Cleavage experiments were also carried out with lignin sulfonates prepared from Western hemlock, Douglas fir and Sitka spruce woods and the results are given in Fig. 2 and Table III along with other results from experiments with the mixed species dialyzable and non-dialyzable lignin sulfonates, MD and MN. The yields of the ether-soluble substances obtained from the various samples range from 40 to more than 50% and the total yields of vanillin, vanillic acid and acetovanillone amount to 25 to about 30%. These values are in the same range as those found by Pearl and Beyer,[3,4] and by Leopold,[5] who studied the nitrobenzene oxidation of lignin sulfonates. The total yields obtained from the hemlock, spruce and dialyzable mixed species lignin sulfonates are believed to be significantly higher than those obtained from the Douglas fir and the non-dialyzable lignin mixed species samples. This may arise because of the presence in these latter preparations of a higher proportion of linkages resistant to cleavage.

In the electrophoresis patterns there were evident several peaks which could not be identified with substances known to be lignin cleavage products and available to us. Each of these substances was designated by a letter, *e.g.*, A, B, F, etc., as indicated on Figs. 1 and 2, and was characterized in a preliminary manner by measurement of electrophoretic mobility (Table V) and by absorption spectrum (Fig. 3). Extinction coefficients of these compounds are unknown so that the proportions in which they were observed are represented in Table IV in units of percentage of the total ultraviolet absorption of the substances in the fraction examined by electrophoresis. Some of these substances may be those recently identified by Pearl and Beyer[3,4] or by Leopold,[5] and others may be new products of the alkaline cleavage of lignins. The substance B has recently been shown to be identical with vanilloylformic acid.[11]

In similar experiments with lignin sulfonates from maple (*Acer macrophyllum*), Ryan[12] with one of the authors has used the electrophoresis technique to establish the presence in the reaction product mixture of vanillin, vanillic acid, syringaldehyde and syringic acid. Several other substances were observed which were not identified, although evidence was obtained for the presence of acetovanillone and possibly syringoylformic acid.

The authors appreciate the gift from Dr. I. A. Pearl, Institute of Paper Chemistry, Appleton, Wisconsin, of the substances DV, 5CV, DVA and 5CVA, and also the gift from Dr. C. Russell, Northern Regional Research Laboratory, Peoria, Illinois of the substances 5AV, FA, 5AVA, 5CV, 5FVA, DVA and 5CVA as listed in Table V. They are grateful to Mr. D. W. Glennie for help in preparation of this manuscript.

[11] D. W. Glennie, H. Techlenberg, E. Reaville and J. L. McCarthy, THIS JOURNAL, 77, 2409 (1955).

[12] A. S. Ryan, M.S. Thesis, University of Washington, 1950.

SEATTLE, WASHINGTON

[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY AND CHEMICAL ENGINEERING, UNIVERSITY OF WASHINGTON]

Lignin. V. Vanilloylformic Acid from Alkaline Cleavage of Lignin Sulfonates[1,2]

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Alkaline cleavage of lignin sulfonates in the presence of cupric oxide yields a complex mixture of phenolic substances and partial separation of the components of this mixture has been achieved by means of anion-exchange resins. Some known cleavage products have been separated. In addition, a substance apparently heretofore unrecognized as a cleavage product has been isolated and shown to be vanilloylformic acid by comparison with authentic samples. Two methods for the synthesis of vanilloylformic acid have been developed to supplement those previously described in the literature.

Introduction

Previous studies[4-6] of the alkaline cleavage of lignin sulfonates in the presence of various oxidants have shown the reaction products to include low molecular weight phenols such as guaiacol, vanillin, acetovanillone, vanillic acid, 5-carboxyvanillin, 5-carboxyvanillic acid, 5-formylvanillin and 5-formylvanillic acid. These and other products of the reaction are obtained in complex mixtures and electrophoretic examinations of these mixtures have led to the detection in this Laboratory of several apparently new lignin cleavage products which have been characterized by electrophoretic mobility and ultraviolet absorption.[1] One of these has now been isolated in crystalline form and the object of the present paper is to describe the method used to accomplish its separation and to report evidence for its identity with vanilloylformic acid.

