we have shown that ascorbic acid oxidase is a copper–protein enzyme similar to tyrosinase (polyphenol oxidase), we thought it of interest to try its effect upon the above substrates, although some investigators[4,5] already have shown that the enzyme does not catalyze the oxidation of phenols. Using one of our preparations of highest purity (600 units per mg.) we found that it was about 520 times more active toward ascorbic acid than toward catechol and about 335 times more active toward its own substrate than toward hydroquinone. No activity toward *p*-cresol was obtained when an amount of ascorbic acid oxidase as high as 846 units was used. These activities are extremely small and to all intents and purposes it may be concluded that the enzyme does not catalyze the oxidation of these substances except at high concentrations.

Summary

1. A method has been described for the preparation of a highly purified ascorbic acid oxidase having an activity of 600 to 630 units per mg. dry weight.

2. At high concentrations the enzyme was green, at lower concentrations blue or bluish-green.

3. The ascorbic acid oxidase was found to be a copper-protein compound, containing 0.15% of copper.

4. The purest enzyme preparations obtained had an activity of 432 units per γ of copper.

5. Manganese seems to play no part as activator for this enzyme.

6. The purest enzyme preparation was found to have lost 99.65% of the peroxidase accompanying the enzyme in the crude juice and may be considered practically free of this enzyme.

7. Ascorbic acid oxidase has no action toward *p*-cresol and only shows a slight action toward catechol and hydroquinone at comparatively high concentrations.

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[CONTRIBUTION FROM THE DIVISION OF INDUSTRIAL AND CELLULOSE CHEMISTRY, MCGILL UNIVERSITY]

Studies on Lignin and Related Compounds. XLVII. The Synthesis of Xylosides Related to Lignin Plant Constituents

BY J. H. FISHER, W. LINCOLN HAWKINS AND HAROLD HIBBERT

The question of whether lignin is in chemical combination with a carbohydrate constituent in the plant has been a matter of speculation for many years. Some workers[1] believe that lignin is present as an incrustant in the cell wall and is not in chemical union with any other product. Evidence[1] from a study of X-ray patterns of pure cotton cellulose and cellulose from wood appears to indicate the absence of a chemical union. However, the possibility of the existence of a lignin-hemicellulose complex as suggested by Harris, Sherrard and Mitchell[2] and later supported by Norman and Shrikhande[3] is not excluded.

Recent studies on the enzymatic degradation of wood by Ploetz[4] provide further evidence for the presence of a lignin carbohydrate complex. Bailey[5] in work dealing with lignin extracted by the use of butyl alcohol obtains evidence indicating a chemical combination of part of the lignin with cellulose in certain woods. Norman[6] and also Hägglund[7] have presented comprehensive reviews concerning the relationship between lignin and cellulose in the cell wall.

Possible Mode of Combination.—The two principal carbohydrate constituents of the cell wall, cellulose and xylan, may be combined with the lignin through ether, ester or glycosidic bonds, that of the last-named occurring most frequently in natural products. To obtain further information on the nature of the lignin union in wood, a number of glycosides have been synthesized from products presumably closely related to lignin.

Although the structure of "protolignin" is unknown, recent studies on the ethanolysis of wood[8] and the hydrogenation of certain isolated lignins[9]

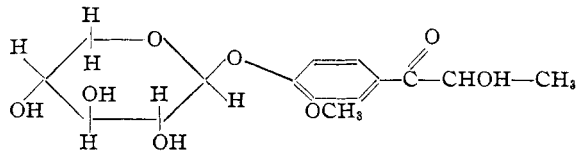
(1) Freudenberg, *J. Chem. Education*, **9**, 1171 (1932).
(2) Harris, Sherrard and Mitchell, THIS JOURNAL, **56**, 889 (1934).
(3) Norman and Shrikhande, *Biochem. J.*, **29**, 2259 (1935).
(4) Ploetz, *Ber.*, **73**, 57, 61, 74 (1940).
(5) Bailey, *Paper Trade J.*, **110**, No. 1, 29 (1940).
(6) Norman, "Biochemistry of Cellulose, Polyuronides, Lignin," Clarendon Press, Oxford, 1937, pp. 59–62.
(7) Hägglund, "Holzchemie," Akademische Verlagsgesellschaft, Leipzig, 1939, pp. 206–212.
(8) Cramer, Hunter and Hibbert, THIS JOURNAL, **61**, 509 (1939); Hunter, Cramer and Hibbert, *ibid.*, **61**, 516 (1939).
(9) Harris, D'Ianni and Adkins, *ibid.*, **60**, 1467 (1938).

