time that acetovanillone has been obtained from a lignin sulfonic acid prepared from a hard wood.

An increase in solubility in aqueous bisulfite solution and a decrease in methoxyl content of formic acid lignin with increasing ozonization have been shown to take place.

MONTREAL, CANADA RECEIVED AUGUST 4, 1939

[CONTRIBUTION FROM THE U. S. DEPT. OF AGRICULTURE, FRUIT AND VEGETABLE BY-PRODUCTS LABORATORY,[1] AND THE DEPARTMENT OF CHEMISTRY OF THE STATE COLLEGE OF WASHINGTON]

Enzymic Preparation of *d*-Galacturonic Acid[2]

BY H. H. MOTTERN AND H. L. COLE

d-Galacturonic acid has been available only in limited quantities. Three methods have been used for its preparation: synthesis from galactose,[3] acid hydrolysis of pectic substances,[4] and enzymic hydrolysis of pectic substances.[4b,5] Although materials such as pectin with a galacturonic acid content of approximately 70%, are readily available, because of the lack of definite knowledge concerning the nature of the starting materials, complicated procedures, and low yields, *d*-galacturonic acid has remained a rare chemical.

Because of recent interest in the possible biological significance[6] of *d*-galacturonic acid, it is desirable to have a method whereby *d*-galacturonic acid can be prepared free from contamination with heavy metals and in quantity at nominal cost. A method which is the subject of this report has now been developed which for the first time makes it possible to prepare readily large or small quantities of the pure acid. A commercial pectinase preparation converts commercially available polygalacturonic acid[7] into galacturonic acid in good yields. This method is not subject to interference by side reactions such as decarboxylation to the acid and the formation of furan derivatives, which interfere with crystallization.[8] Disadvantages of Ehrlich's methods[4b,5] were that they employed the use of a little known "pectolsaure," and an enzyme from a special mold described as "*Penicillium Ehrlicii*."

(1) Food Research Division Contribution No. 418.

(2) Abstracted from a portion of a thesis submitted to the Graduate School of the State College of Washington by H. H. Mottern in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

(3) Sell and Link, THIS JOURNAL, **60**, 1813 (1938).

(4) (a) Morell, Bauer and Link, *J. Biol. Chem.*, **105**, 15 (1934); (b) Ehrlich and Guttmann, *Biochem. Z.*, **259**, 100 (1933).

(5) Ehrlich, Guttmann and Haensel, *ibid.*, **281**, 93 (1935).

(6) Manville, Bradway and McMinnis, *Am. J. Digestive Diseases Nutrition*, **3**, 570 (1936); Sullivan and Manville, *Am. J. Pub. Health*, **27**, 1108 (1937).

(7) Obtained from California Fruit Growers Exchange, Products Division, Ontario, California.

(8) Link and Niemann, THIS JOURNAL, **62**, 2474 (1930).

Experimental Part

Preparation of Polygalacturonic Acid.—One hundred grams of powdered apple or citrus pectin[9] was suspended in 250 cc. of 50% ethanol in a mixing bowl of 2 liters capacity which was provided with an egg-beater type stirrer. Since adequate stirring could not be accomplished with a common motor-driven laboratory stirrer it was necessary to use the egg-beater type. When the pectin had been thoroughly wetted by the ethanol and was uniformly dispersed, 10 g. of calcium chloride[10] dissolved in 250 cc. of water was added. The mixture was stirred until the pectin had taken up the aqueous solution and then 250 cc. of 3 *N* sodium hydroxide solution measured ready for use was added promptly. Stirring was continued until the mixture formed a smooth pasty mass. It was permitted to stand for fifteen minutes, after which any clear supernatant liquor was decanted. The mixture was neutralized with hydrochloric acid and sufficient excess added to give a concentration of about 1.5%, and boiled for ten minutes while being vigorously stirred. The polygalacturonic acid was filtered with suction and washed with water containing 10 cc. of concentrated hydrochloric acid per liter. The hydrochloric acid was removed by washing with 50% ethanol. The product was dried by washing several times with ethanol and then with ethyl ether. Yields of 75–80 g. were obtained. Polygalacturonic acid in the moist state may be used directly for the preparation of galacturonic acid.

Enzymic Hydrolysis of Polygalacturonic Acid to Galacturonic Acid.—Seventy-five grams of dry polygalacturonic acid (or the equivalent of the moist preparation obtained as described above) was suspended in 750 cc. of water, which had been warmed to 40°, in a mixing bowl which was provided with an egg-beater type stirrer and while stirring 75 cc. of 3 *N* sodium hydroxide solution was added. The mixture formed a smooth viscous mass. After dispersion the *p*H was adjusted to between 3.7 and 4.0 by the addition of measured amounts of 3 *N* sodium hydroxide or 3 *N* sulfuric acid solutions. To the mixture was then added 3.75 g. of "Pectinol 100D."[11] The mixture was

(9) Ordinary commercial fast-setting powdered pectin was satisfactory without purification.

(10) The calcium salt was used to control the degree of swelling of the pectic acid particles so that a grainy precipitate was obtained which could be satisfactorily filtered and washed.