Discussion

Alkaline cleavage of the lignin sulfonates was carried out with a sodium hydroxide solution in the presence of cupric oxide under conditions similar to those employed by Pearl.[7] There resulted a dark colored solution which was filtered to remove brown solids, acidified, then extracted to obtain total ether-soluble products amounting to 22.7% of the lignin, assuming it to contain 14.5% methoxyl. The electrophoresis pattern[8] for these prod-

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[1] For Paper IV of this series, see R. E. Davis, E. T. Reaville, Q. P. Peniston and Joseph L. McCarthy, THIS JOURNAL, 77, 2405 (1955).

[2] Presented in part at Pacific Northwest Regional meetings of the American Chemical Society at Richland, Washington (1954) and Pullman, Washington (1953).

[3] Hooker Electrochemical Company Research Fellow.

[4] F. E. Brauns, "The Chemistry of Lignin," Academic Press Inc., New York, N. Y., 1952, pp. 546–562.

[5] I. A. Pearl, THIS JOURNAL, 72, 2309 (1950).

[6] B. Leopold, *Acta Chem. Scand.*, 6, 38 (1952).

[7] I. A. Pearl and D. L. Beyer, *TAPPI*, 33, 544 (1950).

[8] Q. P. Peniston, H. D. Agar and J. L. McCarthy, *Anal. Chem.*, 23, 994 (1951).

ucts is shown in Fig. 1a and the pattern for this same preparation after adding synthetic vanilloylformic acid is shown in Fig. 1b.

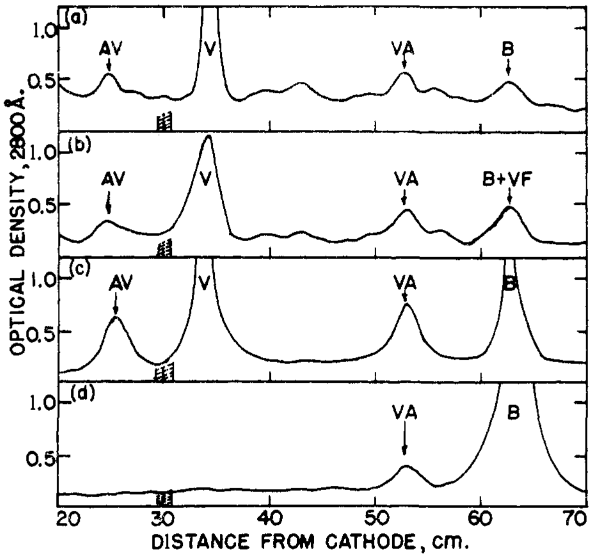

Fig. 1.—Electrophoresis patterns: (a) total ether extract from cupric oxide oxidation of lignin sulfonates; (b) total ether extract from cupric oxide oxidation of lignin sulfonates with synthetic vanilloylformic acid added; (c) first one third extracted of total ether extract; (d) substances eluted from anion-exchange column. Letters show position in electrophoresis tube of acetovanillone, AV; vanillin, V; vanillic acid, VA; substance B and vanilloylformic acid, VF. Cross-hatching at 30 cm. indicates initial position of sample.

The ether extraction was conducted stepwise and only the first 33% of the total ether-soluble material was used for the separation because the electrophoresis pattern for the next about 1% of material extracted (Fig. 1c) showed the presence of only vanillin, acetovanillone, vanillic acid and also an unidentified substance which was designated as B,[1] and has now been found to be vanilloylformic acid. The one third of the ether-extracted material was dissolved in water, the ether was removed, and the solution was passed through a Duolite A-2 anion-exchange resin column. Examination of the eluant showed partial separation of vanillin, acetovanillone, vanillic acid and B which appeared in the order named. A cut containing mostly B with some vanillic acid was taken, examined by electrophoresis (Fig. 1d), and refractionated by adding it to an anion-exchange resin which had been treated with sodium bisulfite solution and then eluting with a dilute sodium bisulfite solution. Electrophoretic examination showed absence of the vanillic acid in the eluant cut which was acidified and extracted with ether to obtain B as very small pale yellow plates. After purification by recrystallization from dry benzene, B melted at nearly the same temperature as authentic vanilloylformic acid, and a mixture of the two gave no melting point depression. Identification of B as vanilloylformic acid is also supported by comparison of the results of measurements of extinction coefficients (Table I) and electrophoretic mobilities (Fig. 1), and by comparison of the properties of the derivatives described below.

