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On the Isolation of the Allergenically Active Components of the Toxic Principle of Poison Ivy

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The olefinic components of the poison ivy principle have been separated for the first time from natural sources in their allergenically active (free phenolic) form by a process involving benzylation, chromatography, and debenzylation. The composition of poison ivy "urushiol" has been estimated. A method is described for the cleavage of benzyl ethers in molecules containing multiple olefinic bonds, each separated by only one methylene group. The procedure results in debenzylation without alteration in the position or geometrical configuration of the olefinic bonds.

Poison ivy "urushiol" is made up of four components having the carbon skeleton of 3-pentadecylcatechol, and differing from each other only in the degree of unsaturation in the 15-carbon side chain.[2,3] The saturated and minor component, 3-pentadecylcatechol (Hydrourushiol), is a stable and colorless crystalline solid that has been available synthetically for some time[4] and has been extensively used in clinical studies.[5-10] The three olefinic components (mono-, di-, and triolefin), which make up approximately 95–97% of the urushiol principle, are labile oily substances that show marked sensitivity to air oxidation and polymerization. Except for the monoolefin, a very small amount of which was made available by synthesis several years ago,[11] the olefinic components of urushiol have not been available in pure form for clinical study. They were isolated from the natural principle, and their structures were determined, in the form of their dimethyl ether derivatives, which are not allergenically active.[2] It has not been possible to convert these methyl ethers back to the free and active catechols because the acid condition required for the hydrolysis causes rapid and extensive polymerization of such alkenyl phenols.

The synthesis of the monoolefinic component in the free phenolic (allergenically active) form was achieved *via* a dibenzyl ether intermediate which was subsequently debenzylated under conditions which did not structurally alter the olefinic side chain.[11] The present investigation was undertaken to explore the possibility that a similar procedure of benzylation and debenzylation might be employed as part of a chromatographic method for isolating, from the plant extract, each of the olefinic components of urushiol in allergenically active form.

Preliminary experiments on the benzylation and chromatography of cardanol[12,13] gave promising results. Consequently, crude poison ivy "urushiol" was treated with benzyl bromide following the procedure previously described[14] and was separated into its components by repeated column chromatography on grade I alumina.[15] The components were hydrogenated, and their unsaturation values were plotted against their respective refractive indices, showing a linear relationship.[15] Chromatographically pure samples of the dibenzyl ethers of the monoolefinic (II), the diolefinic (III), and the triolefinic components (IV) were obtained, and their relative concentrations were determined to be: monoolefin (and saturated component), about 12%; diolefin, about 64%; and triolefin, about 23% (Table I).

TABLE I

HYDROGENATION OF THE CHROMATOGRAPHICALLY SEPARATED DIBENZYL ETHER COMPONENTS OF BENZYLATED "URUSHIOL"[a]

Component	n^{25}D, deg.	Estd. amt., %	Moles of H_2 —absorbed— Total	Olefinic	Unsaturation value[b]
Saturated	1.5315[c]	2	2.00[d]	...	...
Monoolefin	1.5362	10	2.91	0.91	0.09
Diolefin	1.5455	64	4.06	2.06	1.32
Triolefin	1.5551	23	4.86	2.86	0.66

[a] Original n^{25}D 1.5460, d.b.v. 1.92 ± 0.05. [b] Contribution to the approximate d.b.v. of 2 of the original benzylated "urushiol" sample as calculated on the basis of amounts of each component estimated from the chromatogram. [c] Since dibenzylhydrourushiol is a solid at room temperature (m.p. 58–59°), its refractive index at 25° was obtained by extrapolating values obtained using molten and supercooled material between 40 and 70°. [d] For the debenzylation reaction 2.00 moles of H_2 is theoretically required.

Because of its very low concentration in the "urushiol" mixture, it was not convenient to obtain a chromatographic fraction corresponding to the saturated

(1) (a) This paper is based on a portion of the thesis submitted by Kenneth H. Markiewitz to Columbia University in partial fulfillment of the requirements for the Ph.D. degree in chemistry. (b) To whom inquiries should be made.

