In the preparation of 2-cyclohexylaminoethyl methanesulfonate hydrochloride by this procedure about 10% of N-methanesulfonyl-2-cyclohexylaminoethyl methanesulfonate (II) was isolated by adding the ether-insoluble material to cold water, separating the solid that was insoluble in water, and recrystallizing from ethanol; m.p. 76–77.6°. This product was identical (mixed melting point) with an authentic sample of II prepared in 24% yield from 2-cyclohexylaminoethanol, methanesulfonyl chloride and aqueous sodium hydroxide by a Schotten–Baumann procedure and recrystallized from ethanol; m.p. 77.2–77.6°.

*Anal.* Calcd. for $C_{10}H_{21}NO_5S_2$: C, 40.12; H, 7.07. Found: C, 40.16; H, 7.04.

**Procedure C.**—The following preparation of 2-dimethylaminoethyl methanesulfonate hydrochloride illustrates procedure C. The sodium alkoxide was prepared from 7.3 g. (0.082 mole) of 2-dimethylaminoethanol and sodium hydride (3.0 g., 0.123 mole) in 150 ml. of dry ether as in procedure B, with refluxing for four hours to complete the reaction. A solution of methanesulfonyl chloride (9.4 g., 0.082 mole) in 150 ml. of dry ether was placed in a 500-ml. three-necked flask fitted with a sealed stirrer, a dropping funnel and a thermometer extending into the liquid. The solution was cooled to −8° with Dry Ice and trichloroethylene, and the suspension of the sodium alkoxide was added dropwise over a period of 40 minutes at −8 to −5°. Precipitation of sodium chloride occurred immediately, and the mixture was stirred at −8 to −5° for one hour after the addition was completed. The product (Table I) was isolated as the hydrochloride in the manner described under procedure B.

About 10% of N-methanesulfonyl-1-cyclohexylamino-2-propyl methanesulfonate (III) was isolated as a by-product in the preparation of 1-cyclohexylamino-2-propyl methanesulfonate hydrochloride by this procedure. The ether-insoluble solid was combined with material obtained by concentrating the ethereal filtrate from the ester hydrochloride, washed with water, and recrystallized from methanol to a constant melting point of 100.8–101.8°.

*Anal.* Calcd. for $C_{11}H_{23}NO_5S_2$: C, 42.15; H, 7.40; N, 4.47. Found: C, 42.14; H, 7.35; N, 4.50.

**Tetraethylpiperazinium Dichloride** (I).—A solution of 2.6 g. of methanesulfonyl chloride in 5 ml. of dry ether was added to 2.0 g. of 2-diethylaminoethanol without cooling, producing an exothermic reaction that raised the temperature to a maximum of 82°. The resulting oil was dissolved in absolute ethanol, and the solution was saturated with dry hydrogen chloride, with cooling in ice. Addition of dry ether precipitated tetraethylpiperazinium dichloride (I); 1.4 g. (60%), m.p. 274–275° (dec.) (ref. 2 reports m.p. 277°). The dichloride was characterized by conversion to the dipicrate, which after recrystallization from aqueous ethanol melted at 267° (dec.). Since ref. 2 reports m.p. 277° for the dipicrate, an authentic sample of the dipicrate (identical according to mixed melting point with the sample derived from I) was prepared from tetraethylpiperazinium di-*p*-toluenesulfonate[2]; m.p. 268° (dec.).

*Anal.* Calcd. for $C_{24}H_{32}N_8O_{14}$: C, 43.90; H, 4.91; N, 17.07. Found: C, 44.03; H, 5.11; N, 17.16.

**Rearrangement of 2-Cyclohexylaminoethyl *p*-Toluenesulfonate Hydrochloride (IV) to the *p*-Toluenesulfonic Acid Salt of 2-Cyclohexylaminoethyl Chloride (V).**—2-Cyclohexylaminoethyl *p*-toluenesulfonate hydrochloride (IV) (2.0 g.) was melted by heating in a bath at 130–150° for 10 minutes. On cooling, the melt solidified; 1.7 g., m.p. 147–150°. Three crystallizations from acetone yielded 1.4 g. of the isomeric *p*-toluenesulfonic acid salt of 2-cyclohexylaminoethyl chloride (V) with a constant melting point of 151–151.6°.