have indicated the fundamental building units are C_6-C_3 derivatives. Ethanolysis experiments have been highly informative in that, contrary to those carried out on high pressure hydrogenation, the functional groups of the aromatic nuclei as well as those of the aliphatic side chains remain intact. Thus it has been possible to isolate such C_6-C_3 lignin building units as α-ethoxypropiovanillone and α-ethoxypropiosyringone. These compounds are considered to be derived from etherification of the corresponding α-hydroxy compounds, their dismutation isomers or their ene-diol forms[10] during ethanolysis. Recently Brickman, Hawkins and Hibbert[11] have isolated the corresponding diketones, vanilloyl and syringoyl methyl ketones, from the ethanolysis products of wood.

The manner in which the building units may be combined in protolignin has been dealt with in a previous communication,[10] in which it has been shown that, in such polymers, there may be primary, secondary or tertiary aliphatic and phenolic hydroxyl groups present so that the carbohydrate material of the cell wall may be combined through any or all of these hydroxyls.

Degradation of lignin sulfonic acids has yielded other aromatic compounds containing the guaiacyl and syringyl nuclei, such as guaiacol,[12] acetovanillone,[13] acetosyringone,[14] and pyrogallol 1,3-dimethyl ether.[15]

In view of the isolation of these derivatives as part of the lignin complex, the synthesis of the following xylosides was undertaken as the first stage in a study of possible glycosidic linkages in protolignin: (1) α-hydroxypropiovanillone β-*d*-xyloside:



(2) α-hydroxypropiosyringone β-*d*-xyloside, (3) guaiacol β-*d*-xyloside, and (4) acetovanillone β-*d*-xyloside.

An extensive investigation of the properties of these xylosides, including a quantitative determination of their stability under a variety of conditions, is in progress. The acidity of the phenolic hydroxyl groups of the phenols in question is to be determined and the stability of the xylosides correlated with these values. The results of this investigation, and the conclusions which may be drawn therefrom regarding the plausibility of a phenolic glycosidic link between lignin and carbohydrate will be presented in a subsequent communication. Such results may possibly have a marked bearing on the mechanism of sulfite pulp manufacture.

Syntheses of Acetylated Xylosides.—The acetylated forms of the above xylosides were synthesized by condensation of acetobromoxylose with the phenol by a modification of Helferich's process.[16] Attempted synthesis of the acetylated xylosides of α-acetoxypropiovanillone and α-acetoxypropiosyringone by his method resulted in very low yields (13%), even when a large excess of acetobromoxylose was used. By (1) substitution of the potassium for the sodium salt of the phenol, (2) operating at 0° throughout the reaction, and (3) carefully controlling the *p*H of the reaction medium during addition of the reagents, it was possible to double the yield, an important point in view of the difficulties involved in the preparation of the phenolic products.

In the Helferich reaction the salt of the phenol used and the water present in the reaction mixture, both react with acetobromoxylose. Since the phenol reacts only in the form of its salt, the yield of the xyloside depends on the proportion of this salt present throughout the reaction. In Helferich's method the aqueous solution of the sodium salt of the phenol is mixed with an acetone solution of the acetobromoxylose and allowed to stand at room temperature. Part of the acetobromoxylose reacts with the water present producing hydrogen bromide which in turn liberates the phenol from its salt, resulting in an increasing amount of the free phenol. By adding alkali throughout the reaction this is prevented and a maximum amount of phenol available for condensation is obtained, thus ensuring maximum utilization of the acetobromoxylose. Excess of alkali is to be avoided in order to prevent decomposition of the acetobromoxylose and deacetylation of the xyloside as well as fission of the newly formed xylosidic union. Sufficient alkali is added to keep 90% of the phenol in the form of its potassium salt. The exact proportion of phenol as

(10) Hibbert, THIS JOURNAL, **61**, 725 (1939).
(11) Brickman, Hawkins and Hibbert, *ibid.*, **62**, 986 (1940).
(12) Leger and Hibbert, *Can. J. Research*, **B16**, 68 (1938).
(13) Buckland, Tomlinson and Hibbert, *ibid.*, **B16**, 54 (1938).
(14) Leger and Hibbert, THIS JOURNAL, **60**, 565 (1938).
(15) Leger and Hibbert, *Can. J. Research*, **B16**, 151 (1938).
(16) Helferich, *Ann.*, **520**, 156 (1936).

potassium salt required to give a maximum yield will depend on the acidity of the phenolic group and the stability of the resulting xylosidic bond.