(2) W. F. Symes and C. R. Dawson, *J. Am. Chem. Soc.*, **76**, 2959 (1954).

(3) C. R. Dawson, *Trans. N. Y. Acad. Sci.*, **18**, 427 (1956).

(4) C. R. Dawson, D. Wasserman, and H. Keil, *J. Am. Chem. Soc.*, **68**, 534 (1946).

(5) H. Keil, D. Wasserman, and C. R. Dawson, *J. Exptl. Med.*, **80**, 275 (1944).

(6) H. Keil, D. Wasserman, and C. R. Dawson, *J. Allergy*, **16**, 275 (1945).

(7) H. Keil, D. Wasserman, and C. R. Dawson, *Ind. Med.*, **14**, 285 (1945).

(8) A. M. Kligman, *A. M. A. Arch. Dermatol.*, **77**, 149 (1958); **78**, 47 (1958).

(9) W. L. Epstein and A. M. Kligman, *J. Invest. Dermatol.*, **28**, 291 (1957).

(10) S. Srinivasan, H. Baer, R. Bowser, and A. Karman, *Anal. Biochem.*, **6**, 234 (1963).

(11) B. Loev and C. R. Dawson, *J. Org. Chem.*, **24**, 980 (1959).

(12) All of the components of cardanol, the alkenyl monophenol of cashew nut shell liquid, have the carbon skeleton of 3-pentadecylphenol, and differ only from each other in the olefinic character of the normal 15-carbon side chain.

(13) S. V. Sunthankar, unpublished observations, Columbia University, 1953.

(14) B. Loev and C. R. Dawson, *J. Am. Chem. Soc.*, **78**, 6095 (1956).

(15) W. F. Symes and C. R. Dawson, *ibid.*, **75**, 4952 (1953).

component. The presence of this component in the original "urushiol" mixture was demonstrated, however, when, in the course of a low-temperature fractional crystallization of the debenzylated monoolefinic component, a small amount (1.7%) of hydrourushiol was isolated.

It has been observed in these laboratories that the different olefinic components of poison ivy "urushiol," Japanese Lac urushiol, cardanol, etc., vary considerably in their stability, the more highly unsaturated components being the least stable. Therefore, the composition values given above for the tri- and diolefinic components of the benzylated urushiol mixture may be somewhat lower than those that would be found in the urushiol as it exists in the plant.

A synthetic sample of the dibenzyl ether of hydrourushiol and the isolated dibenzyl ethers of the mono-, di-, and triolefinic components were found to have almost identical ultraviolet spectra in regard to the position and magnitude of their absorption maxima and minima. This fact, as pointed out elsewhere,[2] precludes the existence of conjugation in the di- and triolefinic components of the chromatographically separated dibenzyl ethers.

It is of interest to contrast the alumina-stable character of the nonconjugated *cis* double bond system (8,-11,14) of the triolefinic component of poison ivy "urushiol" with that of the olefinic system of the triolefinic component of Japanese Lac. The Lac olefin contains a conjugated diene system (8,11,13),[16] which has been found to rearrange into a completely conjugated system in the presence of alumina.[17]

In the process of working up the dibenzyl ethers, a yellowish green color rapidly developed unless the temperature was kept below 45° and essentially oxygen-free conditions were maintained. Such colored oxidation products were found to be low in unsaturation and to possess some carbonyl function (infrared). Furthermore, the vinyl infrared absorption characteristic of the pure triolefinic component was found to be considerably reduced in intensity.