*Anal.* Calcd. for $C_{15}H_{24}NO_3SCl$: C, 53.96; H, 7.25; N, 4.20. Found: C, 53.93; H, 7.26; N, 4.36.

The rearrangement product V was soluble in water, and gave a negative test for chloride ion with aqueous silver nitrate. A solution of 0.92 g. of V in 20 ml. of water became turbid upon addition of a slight excess of sodium hydroxide, and extraction with ether followed by drying the extract over calcium chloride and introduction of dry hydrogen chloride precipitated 0.425 g. of 2-cyclohexylaminoethyl chloride hydrochloride, m.p. and mixed m.p. with a known sample,[10] 217.4–218.8°. The sodium *p*-toluenesulfonate present in the aqueous solution was identified by conversion into the *p*-toluenesulfonic acid salts of *p*-nitrobenzamidine and *p*-toluidine, which were identical in melting point and mixed melting point with authentic samples. The rearrangement product V also was characterized by direct comparison with an authentic sample (identical by melting point and mixed melting point) prepared from 2-cyclohexylaminoethyl chloride and *p*-toluenesulfonic acid.

CAMBRIDGE, MASSACHUSETTS RECEIVED AUGUST 8, 1951

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[CONTRIBUTION FROM THE INSTITUTE OF PAPER CHEMISTRY]

# Studies on Lignin and Related Products. VII.[1] The Isolation of Certain Compounds from Lignin Oxidation Mixtures by Chromatographic Techniques[2,3]

BY IRWIN A. PEARL AND EDGAR E. DICKEY

Because the complex nature of fractions obtained by oxidizing lignosulfonate materials with cupric oxide and alkali precluded their complete analysis by heretofore employed procedures, recourse was made to adsorption chromatography. By means of this technique, the sodium bisulfite-soluble and the alkali-soluble fractions from such oxidations have yielded, in addition to previously isolated compounds, the following new oxidation products: 4,4′-dihydroxy-3,3′-dimethoxychalcone, 4,4′-dihydroxy-3,3′-dimethoxybenzil, 4,4′-dihydroxy-3,3′-dimethoxybenzophenone, and an unidentified isomer of dehydrodivanillin which is probably a dihydroxydimethoxyformylbenzophenone. Although the isolation of these products can be explained by assuming their resynthesis from vanillin or the like originally formed by degradation of the lignin, there is also a possibility that some of the $C_6$–$C_3$ units in the lignin structure are linked together through the α-carbon atoms.

In a recent communication[4] the isolation and identification of vanillin, guaiacol, acetovanillone, vanillic acid, 5-carboxyvanillin (I), 5-carboxyvanillic acid (II), dehydrodivanillin (III) and dehydrodivanillic acid (IV) from alkaline cupric oxide oxidations of fermented (*Torulopsis utilis*) spent sulfite liquor was reported. These isolations were effected by chemical separations and fractionations by means of solubility differences in various solvents and solvent combinations. During the above work it became obvious that the complex nature of the various fractions precluded their complete analysis by these two methods alone. Recourse was made to adsorption chromatography, a technique which recently was demonstrated to give exceptionally good results in the

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(1) For paper VI of this series, see *Tappi*, **33**, 544 (1950).

(2) Presented before the Division on Plant Cell-Wall Constituents at the XIIth International Congress of Pure and Applied Chemistry, New York, N. Y., September 10–13, 1951.