α-Acetoxypropiovanillone and α-acetoxypropiosyringone contain asymmetric carbon centers. Condensation of these racemic compounds with optically active acetobromo-*d*-xylose should give rise to isomers of slightly different properties. The difficulties encountered in the purification of the acetylated xylosides of the above two phenols, especially the latter, would indicate that this is the case.

Deacetyla ion of Acetylated Xylosides.—The Zemplén method[17] proved to be the most satisfactory process for deacetylation. Guaiacol triacetyl β-*d*-xyloside and acetovanillone triacetyl β-*d*-xyloside were successfully deacetylated by Mauthner's aqueous ammonia method[18] with yields of 85–90%. The former xyloside also was deacetylated by the anhydrous methanolic ammonia method.[19] However, the triacetyl xylosides of α-acetoxypropiovanillone and of α-acetoxypropiosyringone are unstable under both these conditions. The β-*d*-xyloside of α-hydroxypropiosyringone proved to be remarkably unstable. Mere solution in pure hydroxylic solvents, such as ethanol, acetone, dioxane or water, at 45° resulted in decomposition as indicated by a marked decrease in the melting point.

Experimental

α-Acetoxypropiovanillone.—α-Bromopropiovanillone[20] (24 g.), glacial acetic acid (144 cc.) and anhydrous potassium acetate (62 g.) were heated together on the steam-bath at 100° for six hours. The solution was poured into cold water (1152 cc.) with stirring, and the product allowed to crystallize at 0° for thirty-six hours. It was recrystallized from carbon tetrachloride in the form of fine needles; yield, 13.3 g. (60%); m. p. 105–106°. *Anal.* Calcd. for $C_{12}H_{14}O_5$: C, 60.48; H, 5.93; OCH_3, 13.0. Found: C, 60.45; H, 6.02; OCH_3, 13.0.

Triacetyl β-*d*-Xyloside of α-Acetoxypropiovanillone.—This was obtained by a *p*H controlled modification of the Helferich method.[16] The *p*H of an aqueous acetone solution of α-acetoxypropiovanillone containing 90% of an equivalent amount of potassium hydroxide was found to be approximately 9.0. The reaction was carried out under nitrogen at 0°.

A trace of phenolphthalein and 2.38 g. of α-acetoxypropiovanillone (0.01 mole) were dissolved at room temperature in a mixture of 8 cc. of acetone and 13.85 cc. of 0.724 *N* aqueous potassium hydroxide (0.01 mole). Acetobromoxylose (6.80 g., 0.02 mole) in 32 cc. of anhydrous acetone was then added to the above solution, and the flask shaken to ensure rapid admixture of the two solutions. In a few moments the *p*H of the resulting solution dropped below 9.0, as indicated by the color change. The dropwise addition of potassium hydroxide was begun at a rate sufficient to maintain the *p*H at 9.0. When 13.85 cc. of potassium hydroxide (0.724 *N*) had been added (twenty minutes) the reaction mixture was set aside for three hours, when the *p*H was approximately 7.0. The mixture was extracted thoroughly with benzene (150 cc.), the benzene extract shaken quickly with two 200-cc. portions of sodium carbonate (2%) at 5°, washed with water and dried for a few minutes with calcium chloride. The solution was evaporated at 40° under reduced pressure. Recrystallization of the residue twice from 50% aqueous ethanol (127 cc.) yielded 1.08 g. (25%) of fine needle-shaped crystals: m. p. 149.4–149.7°. *Anal.* Calcd. for $C_{28}H_{28}O_{12}$: C, 55.62; H, 5.69; OCH_3, 6.26; $COCH_3$, 34.7. Found: C, 55.50; H, 5.76; OCH_3, 6.20; $COCH_3$, 35.5.[21]

In the above reaction only 0.48 g. of α-acetoxypropiovanillone could be recovered unchanged.