Prior work in these laboratories has shown that the benzyl ethers of alkenylphenols are cleaved readily by sodium and butanol without changing the position or geometrical configuration of an isolated double bond.[11,14] However, a new procedure had to be developed in the present investigation when the sodium–butanol cleavage method was found to be unsatisfactory for alkenylphenols having multiple olefinic bonds, each separated by only one methylene group. When a chromatographed sample of benzylated "urushiol" having a double bond value (d.b.v.) of 1.75 and consisting of approximately 75% of the diolefinic and 25% of the monoolefinic component was treated with sodium and butanol, the d.b.v. of the cleavage products dropped to 1.5. Furthermore, ultraviolet and infrared spectral analysis revealed that approximately one-third of the material now contained a conjugated diene system within the 15-carbon side chain. The reaction conditions employed (2 hr. refluxing at 120°) are not very different from those that have been found to result in optimum conjugation of the polyolefinic components of ethylcardanol, *i.e.*, strong base at 140° for 20 min.[18]

The alkali isomerization of olefins containing multiple double bonds separated from each other only by one methylene group has been explained in terms of the abstraction of a hydrogen from the methylene group activated by the two adjacent centers of unsaturation.[19] The absence of such a methylene group in the monoolefin presumably accounts for its stability under the same conditions.[11,14] It is also known[20] that conjugated diene systems are reduced to monoolefins by metallic sodium. Thus the observed loss in unsaturation was very likely due to the reduction of diolefinic material which had become conjugated under the conditions of the experiment.

In view of the above results it became necessary to search for a milder way of accomplishing the debenzylation process, and attention was turned to the use of sodium–potassium alloy dispersions in ligroin. This reagent had been successfully used under mild conditions for the cleavage of diphenyl ether[21] and a variety of methyl ethers.[22] Before applying this method of debenzylation to the dibenzyl ethers of the olefinic components of poison ivy "urushiol," a sample of benzylhydrocardanol was treated with a 2 molar excess of the Na–K alloy dispersed in petroleum ether at 35° for 5 min. A quantitative yield of hydrocardanol and toluene was obtained. The dibenzyl ether of hydrourushiol was also found to be quantitatively cleaved by treatment with a 2 molar excess of the Na–K alloy dispersed in ligroin at 75° for 5 min. When the same conditions were applied to the "urushiol" dibenzyl ethers the debenzylated olefinic components were obtained in good yield without detectable modification of the olefinic unsaturation.

The products obtained from the debenzylation of the "urushiol" dibenzyl ethers were orange oils that were immediately purified by molecular distillation. The resulting slightly yellow oils were obtained in good yield and without loss in unsaturation, a point worth noting in view of the known thermal instability of alkenylphenols.[11] The di- and triolefinic products were immediately examined spectrally for evidence of isomerization or conjugation of the olefinic systems,[15] and none was found. The triolefinic component showed strong vinyl absorption at 10.1 and 11.0 μ corresponding to a terminal olefinic bond.

Little is known about the mechanism of the reaction or the nature of the reaction complex involved in the cleavage of ethers by Na–K alloy. However, it appears that no actual bond breaking occurs until a source of protons (such as an alcohol) is added after the formation of the complex. When gaseous sulfur dioxide was introduced into the cleavage reaction mixture prior to the addition of the proton source, the dibenzyl ether was recovered unchanged.

Because of the necessity of making many determinations of double bond values on very limited amounts of chromatographically purified olefins, a microhydrogenation method was developed, based on the use of

(16) S. V. Sunthankar and C. R. Dawson, *J. Am. Chem. Soc.* **76**, 5070 (1954).

(17) K. H. Markiewitz, unpublished observations.

(18) P. Monnikendam, Ph.D. Dissertation, Columbia University, 1957.

(19) P. L. Nichols, S. F. Herb, and R. W. Riemenschneider, *J. Am. Chem. Soc.*, **73**, 247 (1951); A. M. Abu-Nasr and R. T. Holman, *J. Am. Oil Chemists' Soc.*, **32**, 414 (1955).

(20) A. Klages, *Ber.*, **35**, 2633, 2646 (1902).

(21) E. Mueller and W. Bunge, *ibid.*, **69**, 2171 (1936).

(22) K. Ziegler and B. Schnell, *Ann.*, **437**, 227 (1924).

the Warburg constant volume respirometer.[23] The method proved to be most satisfactory even for samples as small as 1–2 mg.