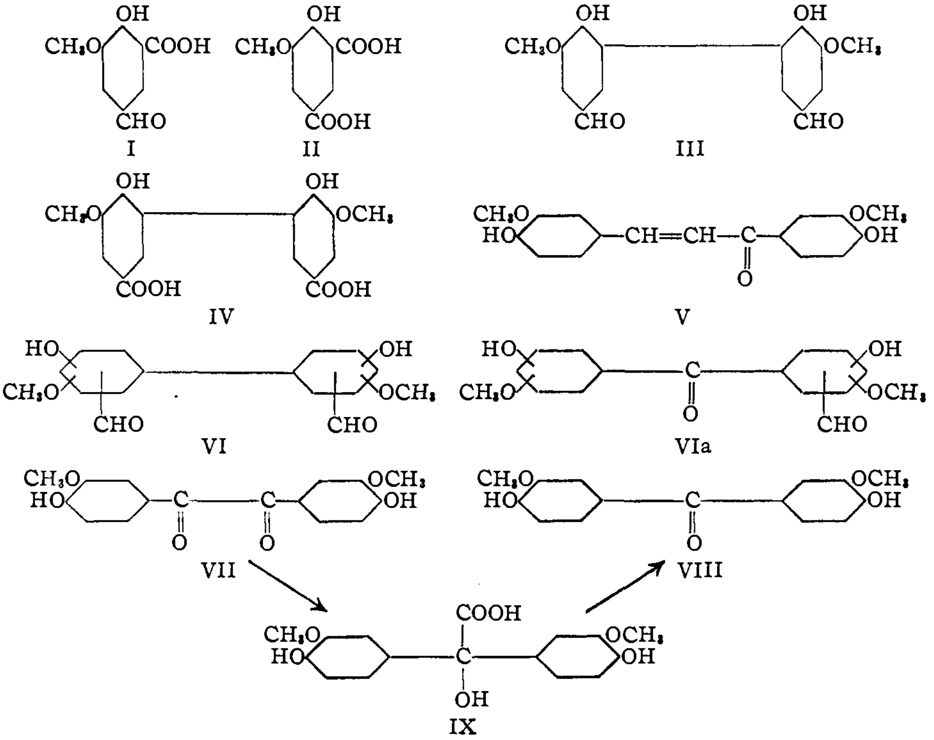
(3) This paper represents a portion of the results obtained in the research program sponsored by the Sulphite Pulp Manufacturers' Research League and conducted for the League by The Institute of Paper Chemistry. Acknowledgment is made by the Institute for permission on the part of the League to publish these results.

(4) I. A. Pearl, THIS JOURNAL, **72**, 2309 (1950).

separation of vanillin and syringaldehyde.[5] The present paper describes the use of adsorption chromatographic techniques for the separation and isolation of a number of compounds from the bisulfite-soluble and alkali-soluble fractions of an alkaline cupric oxide–lignosulfonate reaction mixture.

A cupric oxide–alkali oxidized fermented spent sulfite liquor was saturated with carbon dioxide and extracted with trichloroethylene. The trichloroethylene extract was fractionated into 21% sodium bisulfite, 5% sodium hydroxide and neutral fractions.

A benzene solution of the sodium bisulfite-soluble fraction was chromatographed on acid-washed Magnesol[5] and developed with 100:1 benzene–ethanol. The column was streaked with three of the reagents described previously[5]—namely, 2,4-dinitrophenylhydrazine, ferric chloride and potassium permanganate. These streaks, together with visible bands, indicated zones which were separated and individually eluted to yield vanillin as the chief product and smaller amounts of acetovanillone, 4,4'-dihydroxy-3,3'-dimethoxychalcone (V) (0.08%), melting at 118–120°, and a bisulfite-soluble compound (VI) (0.1%), melting at 178.5–179.5°, the analysis of which corresponds with that of III; VI gives a diacetate melting at 135–136°; the analysis corresponds with that for the diacetate of III. Apparently VI is an isomer of III or one of the isomeric dihydroxydimethoxyformyl diphenyl ketones (VIa). Solubility and melting point indicate the latter.



A benzene solution of the alkali-soluble fraction was chromatographed in the same manner to yield guaiacol, acetovanillone, a little vanillin, vanillil (4,4'-dihydroxy-3,3'-dimethoxybenzil) (VII) (0.1%), melting at 230–231.5° and 4,4'-dihydroxy-3,3'-dimethoxybenzophenone (VIII) (0.1%), melting at 155–156°.

V was identified by analysis and identity was established of both the parent compound and its diacetate with authentic 4,4'-dihydroxy-3,3'-dimethoxychalcone and its diacetate prepared by the condensation of vanillin and acetovanillone in the presence of hydrochloric acid in accordance with the method of Vorländer[6] for the preparation of 4,4'-dihydroxychalcone. Ultraviolet absorption spectra were also identical. The structure of VII was also established by the identity of the compound and its diacetate with authentic compounds prepared synthetically. Vanillil was synthesized by the oxidation with cupric oxide in acetic acid of 4,4'-dihydroxy-3,3'-dimethoxyhydrobenzoin (hydrovanilloin) which, in turn, was prepared by the electrolytic reduction of vanillin.[7] Vanillil was recently isolated from oxidized spent sulfite liquor by Johnson and Marshall,[8] who reported a melting point of 227–228°.

