), sulfuric acid (1 ml of 0.5 *N*), copper sulfate pentahydrate (0.6 g), and isopropanol (12 ml). The solution was heated under reflux for 1 h, then it was concentrated to about 5 ml with the formation of brown-colored crystals. These were recrystallized from water to give white needles, m.p. 195–196 °C, mixture m.p. with the osatriazole (11) prepared from D-glucose showed no depression.

Emulsin (25 mg) was dissolved in distilled water (50 ml) containing compound **1** (300 mg). After the reaction mixture had been left at room temperature for 22 h, a determination of copper reducing power, carried out according to the Somogyi method (10) on a 0.5 ml aliquot, was equivalent to 1.07 mg of D-glucose. After 45 h this value had increased to 1.22 mg. Since the theoretical amount of D-glucose present on complete hydrolysis would be 1.28 mg for a 0.5 ml aliquot (assuming a molecular weight of 420 for **1**) the hydrolysis was considered to be complete. Chromatographic analysis of the hydrolysate showed that an appreciable amount of compound **1** (R_f 0.40) was still present. In addition, a new spot (R_f 0.79) was observed which produced the same fluorescence as compound **1**. The reaction mixture was therefore extracted continuously for several hours with chloroform, during which time material crystallized out on the flask at the surface of the chloroform. The addition of petroleum ether until the chloroform solution became slightly turbid, caused material (**4**) to separate in the form of flaky brown crystals, 150 mg.

Reactions of the Aglycone (4)

Purification was by recrystallization from ethyl acetate–petroleum ether. The recrystallized aglycone was in the form of near-white clusters, optically inactive, m.p. 182–183 °C. Ultraviolet spectrum (methanol): λ_{max} 325 and shoulder at 300 mμ (log ε 4.37 and 4.27).

Anal. Calcd. for $C_{14}H_{11}O_3(OCH_3)$, (mol. wt., 258): C, 69.7; H, 5.46; OCH_3, 12.0. Found (mol. wt., 251; Rast): C, 69.7, 69.7; H, 5.62, 5.65; OCH_3, 11.9, 12.0.

The aglycone in ethanol was hydrogenated with palladium–charcoal catalyst in the apparatus described by Siggia (9). Hydrogen consumed was equivalent to 0.93 and 0.95 moles per mole of aglycone. The reaction mixtures from these hydrogenations were filtered to remove the catalyst, and the filtrates from two runs, containing originally 72 mg of aglycone, were combined. After evaporation of the solvent, the residue was crystallized from benzene to yield white needles, m.p. 152–153.5 °C. Ultraviolet spectrum (methanol): λ_{max} 280 mμ (log ε 3.64).

Anal. Calcd. for $C_{14}H_{10}(OH)_3(OCH_3)$: C, 69.2; H, 6.20; OCH_3, 11.9. Found: C, 69.5, 69.2; H, 6.25, 6.47; OCH_3, 11.8, 11.7.

The aglycone (0.1 g) was dissolved in pyridine (3 ml) – acetic anhydride (2 ml) and the reaction mixture was left at room temperature overnight. The reaction mixture was worked up as usual and the product was recrystallized from ethanol–water to yield the triacetate of **4** as coarse needles, m.p. 103–104 °C. Ultraviolet spectrum (methanol): λ_{max} 315 with shoulder at 300 mμ (log ε 4.35 and 4.34).

Anal. Calcd. for $C_{14}H_8O_3(C_2H_3O)_3(OCH_3)$: OCH_3, 8.08; acetyl, 33.6. Found: OCH_3, 7.96, 7.91; acetyl, 33.1, 34.0.

The aglycone (0.1 g) was dissolved in anhydrous pyridine (2 ml) and benzoyl chloride (0.5 ml). The reaction mixture was warmed over a low flame for 1 min, and then poured into cold water (20 ml). The product, the tribenzoate of **4**, was worked up as above and was recrystallized from a chloroform–ethanol mixture to needles, yield 0.16 g, m.p. 108.5–110 °C.

Anal. Calcd. for $C_{14}H_8O_3(C_6H_5CO)_3(OCH_3)$: C, 75.8; H, 4.59; OCH_3, 5.44. Found: C, 75.4, 75.3; H, 5.02, 4.95; OCH_3, 5.40, 5.62.

Dimethyl sulfate (2 ml) was added to a solution of the aglycone (0.2 g) in ethanol (2 ml). A solution of sodium hydroxide (40%) was added, in drops, until the reaction mixture was alkaline. The mixture was cooled and it was diluted with water, whereupon a golden-yellow oil separated, and after several days the tri-*O*-methyl ether of **4** crystallized as pale-yellow clusters of needles, yield 0.10 g, m.p. 66.5–68 °C. Ultraviolet spectrum (methanol): λ_{max} 320 and shoulder at 305 mμ (log ε 4.31 and 4.28).

Anal. Calcd. for $C_{14}H_8(OCH_3)_4$: C, 72.0; H, 6.66; OCH_3, 41.3. Found: C, 72.1, 72.1; H, 6.73, 6.80; OCH_3, 40.3, 40.6.