(11) This preparation is "Pectinase 46 AP" standardized by the addition of diatomaceous earth. It was obtained from Röhm and Haas Co., Bristol, Penna.

next placed in a bottle and sufficient toluene was added to prevent mold growth. The bottle, stoppered with cork, was kept at a temperature of 35–40° for ten days.

At the end of the hydrolysis period[12] (usually less than ten days) the calculated amount of dilute sulfuric acid was added to set free the galacturonic acid from its sodium salt. The hydrolyzate was clarified by filtration using infusorial earth as a filter aid and was concentrated to a thick sirup under vacuum. The sirup was purified by the addition of five volumes of 95% ethanol and three volumes of ethyl ether, which precipitated sodium sulfate, ethanol insoluble fractions of the enzyme preparation, and any unhydrolyzed polygalacturonic acid. After standing for twelve hours the ethanol–ether solution of galacturonic acid was decanted from the residue and evaporated to a thick sirup under vacuum. Crystallization,[13] which required approximately twenty-four hours for completion, was hastened by adding small amounts of 95% ethanol and seeding with a few crystals of galacturonic acid. The acid was triturated with cold 90% ethanol, filtered, and washed with ethanol. It was purified further by dissolving in 50% ethanol, treating with charcoal, filtering, and recrystallizing after the addition of sufficient ethanol to make the ethanol concentration 75%. The yield from 75 g. of polygalacturonic acid was 20 g. of the purified product. The m. p. was 113–115° (uncorr.).[14]

(12) Indicated by treating a test portion with fresh enzyme for twenty-four hours and showing no increased reduction of Fehling's solution over previous samples.

(13) Direct crystallization of α-*d*-galacturonic acid from the purified hydrolyzate depends upon the use of starting materials which will not yield substances such as sugars interfering with crystallization.

Acknowledgment.—The writers are indebted to the California Fruit Growers Exchange, Research Department, Ontario, California for citrus pectin, to General Foods Corporation, New York City, for apple pectin, and to Röhm and Haas Co., Bristol, Penna., for pectinase preparations. Acknowledgment is also made to Dr. I. A. Manville, University of Oregon School of Medicine, for helpful suggestions and criticisms.

Summary

A method has been developed for the quantity preparation of *d*-galacturonic acid by the action of a pectinase enzyme on polygalacturonic acid.

(14) Link and Nedden, *J. Biol. Chem.*, **94**, 307 (1931).

PULLMAN, WASHINGTON RECEIVED APRIL 15, 1939

[CONTRIBUTION FROM THE BAILEY CHEMICAL LABORATORY OF THE UNIVERSITY OF KANSAS]

Iodo Derivatives of Phenyl Ether. II. Studies in Orientation

BY R. Q. BREWSTER AND H. S. CHOGUILL

Substances containing two or more benzene rings offer numerous problems in orientation. A given substituent may enter one of the nuclei at a certain position while another substituent may take an entirely different location. The experiments here reported were undertaken in order to obtain more information on these orientation effects in the phenyl ether series. Such knowledge was desirable in the continuation of a study of the iodinated derivatives of phenyl ether[1] which has been conducted in this Laboratory. Much work upon this subject has been done by several other investigators[2] using particularly those phenyl ether derivatives containing substituents of halogens, acetamino, nitro and to some extent methoxy groups. The present work is concerned primarily with the derivatives of 2-methoxy- and 4-methoxyphenyl ether. Since the methoxy radical is one of the more strongly directing groups, it would be expected that 4-methoxyphenyl ether would undergo substitution at position 3 and so it does upon nitration, 3-nitro-4-methoxyphenyl ether being the sole product.[2c] We find that halogenation however introduces the halogen atom at position 4′. If that position should be occupied, the halogen attaches itself to the carbon atom at 3 unless the substituent at 4′ is a group such as hydroxy or amino which exceeds the methoxy group in orienting power. Likewise the methoxy group is the controlling factor in the nitration of 2-methoxyphenyl ether as the product obtained is 5-nitro-2-methoxyphenyl ether, yet halogens substitute at position 4′ and take position 5 only if 4′ is occupied. In the halogenation of 4-nitrophenyl ether or 2-nitrophenyl ether, McCombie, Macmillan and Scarborough[2b] showed that a halogen enters at 4′, *i. e.*, para to the ether oxygen and always in the non-nitrated nucleus. The strong preference of a halogen for a position para to the ether oxygen is shown in the iodination of 4-methoxy-2′-nitrophenyl ether where the iodine takes position 4′ (Compound XIII) in spite of the presence of a nitro group in the same ring. However, in the iodination of 2-methoxy-4-nitro-

(1) Brewster and Strain, THIS JOURNAL, **56**, 117 (1934).

(2) (a) Scarborough and colleagues, *J. Chem. Soc.*, 2361 (1929); (b) *ibid.*, 529 (1931); (c) Lea and Robinson, *ibid.*, 411 (1926); (d) Oesterlin, *Monatsh.*, **57**, 31 (1931); (e) Raiford, Thiessen and Wernert, THIS JOURNAL, **52**, 1205 (1930).