The presence of VIII is easily accounted for by assuming a benzilic acid rearrangement of VII to form vanillilic acid (IX) which, upon oxidative decarboxylation, yields the ketone VIII. VIII could not be isolated from its band directly, but its diacetate was easily purified and then hydrolyzed to the parent compound.

The ultraviolet absorption spectra of these compounds in ethanol were determined and are pictured in Fig. 1. The insolubility of III in ethanol precluded the determination of its absorption spectra in this solvent; therefore, alkaline ethanol was employed. The ultraviolet curve of VII obtained in ethanol did not correspond with that obtained by Johnson and Marshall.[8] However, the ultraviolet curve determined in alkaline ethanol was identical with that reported by them. Ultraviolet curves in alkaline ethanol are pictured in Fig. 2.

Johnson and Marshall have tentatively concluded that vanillil results by oxidation of vanilloin

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(5) I. A. Pearl and E. E. Dickey, *ibid.*, **73**, 863 (1951).

(6) D. Vorländer, *Ber.*, **58**, 128 (1925).

(7) I. A. Pearl, in press.

(8) A. M. Johnson and H. B. Marshall, paper presented before the Division of Cellulose Chemistry at the 118th Meeting of the American Chemical Society, Chicago, Ill., Sept. 3–8, 1950.

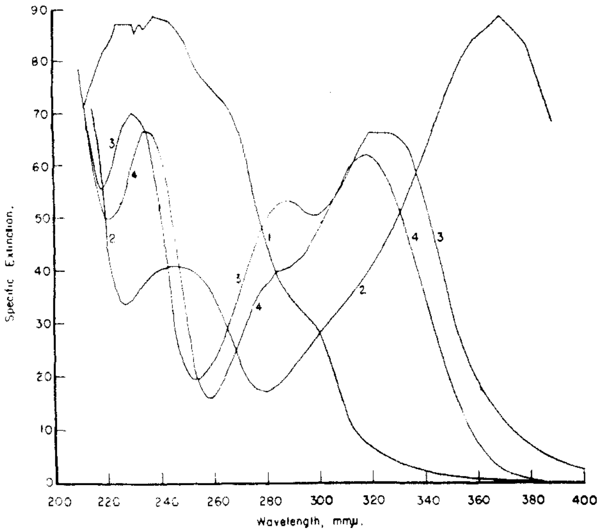

Fig. 1.—Ultraviolet absorption spectra in 95% ethanol: 1, IV; 2, V; 3, VII; 4, VIII.

first formed by the benzoin condensation of vanillin in the complex lignin reaction mixture. The nonexistence of benzoin-type catalysts in this reaction mixture, together with our experience that vanilloin cannot exist in alkaline solution, lead us to question such a conclusion. The isolation of VII, VIII and 4,4'-dihydroxy-3,3'-dimethoxystilbene[9] from lignosulfonate reaction mixtures indicates the possibility of a linkage between the α-carbon atoms of two $C_6$–$C_3$ units in at least a part of the complex lignin structure.