Oxidations

The aglycone benzoate (1.1 g) was dissolved in acetic acid (50 ml) and maintained at 60–70 °C. Chromium trioxide (1.8 g) dissolved in acetic acid (60 ml) was slowly added to the solution over a period of 1 h. After the reaction was complete, a small volume of methanol was

added. The reaction mixture was evaporated almost to dryness under reduced pressure, then mixed with water (100 ml) and extracted four times with ether in a separatory funnel. The ether solution was washed twice with hydrochloric acid (5%), then twice with water, and finally it was dried over anhydrous sodium sulfate. Evaporation of the ether left crude, yellow crystals, which were recrystallized from acetic acid to yellow-white crystals, yield 0.185 g, m.p. 224–232 °C. α-Resorcylic acid was synthesized (12) and benzoylated. The product had m.p. 231–233 °C, mixture m.p. undepressed with the above. Their X-ray powder diffraction spectra were identical.

Compound **2** (2.65 g) was dissolved in acetic acid (100 ml) and the solution was heated in a water bath to about 63–64 °C. Chromium trioxide (3.0 g) dissolved in acetic acid (200 ml) was added with stirring over a period of 1 h. The mixture was worked up as described above to yield the ether extract which was extracted with 6% aqueous sodium bicarbonate solution. The bicarbonate extract was acidified to liberate organic acids and then re-extracted with ether; this ether extract was dried over anhydrous sodium sulfate. After being filtered, the ether extract was evaporated to dryness and the resultant oil kept *in vacuo* for three days, until partial crystallization occurred, and the uncrystallized portion was removed by washing quickly with ethanol (15 ml) and filtering. The product was recrystallized from ethanol to yield yellow-white crystals, 0.43 g, m.p. 189–191 °C. The product (0.43 g) was dissolved in water (10 ml) containing sodium hydroxide (250 mg). After standing at room temperature for 2 h, the solution was neutralized with sulfuric acid (10 ml of 5%) and it was heated under reflux for 2 h. The mixture was then cooled to room temperature, saturated with sodium chloride, and extracted with ether. The ether extract was dried and evaporated to yield 0.10 g of crude product, m.p. 221–229 °C. When recrystallized from acetic acid, the product had m.p. 237–239 °C, and showed no depression in m.p. when admixed with synthetic α-resorcylic acid. The aqueous layer was deionized and examined by paper chromatography. Only one spot with the characteristic color reactions and R_f value of D-glucose was found.

The ethanol reserved from the uncrystallized portion of the original product yielded an oil on evaporation. This oil partially dissolved in hot water, leaving a dark-brown gum which was removed on the filter. The filtrate deposited a fluffy white solid (100 mg) which after three recrystallizations from ethanol had m.p. 138–143 °C (acetylvanillic acid melted at 145–147 °C). The water-insoluble gum was dissolved in ethanol (10 ml) and water (5 ml) and barium hydroxide octahydrate (2 g) was added. The mixture was allowed to stand at room temperature overnight. The brown precipitate which formed was removed on a filter and was dissolved in dilute hydrochloric acid. The acidified solution was filtered and extracted with ether. When dried and evaporated, the ether extract yielded 0.10 g of product, m.p. 198.5–203 °C, raised to 206–208 °C by recrystallization from water, mixture m.p. with vanillic acid 207–209 °C. A sample of this product was acetylated. The acetate had m.p. 147–149 °C, mixture m.p. with acetylvanillic acid undepressed. The X-ray patterns of vanillic acid and the isolated acid, m.p. 206–208 °C, were taken and proved to be identical.

3,4′,5-Triacetoxy-3′-methoxystilbene-α-carboxylic acid (3)

Sodium 3,5-dihydroxyphenylacetate (5.9 g), vanillin (4.2 g) and acetic anhydride were heated in a sealed tube for 8 h at about 175 °C. The mixture was cooled and poured into ice water (500 g). The viscous brown oil which separated was washed with water and then dissolved in ethyl acetate (200 ml). Acidification of the sodium bicarbonate extract of the ethyl acetate yielded 0.5 g of the product, m.p. 220–221 °C, recrystallized from methanol. Ultraviolet spectrum (ethanol): λ_{max} 283 and 310 mμ (log ε 4.38 and 4.18). Infrared (i.r.) spectrum (KBr): ν_{max} 1770, 1680, 1615 cm^{-1}.

Anal. Calcd. for $C_{22}H_{20}O_9$: C, 61.7; H, 4.71. Found: C, 61.5; H, 4.82.