Experimental[23–25]

Poison ivy "urushiol" previously extracted from the plant[26] had been stored in a brown bottle for about 2 years.[27] Its black color and high viscosity indicated the presence of oxidation and polymerization products accumulated mainly during the storage period.

Dibenzyl "Urushiol" (I).—A mixture of 29.1 g. of crude "urushiol," 25 ml. of benzyl bromide, and 30 g. of potassium carbonate in 50 ml. of acetone was refluxed for 15 hr. under an atmosphere of nitrogen. Filtration of the reaction mixture, followed by vacuum distillation of solvent and unreacted benzyl bromide left 33 g. of a viscous black oil. This oil was put on a column of grade I alumina (1100 g.) and washed with ligroin to remove unadsorbed products. The column was then thoroughly extracted with anhydrous diethyl ether to yield 16 g. of a yellow oil (I), $n^{25}D$ 1.5460, having a double bond value (d.b.v.) of 1.92 ± 0.05 (absorbed 3.92 moles of hydrogen). Its ultraviolet spectrum was identical with that of a synthetic sample of the dibenzyl ether of "hydrourushiol." The hydrogenated material was identified as 3-pentadecylcatechol (hydrourushiol) by its ultraviolet spectrum and melting point. Similar results were obtained in several runs, and it appears that the benzylation reaction went in about 75% yield.[28] Deactivation of the alumina column with water, and subsequent extraction with diethyl ether yielded 10.2 g. of a black oil, $n^{25}D$ 1.538, which was not further investigated except that its ultraviolet spectrum indicated mainly oxidation products.

Chromatographic Separation of I.—A 14-g. sample of I in 200 ml. of ligroin (b.p. 64–68°) was passed through a column of 1540 g. of grade I alumina. The column was fully developed under an atmosphere of nitrogen with a 12.5% solution of anhydrous diethyl ether in ligroin. The alumina was extruded and sectioned into 20 equal fractions, and each fraction was extracted with anhydrous diethyl ether. The solvent was removed by careful heating up to 60° under nitrogen; total recovery, 84.5%. The residues were combined according to their refractive indices: (A) 1.53 g., $n^{25}D$ 1.5365–1.5401 (13%), monoolefinic; (B) 6.48 g., $n^{25}D$ 1.5432–1.5490 (55.7%), diolefinic; (C) 2.75 g., $n^{25}D$ 1.5515–1.5570 (23.5%), triolefinic; and (D) 0.89 g., $n^{25}D$ 1.5595–1.5620 (7.6%), decomposition products. The olefinic components thus obtained from several similar chromatographic separations of I were combined and rechromatographed repeatedly until satisfactorily pure samples of each of the olefins were obtained.

In this manner a monoolefinic component (II) was obtained from fraction A ($n^{25}D$ 1.5362). A sample of $n^{25}D$ 1.5353 absorbed 2.91 moles of hydrogen (over Pd–C) corresponding to a d.b.v. of 0.91 (see Table I). The reduction product was pure hydrourushiol, identified by melting point and ultraviolet spectrum.

Anal. Calcd. for one double bond $C_{35}H_{46}O_2$: C, 84.41; H, 9.17. Found: C, 84.77; H, 9.29.

The diolefinic component (III) was obtained from fraction B ($n^{25}D$ 1.5455). A sample of $n^{25}D$ 1.5459 absorbed 4.06 moles of hydrogen (over Pd–C) corresponding to a d.b.v. of 2.06 and gave pure hydrourushiol.

Anal. Calcd. for two double bonds $C_{35}H_{44}O_2$: C, 84.63; H, 8.93. Found: C, 84.98; H, 8.69.

Repeated chromatographic purification of fraction C using a less polar solvent system (4.5% anhydrous diethyl ether in ligroin) gave IV, the triolefinic component ($n^{25}D$ 1.5551). On catalytic reduction it absorbed 4.86 moles of hydrogen and gave pure hydrourushiol (97% of theoretical for three double bonds).