α-Acetoxypropiosyringone.—This was synthesized by the method of Hunter, Cramer and Hibbert.[8] One of the steps in this synthesis involves the dehydration of 3,4,5-trimethoxyphenyldiethylcarbinol to the corresponding pentene. It was found convenient to effect this dehydration by heating with a trace of iodine at 100° for one hour.[22] This method gives a purer pentene derivative and an increase in yield to 60% of 3,4,5-trimethoxypropiophenone on ozonization.

Triacetyl β-*d*-Xyloside of α-Acetoxypropiosyringone.—This was synthesized by the same procedure as used with α-acetoxypropiovanillone. The crude product (m. p. 100°) was very difficult to purify, and this was effected by recrystallization from 33% aqueous ethanol. Six recrystallizations were necessary before a product in the form of small flaky crystals and of constant melting point (128.6–128.8°) was obtained, yield 25%. *Anal.* Calcd. for $C_{24}H_{30}O_{13}$: C, 54.73; H, 5.74; OCH_3, 11.78; $COCH_3$, 32.7. Found: C, 54.70; H, 5.75; OCH_3, 11.8; $COCH_3$, 34.1.[21]

Guaiacol Triacetyl β-*d*-Xyloside.—This was prepared, as above, by using equimolecular quantities of acetobromoxylose and potassium guaiacolate and omitting *p*H control of the reaction mixture. The xyloside crystallized from 50% aqueous ethanol in large rhombic crystals; yield 43%; m. p. 139.8–140.0°. *Anal.* Calcd. for $C_{18}H_{22}O_9$: C, 56.51; H, 5.81; OCH_3, 8.1; $COCH_3$, 33.8. Found: C, 56.50; H, 5.82; OCH_3, 8.0; $COCH_3$, 33.9.

Acetovanillone Triacetyl β-*d*-Xyloside.—A similar procedure was employed using a 50% excess of acetovanillone; yield 58%. It crystallized from aqueous acetone in large monoclinic crystals; m. p. 133.3–133.6°. *Anal.* Calcd. for $C_{20}H_{24}O_{10}$: C, 56.58; H, 5.71; OCH_3, 7.31; $COCH_3$, 30.4. Found: C, 56.50; H, 5.83; OCH_3, 7.29; $COCH_3$, 31.5.[21]

β-*d*-Xyloside of α-Hydroxypropiovanillone.—The deacetylation of the acetylated xyloside was carried out by the Zemplén method.[17] The reaction was allowed to pro-

(17) Zemplén, *Ber.*, **62**, 1613 (1929).
(18) Mauthner, *J. prakt. Chem.*, **124**, 313 (1930).
(19) Lucas, *Bull. soc. chim.*, (5) **2**, 1605 (1935).
(20) Cramer and Hibbert, THIS JOURNAL, **61**, 2205 (1939).
(21) High acetyl values were found to be due to production of volatile acids other than those from the acetyl groups.
(22) Hibbert, THIS JOURNAL, **37**, 1748 (1915).

ceed for four hours at 20°, the sodium methylate neutralized by addition of an equivalent amount of acetic acid, and the solvent evaporated at 20° under reduced pressure. The residue crystallized from a mixture of anhydrous methanol and ether (1:1) as dense rhombic crystals; yield 76%; m. p. 193–194.5°, with slight decomposition. *Anal.* Calcd. for $C_{16}H_{20}O_8$: C, 54.85; H, 6.14; OCH_3, 9.45. Found: C, 54.90; H, 6.30; OCH_3, 9.40.

β-d-Xyloside of α-Hydroxypropiosyringone.—Deacetylation was effected as described for the β-d-xyloside of α-hydroxypropiovanillone. The residue obtained after evaporation of the methanol from the reaction mixture had to be purified with utmost care, otherwise decomposition resulted. This was effected by dissolving in a minimum amount of anhydrous methanol at 20° and cooling to −10° when the xyloside crystallized in the form of fine needles; yield 73%; m. p. 149.4–150.0°. *Anal.* Calcd. for $C_{16}H_{22}O_9$: C, 53.60; H, 6.18; OCH_3, 17.32. Found: C, 53.51; H, 6.35; OCH_3, 17.28.