TABLE I

ULTRAVIOLET ASBORPTION BY VANILLOYLFORMIC ACID AND OTHER SUBSTANCES

Compound	In ethanol λmax Å.	In ethanol εmax	In alkaline ethanol λmax Å.	In alkaline ethanol εmax
B	3100	9,700	3500	21,600
Vanilloylformic acid	3100	9,500	3500	23,900
Vanillin[a]	3100	10,800	3530	30,100
Acetovanillone[a]	3030	7,800	3480	22,000
Veratroylformic acid	3080	6,930	3060	7,050
Protocatechuoylformic acid	3120	8,380	3760[b]	13,700[b]
2,4-D[c] of B	4080	31,200	..	....
2,4-D[c] of vanilloylformic acid	4080	29,500	..	....

[a] Calculated from data reported by Lemon.[9] [b] Measured in aqueous alkaline solution. [c] "2,4-D" indicates 2,4-dinitrophenylhydrazone.

Authentic vanilloylformic acid was obtained by synthesis in two ways. In a modification of Bouveault's method,[9] veratroylformic acid was demethylated with aluminum chloride to provide a mixture of the starting material, vanilloylformic acid and protocatechuoylformic acid from which vanilloylformic acid was isolated in only about 1% yield. A more successful synthesis was achieved by use of a modification of the method of Schwartz and Capek[10] employed by them for the preparation of isovanilloylformic acid.

Vanilloylformic acid is perhaps to be expected as an alkaline cleavage product of lignins, since Freudenberg and co-workers have reported[11] the possible presence of veratroylformic acid among methylated products obtained by alkali fusion of hydrochloric acid lignin. They have also recorded[12] the isolation in small yield of veratroylformic acid from spruce lignin treated with alkali, methylated and finally oxidized with permanganate, although in this case the veratroylformic acid may have arisen in part from acetovanillone which was methylated to acetoveratrone and subsequently oxidized.[13] In a study of nitrobenzene oxidation of model lignin compounds, von Wacek and Kratzl[14] suggested that vanilloylformic acid might be present among the products obtained from oxidation of propioguaiacone. However, under their conditions, acetovanillone was almost quantitatively converted to vanillin and, if this conversion proceeds through a vanilloylformic acid intermediate, it can be inferred that vanilloylformic acid is not likely to survive conventional nitrobenzene oxidations of lignins.[4] Thus, the presence of vanilloylformic acid among the cleavage products of lignins would seem to be dependent on the nature and conditions of the cleavage treatment. Possibly the species of wood used may also be of importance,

(9) M. L. Bouveault, *Bull. soc. chim.*, [3] **19**, 76 (1898).

(10) R. Schwartz and V. K. Capek, *Monatsh.*, **83**, [4] 883 (1952).

(11) K. Freudenberg, H. Harder and L. Marker, *Ber.*, **61**, 1760 (1928).

(12) K. Freudenberg, A. Janson, B. Knopf and A. Haag, *ibid.*, **69**, 1415 (1936).

(13) P. Dreyfus and C. Cocuzza, *Gazz. chim. ital.*, **68**, 95 (1938).

(14) A. von Wacek and K. Kratzl, *Ber.*, **76**, 891 (1943).

although it was found in previous work[1] that vanilloylformic acid was obtained from hemlock, Douglas fir and spruce wood lignins.