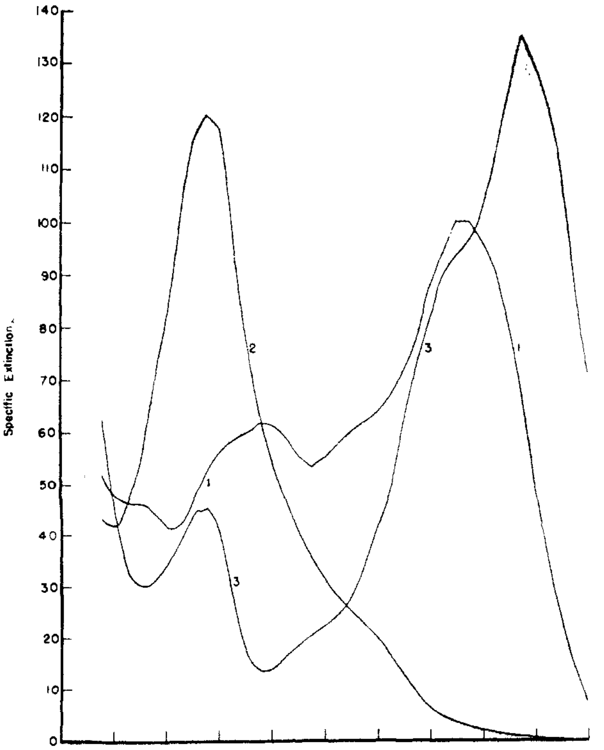

Fig. 2.—Ultraviolet absorption spectra in alkaline ethanol: 1, III; 2, IV, 3, VII.

## Experimental

All melting points are uncorrected.

A *Torulopsis utilis*-fermented spent sulfite liquor of spruce origin was oxidized in a stirring autoclave with cupric oxide and sodium hydroxide as described in a previous paper[1] at 170° for 5 hours with a copper hydrate–lignin molar ratio of 13.5 and a sodium hydroxide–lignin molar ratio of 11.9. Four identical experiments were made in a 5-gallon automatically controlled autoclave. An aliquot of the combined filtered reaction mixtures (32 liters, representing 843 g. of original lignin) was saturated with carbon dioxide and extracted countercurrently with trichloroethylene in a modified Scheibel extraction column.[10] The trichloroethylene was removed by distillation under reduced pressure to give 161 g. of extract (19.0% on the lignin basis). The dry extract was covered with 750 ml. of benzene and filtered. The benzene solution was extracted with 5% sodium hydroxide solution. The benzene was washed with water, dried and distilled to yield a neutral fraction.

The sodium hydroxide solution was saturated with sulfur dioxide and extracted with ether. The ether was dried and distilled to yield 33 g. (3.9%) of alkali-soluble materials. The bisulfite solution was acidified with sulfuric acid, heated and aspirated, cooled, and then extracted with ether. The ether yielded 101 g. of bisulfite-soluble material (12.0%).

**Chromatography of the Bisulfite-soluble Fraction.**—A benzene solution containing 21.79 g. of the bisulfite-soluble material was chromatographed on a column of acid-washed Magnesol[5] (75 mm. in diameter and 280 mm. in length). The chromatogram was developed with 2700 ml. of a mixture of benzene and absolute ethanol (100:1 by volume). After extrusion, the developed chromatogram was streaked with the following streak reagents[5]: 2,4-dinitrophenylhydrazine, ferric chloride and potassium permanganate. The entire procedure was repeated and the corresponding zones were combined. These were eluted with acetone to yield the fractions given in Table I.

TABLE I

CHROMATOGRAPHIC SEPARATION OF BISULFITE-SOLUBLE FRACTION

| Zone | Recovered material g. | % |
|---|---|---|
| F | 0.48 | 1.1 |
| E | 0.05 | 0.1 |
| D | 0.38 | 0.9 |
| C | 0.64 | 1.5 |
| B (bright yellow) | 5.0 | 11.5 |
| A | 24.3 | 55.9 |
| Effluent | 11.0 | 25.3 |
| | 41.85 | 96.3 |

It is apparent that almost all the material in the bisulfite-soluble fraction is in zones B, A and the effluent.

Zone A and the effluent yielded almost pure vanillin upon removal of the acetone.

The bright yellow zone B was rechromatographed in benzene solution on an acid-washed Magnesol column (48 mm. in diameter by 215 mm. in length) and developed with 750 ml. of a mixture of petroleum ether (b.p. 65–110°)–ethanol (50:1 by volume). The extruded column, when streaked and sectioned as before, gave the results in Table II.

TABLE II

CHROMATOGRAPHIC SEPARATION OF ZONE B FROM THE INITIAL CHROMATOGRAM OF THE BISULFITE-SOLUBLE FRACTION

| Zone | Recovered material g. | % |
|---|---|---|
| E | 0.007 | 0.1 |
| D | 0.036 | 0.7 |
| C | 2.00 | 40.0 |
| B | 1.433 | 28.7 |
| A | 1.224 | 24.5 |
| | 4.700 | 94.0 |

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(9) H. Richtzenhain and C. von Hofe, *Ber.*, **72B**, 1890 (1939).