Guaiacol β-d-Xyloside.—This was prepared by deacetylation of the acetylated xyloside by the Zemplén method.[17] The xyloside crystallized from ethyl acetate in rod-shaped crystals, yield 85%, m. p. 175.3–176.0°. *Anal.* Calcd. for $C_{12}H_{16}O_6$: C, 56.23; H, 6.29; OCH_3, 12.10. Found: C, 56.20; H, 6.43; OCH_3, 12.05.

Acetovanillone β-d-Xyloside.—The conditions of deacetylation were as described by Zemplén.[17] The xyloside was recrystallized, first from ethyl acetate, and then from 50% anhydrous methanol–ether, and obtained in the form of very fine hair-like crystals; yield 85%; m. p. 145.2–145.7°. *Anal.* Calcd. for $C_{14}H_{18}O_7$: C, 56.25; H, 6.08; OCH_3, 10.40. Found: C, 56.20; H, 6.41; OCH_3, 10.30.

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Summary

Methods are given for the synthesis of the β-d-xylosides of α-hydroxypropiovanillone, α-hydroxypropiosyringone, guaiacol and acetovanillone, and their corresponding fully acetylated derivatives.

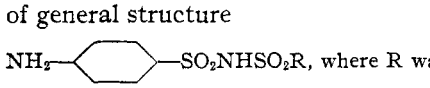
MONTREAL, CANADA RECEIVED MARCH 28, 1940

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE CALCO CHEMICAL DIVISION OF THE AMERICAN CYANAMID COMPANY]

Sulfanilamide Derivatives. VII. N¹-Alkanesulfonylsulfanilamides and Related Compounds[1]

BY M. L. CROSSLEY, E. H. NORTHEY AND MARTIN E. HULTQUIST

We were led to further investigation of sulfanilamide derivatives containing a disulfonamide linkage, $—SO_2NHSO_2—$, by the preliminary pharmacological results on disulfanilamide.[2] Long chain N¹-alkanesulfonylsulfanilamides analogous to the N¹-acylsulfanilamides[3] were of most interest, but a series of compounds was synthesized of general structure



$—SO_2NHSO_2R$, where R was alkane (from 2 to 12 carbons), cycloalkane, and aralkane.

The synthesis of the intermediate sulfonyl chlorides followed the procedure of Treat B. Johnson.[4]

These were treated with N⁴-acetylsulfanilamide in aqueous solution and the resulting N⁴-acetyl-N¹-alkanesulfonylsulfanilamides were hydrolyzed by boiling with sodium hydroxide to remove the N⁴-acetyl group. The resulting N¹-alkanesulfonylsulfanilamides were highly water soluble for the lower members of the series, but became increasingly water insoluble in the higher members. All were strongly acidic and formed neutral sodium salts.

Chemotherapeutic Properties.[5]—Preliminary results in mice against beta-haemolytic streptococci indicate that these compounds as a class are of low chemotherapeutic activity. A complete evaluation of them awaits the final results of the chemotherapeutic study.

Experimental Part

N¹-Butanesulfonylsulfanilamide.—78.3 grams (0.5 mole) of 1-butanesulfonylchloride was added to a slurry of 214 g. (1 mole) of N⁴-acetylsulfanilamide and 400 cc. of water; 50% sodium hydroxide solution was added gradually to maintain a pink spot test on benzoazurine paper (*p*H 11–12) while holding the temperature at 35–40° by addition of ice. The time of adding sodium hydroxide was thirty minutes. When no more sodium hydroxide was consumed after stirring for thirty minutes, the reaction was considered

(1) Presented in part before the Division of Medicinal Chemistry, A. C. S. Meeting, Cincinnati, Ohio, April, 1940.

(2) Crossley, Northey and Hultquist, THIS JOURNAL, **60**, 2222 (1938).

(3) Crossley, Northey and Hultquist, *ibid.*, **61**, 2950 (1939).

(4) Johnson and Sprague, *ibid.*, **58**, 1348 (1936); Sprague and Johnson, *ibid.*, **59**, 1837 (1937); Johnson and Douglass, *ibid.*, **61**, 2549 (1939); U. S. Patent 2,147,346, Feb. 14, 1939.

(5) This phase of the work was carried out under the direction of W. H. Feinstone and will be reported elsewhere.