For the present alkaline cleavage experiments in the presence of cupric oxide, the yield of vanilloylformic acid is estimated from the electrophoresis patterns by the previously described procedure[1] to be about 0.4% of the lignin. From prior results, the yield in the presence of mercuric oxide is indicated to be about 2% of the lignin. However, treatments of pure vanilloylformic acid under conditions similar to those of its formation from lignin sulfonates in this investigation have shown that about 40% disappeared and that an almost corresponding amount of vanillic acid appeared with a possible trace of vanillin, while acetovanillone remained essentially unchanged when treated in this way. It thus appears that under present conditions vanilloylformic acid is one intermediate in the formation of vanillic acid and possibly vanillin from lignin sulfonates while acetovanillone is a stable end-product.

Experimental[15]

Alkaline Cleavage.—Experiments were conducted with a calcium lignin sulfonate preparation consisting of fermented calcium sulfite spent liquor solids which were found by previously described analytical methods[16] to have the following characteristics: 3.97% H_2O, 8.26% OCH_3, 9.53% reducing substances calculated as glucose, 6.62% CaO, 17.75% sulfated ash, and 14.8 mm.^{2}/day for the diffusion coefficient of material absorbing 2800 Å. ultraviolet radiation.[17]

This material (2300 g.) was mixed in 10 liters of water in a stainless steel autoclave with 2010 g. of cupric hydroxide and with enough copper sulfate and sodium hydroxide to yield 2330 g. of cupric hydroxide and 1610 g. of sodium hydroxide. The autoclave, while being rotated at 20 r.p.m., was heated by indirect steam to 178° in one hour, held at this temperature for an additional three hours, allowed to cool for 0.5 hour, and then opened. The resultant dark colored solution was filtered to remove suspended solids which were washed to yield 20 l. of filtrate and washings. A 2-l. aliquot was acidified to *p*H 1 and the solution with precipitated solids was extracted stepwise with ether to obtain 29.5 g. of total ether-soluble material. This yielded an electrophoresis pattern of the type shown in Fig. 1a and, when synthetic vanilloylformic acid was added, the pattern of Fig. 1b was obtained. After extraction of the first 9.4 g. of ether-soluble material, electrophoresis on the next 0.5 g. extracted gave the pattern of Fig. 1c.

Isolation of Vanilloylformic Acid.—The two ether extracts (9.9 g.) were dissolved in 1 l. of water, the ether removed, and the solution passed through a Duolite A-2 anion-exchange resin column consisting of three sections; a top section (9.0 cm. high by 7.0 cm. inside diameter), a middle section (5.0 cm. by 3.0 cm.), and a bottom section (2.3 cm. by 1.5 cm.) packed with resin 100 to 170 in screen-mesh size. Eluant fractions were collected and analyzed electrophoretically and samples were combined to obtain a "cut" of about 8000 o.d.-ml. containing vanilloylformic acid with some vanillic acid as indicated in Fig. 1d. About 6000 o.d.-ml. of this material was flowed through a 7.5 cm. by 1.5 cm. column of Duolite A-2 anion-exchange resin passing a 170 mesh screen which had been saturated with an aqueous sodium bisulfite solution and then washed with distilled water. Vanillic acid and vanilloylformic acid were eluted with 0.2 and 0.5 *N* sodium bisulfite solution, respectively, as indicated by electrophoretic analyses. By combining appropriate fractions, there was obtained about 5650 o.d.-ml. of vanilloylformic acid free of vanillic acid. The bisulfite solution was acidified, aspirated to remove sulfur dioxide, and extracted with ether. The ether solution was dried with sodium sulfate and then evaporated to dryness to yield 117 mg. of a pale yellow waxy solid observed under the microscope to be platelets, and found to have the following elementary composition; C, 54.51% and H, 4.72%.