Anal. Calcd. for three double bonds $C_{35}H_{42}O_2$: C, 84.97; H, 8.56. Found: C, 85.15; H, 8.45.

The results of the hydrogenation experiments are summarized in Table I.

Sodium and Butanol Cleavage of the Dibenzyl Ether of the Diolefinic Component of Poison Ivy "Urushiol" (I).—Following the procedure developed by Loev,[14] 2.0 g. (0.004 mole) of I (d.b.v. 1.78) was dissolved in 50 ml. of butanol. The reaction vessel was flushed with nitrogen. Refluxing was maintained during the addition of 4.0 g. (0.174 g.-atom) of sodium over a period of 1 hr. After cooling, 25 ml. of water containing a little sodium hydrosulfite was added, and the reaction mixture was neutralized with 12 ml. of glacial acetic acid in 95 ml. of water. The nonaqueous layer was salted out, washed with a saturated salt solution containing some bicarbonate and hydrosulfite, and finally washed with distilled water. After drying over magnesium sulfate, it was heated *in vacuo* under nitrogen (below 60°) to remove the butanol. The residue was 950 mg. of a dark orange oil, samples of which gave a dense white precipitate with methanolic lead acetate and a momentary green color changing to black with ferric chloride solution.

The oil, $n^{25}D$ 1.519, d.b.v. 1.5 ± 0.1, was distilled at an oil bath temperature of 150–200° and a pressure of less than 10^{-3} mm. to give 450 mg. of a yellow viscous oil, $n^{25}D$ 1.5115, d.b.v. 1.4 ± 0.1. Its ultraviolet and infrared spectra showed the presence of conjugated olefinic material.

3-Pentadecadienyl-8′,11′-catechol.—To a suspension of sodium–potassium alloy[29] (230 mg. of sodium, 780 mg. of potassium, 0.030 g.-atom) in 15 ml. of ligroin (b.p. 66–68°) was added 1.8 g. (0.0036 mole) of I (d.b.v. 1.9 ± 0.1) in 15 ml. of ligroin. The colorless solution was refluxed and rapidly stirred under nitrogen. It underwent many changes in color, ranging from light blue to yellow to red, and formed a heavy red flocculent precipitate. Precipitation appeared to be complete within 5 min. Thereupon the system was quickly cooled to room temperature. The slow addition, with constant cooling, of 3 ml. of butanol rapidly decomposed the red precipitate and gave a clear yellow solution. It was neutralized by the slow addition of 2 ml. of glacial acetic acid in 10 ml. of ligroin.[30] Finally large amounts of water containing sodium hydrosulfite were added. The nonaqueous layer was separated, washed several times with distilled water, and dried over magnesium sulfate. The resulting light yellow liquid was then heated (45°) *in vacuo* to remove solvent.

The residual orange oil was immediately placed into a cylindrically shaped molecular still having a cold finger. The distillate from the cold finger dropped into a four-tube fraction cutter. The main fraction (600 mg.) was collected at 148–166° (10^{-4} mm.). It was a faintly yellow-colored oil (d.b.v. 1.9 ± 0.1) which solidified at −5°: $n^{25}D$ 1.5178; over-all yield, 75%. The ultraviolet and infrared spectra showed no evidence for conjugated or transisomerized double bonds.

Anal. Calcd. for $C_{21}H_{32}O_2$: C, 79.70; H, 10.19. Found: C, 79.56; H, 10.16.

3-Pentadecylcatechol.—Following the procedure described above, a sample of the dibenzyl ether of the monoolefinic component (d.b.v. 0.92, $n^{25}D$ 1.5375) was cleaved and molecularly distilled (76% yield). The colorless distillate partially crystallized at room temperature. About one-third of the material was crystallized from pentane at −20° as a colorless solid melting at 49–52°. The crystals were mainly hydrourushiol (3-pentadecylcatechol). This was the only time during the separation and cleavage procedures that the existence of the saturated component could be confirmed. It makes up about 2% of the original benzylated "urushiol."