(10) E. G. Scheibel, *Chem. Eng. Progress*, **44**, 681 (1948).

**Isolation of V and VI.**—The material from Zone C of Table II was dissolved in 10% aqueous sodium hydroxide. The deep yellow solution was diluted with an equal volume of water and saturated with sulfur dioxide at room temperature. The yellow oil which separated soon solidified, and was filtered, washed with water, and dried. The crude product melted at 107–111°. Recrystallization from 50% ethanol yielded small yellow crystalline spheres melting at 118–120°, which did not depress a mixed melting point with authentic 4,4′-dihydroxy-3,3′-dimethoxychalcone. The yield was 0.28 g. (0.16% on the lignin basis). The ultraviolet absorption spectra and solubility of this material were identical with the authentic product. The bright yellow crystals dissolved in hot benzene to form a nearly colorless solution, but the yellow crystals were recovered unchanged upon cooling or evaporation of the solvent.

The diacetate was prepared with acetic anhydride and pyridine and was crystallized from 95% ethanol to give crystals melting at 136–137°; this did not depress the melting point of a mixture with authentic 4,4′-diacetoxy-3,3′-dimethoxychalcone.

The aqueous filtrate from which the chalcone was isolated was extracted twice with ether to remove a little remaining chalcone and was then acidified with dilute sulfuric acid and boiled to expel dissolved sulfur dioxide. A dark oil separated and solidified to a crystalline mass after standing overnight. Two recrystallizations from 95% ethanol and one each from 80% acetic acid and acetone gave pale yellow crystals melting at 178.5–179.5°. The yield of VI was 0.46 g.

*Anal.* Calcd. for $C_{16}H_{14}O_6$: C, 63.57; H, 4.67; $CH_3O$, 20.53; mol. wt., 302.3. Found: C, 63.42; H, 4.88; $CH_3O$, 21.1; mol. wt. (Rast), 296.

The diacetate, crystallized from 95% ethanol, melted at 135–136°.

A dilute alcoholic solution of VI formed a dark brown color with ferric chloride, and a dark red amorphous precipitate upon warming with 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid. The 2,4-dinitrophenylhydrazine derivative, upon recrystallizing from nitrobenzene, was obtained as fine nearly black needles melting at 290–295° with decomposition.

**Chromatography of the Alkali-soluble Fraction.**—A benzene solution containing 16.5 g. of the alkali-soluble fraction was chromatographed in a manner identical with that described for the bisulfite-soluble fraction. The chromatogram was developed with 2000 ml. of benzene–ethanol (100:1) and streaked with phloroglucinol reagent[6] and the other reagents noted previously. The results of sectioning of the column are given in Table III. The data in Table III are based on a combination of two identical experiments representing 33.0 g. of starting material.

TABLE III

CHROMATOGRAPHIC SEPARATION OF ALKALI-SOLUBLE FRACTION

| Zone | Recovered material g. | % |
|---|---|---|
| G | 0.728 | 2.2 |
| F | 0.877 | 2.7 |
| E | 1.068 | 3.2 |
| D | 1.600 | 4.8 |
| C | 0.530 | 1.6 |
| B | 6.602 | 20.0 |
| A | 14.258 | 43.2 |
| Effluent | 4.468 | 13.5 |
| | 30.131 | 91.7 |

**Isolation of VII.**—Most of the dark color was adsorbed near the top of the column and the bulk of the fraction was located on the lower part of the column; and the liquid phenols—mainly guaiacol—were washed from the column into the effluent. Zone A consisted of acetovanillone with a little vanillin. Zone B was removed as a bright yellow band; the other bands were removed separately but contained smaller amounts of material. The eluates from zones B, C and D contained crystalline material. These were taken up separately in 95% ethanol and the crystals removed by filtration. All products appeared to be the same substance in different degrees of purity. The melting points ranged from 226 to 231°. All crystals were combined and recrystallized from glacial acetic acid to a maximum melting point of 230–231.5°. A mixed melting point with authentic vanillil (VII) was not depressed. The yield was 0.89 g. (0.1% on the lignin basis).

*Anal.* Calcd. for $C_{16}H_{14}O_6$: C, 63.57; H, 4.67; $CH_3O$, 20.5. Found: C, 63.52; H, 4.73; $CH_3O$, 20.5.