Characterization of Vanilloylformic Acid.—The substance was soluble in water, ethanol and ether but relatively insoluble in cold benzene. From 41 mg. of crude B, 20 mg. of pale yellow granular crystals were obtained from hot dry benzene, m.p. 131.8–132.5°, after drying 24 hours at 60° *in vacuo*. After recrystallization from dry benzene, m.p. 132.0–132.5° and for a mixture of this substance with authentic vanilloylformic acid from veratroylethyl formate, m.p. 131.8–132.8°; reported[18]: 133–134°. Electrophoretic mobility of vanilloylformic acid was found to be 1.06 ± 0.02 cm.^{2}/(volt)(hour) and the same value was found for synthetic vanilloylformic acid. A conductometric titration of 3.58 mg. of vanilloylformic acid gave 170 and 210 as weak and strong acid equivalent weights, the departure from the expected value of 196.1 possibly arising from some decomposition during titration. A 2,4-dinitrophenylhydrazone of vanilloylformic acid was prepared[19] as a red crystalline compound which darkened at 192, softened with apparent decomposition at 207 to 210 and became completely liquid at 230°.

A derivative of vanilloylformic acid with Girard T reagent[20] was formed by refluxing a small amount of vanilloylformic acid with excess reagent in aqueous solution buffered at *p*H 5.2. At higher acidity no derivative was formed. This was determined by extracting the aqueous reaction with ether and examining both layers by electrophoresis, since the Girard derivative is ether insoluble, while vanilloylformic acid is ether soluble. The derivative was not isolated but formation was taken as qualitative evidence for the presence of a carbonyl group probably ketonic.

Formaldehyde-bis-acetovanillone Acetate.—Acetovanillone (33.2 g.) in 100 ml. of absolute ethanol was added to an ethanolic sodium ethoxide (4.6 g. of sodium in 50 ml. of absolute alcohol) solution and a voluminous white precipitate resulted. Then 1 g. of copper powder and 26.8 g. of methylene iodide were added and after 24 hours at reflux about one-half of the solids were in solution. An additional equivalent of methylene iodide was added and refluxing was continued a further 24 hours. On cooling 3.0 g. of orange colored crystals containing copper separated and were filtered off. The filtrate was diluted with 1500 ml. of water, extracted with 300 ml. of chloroform, and then the chloroform solution was extracted with 10% sodium hydroxide solution. Acidification of the aqueous alkaline layer precipitated a yellow-brown solid which was crystallized from ethanol–petroleum ether to give 12.2 g. of acetovanillone, m.p. 114–115°, a 37% recovery. The extracted chloroform solution was dried and evaporated to obtain a dark brown solid which after washing with 100 ml. of ether was a light gray color and amounted to 4.4 g. This was dissolved in 400 ml. of hot ethanol and on cooling, pale gold colored flaky crystals separated, 3.2 g., m.p. 158–160°; after recrystallization from acetonitrile, m.p. 160–161°. Calcd. for $C_{19}H_{20}O_6$: C, 66.27; H, 5.85. Found: C, 65.96; H, 6.22. The infrared spectrum was very similar to that of acetovanillone except a broad peak at 3.0 μ was absent.

Formaldehyde-bis-vanilloylformic Acid Acetal.—A suspension of 2.72 g. of formaldehyde-bis-acetovanillone acetal in 160 ml. of water and 17.5 ml. of 2 *N* sodium hydroxide was maintained at 60–70° with vigorous stirring while 104 ml. of 5% permanganate solution (2% excess) was added over 0.5 hour. After the last addition, stirring was continued 0.75 hour and the mixture allowed to cool. Precipitated manganese dioxide was removed and the pale yellow filtrate acidified with 15 ml. of 6 *N* hydrochloric acid. A flocculent yellow precipitate resulted, was suctioned dry (2.6 g.), and on crystallization from glacial acetic acid gave fine granular white crystals which were washed with a little cold water followed by ethyl acetate, and then dried, m.p. 201–202° with decomposition. Calcd. for $C_{19}H_{16}O_{10}$: C, 56.44; H, 3.99. Found: C, 56.11; H, 3.99. An azine was

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(15) All melting points are uncorrected.

(16) A. E. Markham, Q. P. Peniston and J. L. McCarthy, THIS JOURNAL, **71**, 3599 (1949).