3-Pentadecenyl-8′-catechol.—The mother liquor from the above low-temperature crystallization contained a slightly yellow oil of $n^{25}D$ 1.5115 and d.b.v. 1.1. This material was mainly 3-pentadecenyl-8′-catechol contaminated with a very

(23) Details of the microhydrogenation technique and of other phases of this investigation are described in the dissertation of K. H. Markiewitz, a microfilm of which may be obtained from the Columbia University Library.

(24) Microanalyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y.

(25) All melting points are corrected.

(26) G. A. Hill, V. Mattacotti, and W. D. Graham, *J. Am. Chem. Soc.*, **56**, 2736 (1934).

(27) The original extract was prepared by the Lederle Laboratories Division of the American Cyanamid Co., Pearl River, N. Y.

(28) Hydrourushiol was also benzylated by the above procedure and the dibenzyl ether was obtained in 75% yield.

(29) G. van Rossen Hoogendijk van Bleiswijk, *Z. Anorg. Chem.*, **74**, 152 (1912).

(30) It was subsequently found that the decomposition of the red reaction complex and neutralization of the basic system could be accomplished simultaneously by adding a dilute solution of glacial acetic acid in ligroin.

small amount of the diolefin.[31] The oil was indistinguishable from a synthetic sample of the monoolefinic component[11] by spectral and refractive index data. Catalytic reduction yielded pure hydrourushiol.

3-Pentadecatrienyl-8′,11′,14′-catechol.—A sample of the dibenzyl ether of the triolefinic component (n^{25}D 1.5557, d.b.v. 2.5)[32] was debenzylated and molecularly distilled in 75% yield. The product (mainly 3-pentadecatrienyl-8′,11′,14′-catechol) was a nearly colorless oil (n^{25}D 1.5250)[31] that showed no evidence of conjugation or transisomers on ultraviolet and infrared analysis. Catalytic hydrogenation revealed a d.b.v. of 2.65 and resulted in pure hydrourushiol.

Cleavage of Benzylhydrocardanol.—In order to examine all of the products of the reductive cleavage reaction, a sample of benzylhydrocardanol (the benzyl ether of 3-pentadecylphenol) was debenzylated. The reaction mixture was put immediately onto a column of grade I alumina and washed with pentane. The washings, upon spectrophotometric assay, were found to contain only toluene in an amount corresponding to a quantitative cleavage of the benzyl ether.

The column was thereupon eluted with 95% ethanol and the eluate was assayed in the same manner. It contained only the theoretical amount of 3-pentadecylphenol.

(31) The unsuspected presence of the saturated component resulted in the taking of too large a chromatographic cut for the debenzylation of the "monoolefinic" component. As a consequence this cut contained a small amount of diolefinic material. The refractive index–double bond relationship is linear.[15] Consequently, on the basis of pure hydrourushiol (n^{25}D 1.4990) and the pure diolefin (n^{25}D 1.5178) the pure monoolefin and the triolefin have, respectively, the following n^{25}D values: 1.5084 and 1.5272.

(32) The amount of chromatographically pure triolefin (dibenzyl ether) available was so limited that it was necessary to include some fractions contaminated with diolefinic material for the cleavage experiment.

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An Aldotetraouronic Acid from the Hydrolysis of a Paper Birch 4-*O*-Methylglucuronoxylan[1]

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Partial hydrolysis of a paper birch 4-*O*-methylglucuronoxylan afforded a mixture of oligosaccharides and oligouronides. An aldotetraouronic acid fraction was isolated by preparative paper chromatography and was separated into three components by paper electrophoresis. The structure of the principal component (I) appeared to be *O*-β-D-xylopyranosyl-(1→4)-*O*-[4-*O*-methyl-α-D-glucuronopyranosyl-(1→2)]-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose, based on the isolation of 3-*O*-methyl-D-xylose, 2,3,4-tri-*O*-methyl-D-xylose, 1,2,3,5-tetra-*O*-methylxylitol, and 2,3,4-tri-*O*-methyl-D-glucose in approximately equal molar amounts. This was accomplished through methylation, reduction, and methanolysis of the parent material followed by gas chromatographic analysis. Hypotheses of conformational resistance and steric interference with protonation were used to explain the occurrence of component I in the hydrolysate.