3,4′,5-Trihydroxy-3′-methoxystilbene (4)

The stilbene-α-carboxylic acid (3.9 g), copper powder (4 g) and freshly distilled quinoline (40 ml) were heated to about 185 °C under a nitrogen atmosphere for 3 h. Ethyl acetate (150 ml) was added to the cooled mixture and after filtration the resulting solution was washed several times with dilute hydrochloric acid. The solvent was evaporated *in vacuo* and the residue was treated with 0.2 *N* sodium hydroxide (100 ml) at about 95 °C in a nitrogen atmosphere for 2 h. The solution was cooled and extracted once with ether (25 ml) which was discarded. The aqueous solution was acidified with dilute sulfuric acid and extracted with ethyl acetate. Evaporation of the ethyl acetate left a dark-brown oil (192 mg) which was dissolved in methanol (2 ml) and placed on a column (1 × 30 cm) packed with polyamide powder (Brinkman Instruments). The column was eluted with methanol–water (1:1) to yield finally the product, 30 mg, m.p. 199 °C crystallized from methanol, which gave an orange color with diazotized sulfanilic acid when applied to a thin-layer chromatography plate. Ultraviolet spectrum (ethanol): λ_{max} 285 and 324 mμ (log ε 4.45 and 4.54). Infrared spectrum (KBr): ν_{max} 3300 and 1645 cm^{-1}. Continued elution of the column gave a small amount of a second stilbene (ultraviolet fluorescent) which, however, gave a yellow color with the diazotized sulfanilic acid reagent. The orange color from the desired product was attributed to the guaiacyl nucleus, whereas the yellow color from the second product was attributed to a catechol nucleus produced from the former by demethylation.

The triacetate of this stilbene was prepared by taking the decarboxylation mixture, before it was treated with base, and adding pyridine (5 ml) and acetic anhydride (5 ml); the reaction mixture was kept at room temperature for one day and then worked up in the usual manner. The product was purified on thick-layer plates of silica gel using methylene dichloride as the developing solvent, followed by extraction of the product from the silica gel and recrystallization from methanol, m.p. 105–106 °C. Ultraviolet spectrum (ethanol): λ_{max} 303 and 316 mμ (shoulder at 332 mμ), (log ε of both 4.44). Infrared spectrum (KBr): ν_{max} 1760 cm^{-1}.

Anal. Calcd. for $C_{21}H_{20}O_7$: C, 65.6; H, 5.24. Found: C, 65.5; H, 5.42.

3,4′,5-Trihydroxy-3′-methoxystilbene-3-O-β-D-glucopyranoside Hexaacetate

The stilbene **4** (29 mg), silver oxide (32 mg), and drierite (50 mg) in anhydrous chloroform (5 ml) was treated with

tetra-*O*-acetyl-α-D-glucopyranosyl bromide (21 mg) in anhydrous chloroform (5 ml) for two days at room temperature. Thin-layer chromatographic (t.l.c.) examination of the reaction mixture, on silica gel G, using the developing solvent benzene – ether – ethanol (16:4:1), showed the major product of the reaction (substitution of one of the resorcinol ring hydroxyls) had R_f 0.21, and the minor product (substitution of the guaiacol ring hydroxyl) had R_f 0.13. The major product was separated on a thick layer of silica gel using the above solvent and, after extraction of the product from the silica gel, the product was treated with pyridine (0.5 ml) and acetic anhydride (0.3 ml). The reaction mixture stood at room temperature overnight and was worked up by removing the volatile reagents *in vacuo*. The product was recrystallized from methanol to m.p. 164–165 °C, undepressed on mixing with the natural sample. Similarly, the u.v. and i.r. spectra and t.l.c. behavior of both samples were identical. Ultraviolet spectrum (ethanol): λ_{max} 301 and 319 mμ (shoulder at 332 mμ) (log ε 4.63 and 4.65). Infrared spectrum (KBr): ν_{max} 1740 and 1760 cm^{-1}.

Anal. Calcd. for $C_{33}H_{36}O_{15}$: C, 58.9; H, 5.40. Found: C, 58.9; H, 5.33.

Acknowledgments

We thank the National Research Council of Canada for the awards of a Studentship and a Fellowship to D.H.A., and a bursary and two studentships to J. C. H. Also, we thank F. W. Matthews and Dr. T. K. H. Ruck for the X-ray diffraction spectra and Dr. K. D. Luke for the ultraviolet spectra.

1. G. BILLEK. *In* Progress in the chemistry of organic natural products. Vol. 22. *Edited by* L. Zechmeister. Springer-Verlag, New York. 1964. p. 115.
2. J. CUNNINGHAM, E. HASLAM, and R. D. HAWORTH. J. Chem. Soc. 2875 (1963).
3. W. GRASSMAN and H. ENDRES. J. Chem. Soc. 4579 (1965).
4. D. W. MANSON. Tappi, **43**, 59 (1960).
5. V. D. HARWOOD. Ph.D. Thesis. McGill University, Montreal, Que. 1949.
6. W. E. HILLIS and N. ISHIKURA. J. Chromatog. **32**, 323 (1968).
7. S. KAWAMURA. J. Pharm. Soc. Japan, **58**, 405 (1938).
8. W. GRASSMAN, H. HORMANN, and A. HARTL. Makromol. Chem. **21**, 37 (1957).
9. S. SIGGIA. Quantitative organic analysis via functional groups. John Wiley and Sons, Inc., New York. 1949.
10. M. SOMOGYI. J. Biol. Chem. **160**, 61 (1945).
11. R. M. HANN and C. S. HUDSON. J. Am. Chem. Soc. **66**, 735 (1944).
12. A. W. WESTON and C. M. SUTER. Organic syntheses. Vol. 21. John Wiley and Sons, Inc., New York. 1941. p. 27.