(17) V. F. Felicetta, A. E. Markham, Q. P. Peniston and J. L. McCarthy, *ibid.*, **71**, 2879 (1949).

(18) F. Tiemann, *Ber.*, **24**, 2877 (1891).

(19) R. L. Shriner and R. C. Fuson, "Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1948, p. 171.

(20) Andre Girard, "Organic Syntheses," Coll. Vol. II, A. H. Blatt, Editor, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 85.

prepared with hydrazine giving small yellow rosettes from glacial acetic acid, m.p. 170–171°. Calcd. for $C_{19}H_{16}O_8N_2$: N, 6.80. Found: N, 6.70.

Vanilloylformic Acid.—Hydrolysis of formaldehyde-bis-vanilloylformic acid acetal was carried out by boiling 2.02 g. with 67.5 ml. of 2 *N* hydrochloric acid for 22 hours. On cooling, a small amount of black solid separated and was removed, and the light brown colored solution was extracted with ether. After drying, the ether solution was evaporated to dryness giving a brown oil admixed with pale yellow solid. This residue was taken up in hot dry benzene and on cooling, pale yellow granular crystals separated; yield 0.8 g., m.p. 130–133°. Recrystallization from dry toluene gave pale yellow needles, m.p. 132.5–133°. Calcd. for $C_9H_8O_5$: C, 55.11; H, 4.11; CH_3O, 15.82. Found: C, 55.40; H, 4.20; CH_3O, 15.49.

Improved Vanilloylformic Acid Synthesis.—In a second preparation of vanilloylformic acid similar to that described above, 49.8 g. of acetovanillone was added to 11.7 g. of potassium in absolute ethanol and the solution refluxed with 40.2 g. of methylene iodide and 1 g. of copper powder for 12 hours. Another 40.2 g. of methylene iodide was added and reflux continued for a total of 36 hours. The reaction mixture was worked up as before to give 29.5 g. of chloroform-soluble material, m.p. 159–161°, a 57% yield. No starting material was recovered. Oxidation of the formaldehyde-bis-acetovanillone acetal (29.1 g.) suspended in 1200 ml. of water and 190 ml. of 2 *N* sodium hydroxide as before yielded 24.5 g. of crude formaldehyde-bis-vanilloylformic acid acetal, a 72% yield. Hydrolysis of 20.2 g. of formaldehyde-bis-vanilloylformic acid acetal by refluxing with 600 ml. of 2 *N* hydrochloric acid for 26 hours and workup as before gave 13.2 g. of crude vanilloyformic acid, 67% yield. Recrystallization from dry benzene followed by *n*-hexane–ether gave almost colorless needles, m.p. 131–132°. A conductometric titration on 4.70 mg. of this compound gave values for weak and strong acid equivalents of 190 and 210, respectively. Calcd. for $C_9H_8O_5$: mol. wt., 196.1.

Derivatives of Vanilloylformic Acid.—The 2,4-dinitrophenylhydrazone was prepared[18] as red crystals which darkened at 192°, softened at 207–210° and became completely liquid at 230°. Calcd. for $C_{15}H_{12}N_4O_8$: N, 14.89. Found: N, 14.87. Methylation of synthetic vanilloylformic acid (0.392 g.) with dimethyl sulfate and alkali gave 0.39 g. of pale yellow solid veratroylformic acid, which was recrystallized from dry benzene as almost white granular crystals, m.p. 133–134°, and the melting point of these crystals was not depressed when mixed with authentic veratroylformic acid. Methylation of synthetic vanilloylformic acid (0.196 g.) with diazomethane in ether gave 0.1 g. of ester product and recrystallization from aqueous ethanol gave white crystals of methyl veratroylformate, m.p. 60–61°. Similar treatment of veratroylformic acid gave the ester, m.p. 58–60°, and a mixture of these two products melted at 59–61°.