The 4-*O*-methylglucuronoxylans have been isolated from several hardwoods and all examples of this hemicellulose have essentially the same structure.[2] As illustrated in Figure 1, this polysaccharide consists of a β-1,4-linked xylan chain, substituted randomly at C-2 of the xylose units with 4-*O*-methyl-α-D-glucuronic acid.

Hydrolysis of this hemicellulose in dilute mineral acid yields two series of oligosaccharides: one of β-1,4-linked xylodextrins and a second closely related acidic series. One of the principal acidic oligosaccharides is the crystalline aldotriouronic acid [C(F)D]. All efforts to isolate the isomeric aldotriouronic acid [BC(F)] have failed.[3,4] Two hypotheses have been advanced to account for these observations.

Marchessault and Rånby[5] have suggested that linkage C–D, in Figure 1, has been stabilized through the inductive influence of the uronic acid carboxyl groups. These workers have further suggested simultaneous activation of linkage B–C through the same mechanism. McKee[6] has shown, through study of a 4-*O*-methylglucoxylan, that stabilization of linkage C–D is not dependent on the carboxyl group of unit F. McKee has attributed the resistance to hydrolysis of C–D to the conformational stability of unit C. This stability, induced by the size of the acidic substituent at C-2 of this xylose unit, hinders adoption of a necessary hydrolysis intermediate and in turn stabilizes C–D. McKee's work did not indicate whether or not linkage B–C had been activated, as suggested by Marchessault and Rånby.

In order to test these hypotheses, a 4-*O*-methylglucuronoxylan, isolated from paper birch, was partially hydrolyzed in dilute sulfuric acid. Paper chromatography indicated the usual spectrum of oligosaccharides. The aldotetraouronic acid was isolated by preparative paper chromatography and electrophoresis and was characterized by $[\alpha]^{25}$D 24°, equiv. wt. 604, R_x 0.14 (solvent A), and M_g (mobility) 0.56 (0.1 *M* sodium borate). These data are in general agreement with those of other workers.[3,7]

Three possible tetrasaccharide acid isomers may be expected to occur in the hydrolysate of a 4-*O*-methylglucuronoxylan, on the basis of the supposed structure of this hemicellulose: the linear isomer I [C(F)DE] previously isolated by Timell,[8] the branched isomer II [BC(F)D], and the linear isomer III [ABC(F)]. A reaction sequence involving sodium borohydride reduction, methylation, lithium aluminum hydride reduction, and cleavage of the glycosidic linkages would produce the sugars and sugar alcohols shown in Table I. Anal-

(1) A portion of a thesis submitted in partial fulfillment of the requirements of The Institute of Paper Chemistry for the Ph.D. degree from Lawrence College, Appleton, Wis., June 1964. This work was carried out under the direction of E. E. Dickey.

(2) G. O. Aspinall, *Advan. Carbohydrate Chem.*, **14**, 429 (1959).

(3) J. K. Hamilton and N. S. Thompson, *J. Am. Chem. Soc.*, **79**, 6464 (1957).

(4) H. C. Srivastava, C. T. Bishop, and G. A. Adams, *J. Org. Chem.*, **26**, 3958 (1961).

(5) R. H. Marchessault and B. G. Rånby, *Svensk Papperstid.*, **62**, 230 (1959).

(6) S. C. McKee and E. E. Dickey, *J. Org. Chem.*, **28**, 1561 (1962).

(7) T. E. Timell, *Can. J. Chem.*, **40**, 22 (1962).

(8) T. E. Timell, *Svensk Papperstid.* **65**, 435 (1962).