The diacetate, recrystallized from 95% ethanol, gave white crystals melting at 138–140°.

*Anal.* Calcd. for $C_{20}H_{18}O_8$: C, 62.17; H, 4.70; $CH_3O$, 16.1. Found: C, 62.16; H, 4.75; $CH_3O$, 16.5.

Vanillil is insoluble in water, slightly soluble in hot ethanol, methanol, benzene and cold glacial acetic acid, and soluble in hot glacial acetic acid. It dissolves in aqueous sodium hydroxide to form a bright green solution from which it is precipitated upon acidification, even with carbon dioxide and sulfur dioxide, with the formation of a colorless supernatant liquid. However, upon standing and loss of excess carbon dioxide, bicarbonate solutions slowly turn yellow with accompanying solution of vanillil. Johnson and Marshall[8] noted that vanillil was soluble in sodium bicarbonate, but we have found this to be untrue in the presence of excess carbon dioxide.

**Isolation of VIII.**—The residue obtained after removal of vanillil from zone B of the original chromatogram of the alkali-soluble material was dissolved in benzene and rechromatographed in the same manner. Most of the material was located in a band near the top of the column. All attempts to prepare a crystalline substance from the eluate of this band failed. The resinous product was acetylated with pyridine and acetic anhydride and, after long standing of the acetylated product, a crystalline acetate of VIII was obtained. Recrystallization from 60% ethanol yielded colorless crystals melting at 148–149°.

*Anal.* Calcd. for $C_{19}H_{18}O_7$: C, 63.68; H, 5.06; $CH_3O$, 17.32; mol. wt., 358. Found: C, 63.72; H, 5.18; $CH_3O$, 17.8; mol. wt. (Rast), 358.

The purified acetate was deacetylated by warming in an excess of ethanolic sodium hydroxide. The alkaline solution was acidified with hydrochloric acid to yield a colorless oil which was extracted with ether. Removal of the ether gave a solid which was recrystallized from benzene to give crystals of VIII melting at 155–156°. The yield was 0.753 g. or approximately 0.1% on the lignin basis.

*Anal.* Calcd. for $C_{15}H_{14}O_5$: C, 65.68; H, 5.15; $CH_3O$, 22.6; mol. wt., 274. Found: C, 65.86; H, 5.26; $CH_3O$, 22.5; mol. wt. (Rast), 358.

The reaction of VIII with 2,4-dinitrophenylhydrazine gave an orange-red precipitate melting at 235–237°.

**4,4′-Dihydroxy-3,3′-dimethoxychalcone.**—A solution of 16.6 g. (0.1 mole) of acetovanillone and 15.2 g. (0.1 mole) of vanillin in 300 cc. of absolute ethanol was cooled in an ice–salt-bath and saturated with dry hydrogen chloride gas. Hydrogen chloride introduction was continued, and the deep red solution was allowed to stand. A purple-red precipitate separated. After standing one hour, the mixture was filtered and the precipitate was washed with absolute ethanol. The precipitate was stirred into water, giving 25 g. of yellow product. The precipitate was recrystallized twice from water to give bright yellow crystals of 4,4′-dihydroxy-3,3′-dimethoxychalcone, melting at 118–120°.

*Anal.* Calcd. for $C_{17}H_{16}O_5$: C, 67.99; H, 5.37; $CH_3O$, 20.7. Found: C, 67.97; H, 5.37; $CH_3O$, 20.5.

The diacetate was prepared and crystallized from 50% ethanol to give yellow crystals melting at 136–137°.

*Anal.* Calcd. for $C_{21}H_{20}O_7$: C, 65.62; H, 5.25; $CH_3O$, 16.2. Found: C, 65.78; H, 5.28; $CH_3O$, 16.5.

**Ultraviolet Absorption Spectra.**—Ultraviolet absorption spectra were determined in 95% ethanol with a Beckman model DU spectrophotometer. The alkaline ethanol solvent was prepared by diluting 2.8 ml. of 0.1 *N* alcoholic potassium hydroxide with purified 95% ethanol to 50 ml. All concentrations were approximately 0.02 g. per liter.

APPLETON, WISCONSIN RECEIVED AUGUST 13, 1951