Oxidation of Vanilloylformic Acid.—A typical reaction mixture was vanilloylformic acid (98 mg.), sodium hydroxide (12.4 g.), cupric sulfate pentahydrate (26.9 g.) and water (69 ml.) contained in a 130-ml. stainless steel bomb which was rotated end over end in an oil-bath at 178° for two hours. The resultant mixture was filtered, acidified, ether extracted and an aliquot of the ether soluble solids was analyzed electrophoretically. Yields of vanilloylformic acid and vanillic acid were estimated to be 78 and 13.5 mg., respectively, for the above reaction conditions and 59 and 21 mg. when the reaction time was three hours. Similar treatments of acetovanillone and of acetovanillone–vanillin mixtures produced no vanilloylformic acid detectable by electrophoretic examination of the various ether extracts obtained.

Absorption Spectra.—Ultraviolet absorption spectra were measured with a Beckman Model DU spectrophotometer. Neutral solutions were examined in 95% ethanol. The solvent for alkaline solutions was prepared by diluting 2.8 ml. of 0.1 *N* alcoholic potassium hydroxide with 95% ethanol to 50 ml. as described by Pearl.[21] Protocatechuoylformic acid gave opaque solutions in alkaline ethanol so its extinction coefficient was obtained in an alkaline aqueous solution of the same concentration.

Infrared absorption spectra were measured with a Perkin-Elmer Model 21 Spectrophotometer. Solutions in acetonitrile solvent evaporated films from acetonitrile solutions, and Nujol mulls were used with sodium chloride plates in all cases.

Acknowledgment.—The authors are indebted to Mr. Vincent Felicetta for assistance with certain analytical determinations. The authors appreciated the grant of the Hooker Electrochemical Research Fellowship to D.G.

(21) I. A. Pearl and E. E. Dickey, THIS JOURNAL, **74**, 614 (1952).

SEATTLE, WASHINGTON

[CONTRIBUTION FROM THE NORTHERN UTILIZATION RESEARCH BRANCH[1]]

Factors Affecting Molecular Weight of Enzymatically Synthesized Dextran[2]

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The molecular weight distribution of dextran synthesized by dextransucrase preparations derived from *Leuconostoc mesenteroides* NRRL B-512 can be varied considerably. A bimodal distribution of molecular weight of dextran is generally found. Initial sucrose concentration in reaction mixture affects both yield and molecular weight of polymer synthesized. This may result from the fructose which is produced. Presence of maltose in a reaction mixture leads to synthesis of oligosaccharides and low molecular weight dextran. Low molecular weight dextran also functions as "primer" and can be grown to higher molecular weight polysaccharide. Formation of low modal dextran is enhanced by increase in enzyme concentration at 30° or decrease in reaction temperature. A possible mechanism of enzymatic action operative in dextran synthesis is proposed.

Previous reports[3,4] on synthesis of dextran by the enzyme, dextransucrase,[5] produced by *Leuconostoc mesenteroides* have indicated that certain sugars and sugar derivatives, notably isomaltose, maltose and α-methyl glucoside, function as glucosyl acceptor substrates or "primers." Extension of these findings has revealed that dextran of low molecular weight also acts as acceptor. By adjusting concentrations of such dextran as primer, an appre-

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(1) One of the Branches of the Agricultural Research Service, U. S. Department of Agriculture.

(2) Presented in part before 122nd Meeting of the American Chemical Society, Atlantic City, New Jersey, September, 1952, and 124th Meeting of the American Chemical Society, Chicago, Illinois, September 1953.

(3) H. J. Koepsell, H. M. Tsuchiya, N. N. Hellman, A. Kazenko, E. S. Sharpe, C. A. Hoffman and R. W. Jackson, *Bacteriol. Proc.*, 52nd Meeting, 23 (1952).

(4) H. J. Koepsell, H. M. Tsuchiya, N. N. Hellman, A. Kazenko, C. A. Hoffman, E. S. Sharpe and R. W. Jackson, *J. Biol. Chem.*, **200**, 793 (1953).

(5) It is recognized that more than one enzyme may be involved in synthesis but, for convenience, the term "dextransucrase" will be employed in the singular in this report.