

saturated hydrocarbons in seed coats and the ability of seeds of *Sapindus drummondii* to take a high polish suggest that these alkanes came from the unremoved seed coats rather than from the kernels. Such complications in fat chemistry have been remarked before.⁵

The seed oils of the following relatives of *Sapindus drummondii* have been studied: *Schlichera trijuga*,⁶ *Naphelium lappaceum*,⁶ *Naphelium mutabile*,⁶ *Sapindus trifoliatus*,⁶ *Ungnadia speciosa*,⁶ *Sapindus mukurossi*,⁹ and *Sapindus marginatus*.¹⁰ The iodine number of the seed oil of *Sapindus drummondii* is higher than that of any of the others except *S. marginatus*; this is in agreement with

(8) Schuette, Cowley and Chang, *THIS JOURNAL*, **56**, 2085 (1934).

(9) Kafuku and Hata, *J. Chem. Soc. Japan*, **55**, 369 (1934).

(10) G. S. Jamieson, unpublished work.

the observation that, among related plants, growth in a cooler climate connotes greater unsaturation of the oil. The principal difference between the oil of *S. drummondii* and that of any of its relatives, however, appears to be its lack of *n*-eicosanic (arachidic?) acid.

Summary

1. The physical and chemical characteristics of the seed oil of *Sapindus drummondii* have been determined.

2. The fatty acids present consist chiefly of oleic acid, accompanied by smaller amounts of linoleic, palmitic and stearic acids.

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RECEIVED JULY 20, 1939

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Studies on Lignin and Related Compounds. XLVI. The Action of Ozone on Isolated Lignins¹

BY RODGER M. DORLAND, W. LINCOLN HAWKINS AND HAROLD HIBBERT

The action of ozone on lignin preparations has been studied by a number of workers but the identifiable fragments split off by this treatment have been too small to be of any real value in the elucidation of the structure of lignin. Thus Phillips and Goss² isolated anisic acid in small yield by the action of ozone on methylated alkali lignin obtained from corn cobs. Bell, Hawkins, Wright and Hibbert³ have noted the formation of acetone in the ozonization of birch formic acid lignin. In general it may be said that, when a lignin is subjected to the action of ozone, there results, after treating the resulting product with water, a water-insoluble lignin and an aqueous solution. This latter solution contains mainly a water-soluble, lignin-like material and oxalic acid. The more prolonged the treatment with ozone, the smaller is the resulting water-insoluble and the greater the water-soluble fraction. This action is accompanied by a decrease in the methoxyl content of the ozonized lignin.

In connection with studies being carried out in these Laboratories on the mechanism of the solubilization of lignins by means of aqueous bisulfite solution, it was observed that formic^{4a} and

acetic acid^{4b} lignins are insoluble in aqueous bisulfites but that after a carefully controlled ozonization in formic acid solution they become completely soluble.

The increase in the solubility in sulfite "cooking liquor" and the decrease in the methoxyl content of the lignin with increasing degree of ozonization were studied by carrying out a series of ozonizations on identical samples of birch formic acid lignin using increasing amounts of ozone. The resulting ozonized samples were then analyzed for methoxyl and the quantitative solubility in sulfite "cooking liquor" was determined (Table I).

TABLE I

THE ACTION OF OZONE ON BIRCH FORMIC ACID LIGNIN

G. of O ₃ reacting per 2 g. of lignin	Water-insoluble fraction		Original minus final OCH ₃	% solubility in sulfite liquor of ozonized lignin
	% yield	% OCH ₃		
0.167	95	15.9	2.7	36
.290	88	13.9	4.7	61
.368	68	11.7	6.9	88
.404	72	12.5	6.1	78
.396	73	12.6	6.0	73
.405	65	11.5	7.1	82
.463	60	11.8	6.8	80
.544	53	10.9	7.7	93

These results expressed graphically (Fig. 1) show that both the loss in methoxyl and the increased solubility in sulfite liquor vary directly with the amount of ozone employed. From these data it was possible to calculate the approximate amount

(1) From a thesis submitted to the Faculty of Graduate Studies and Research, McGill University, by Rodger M. Dorland in partial fulfillment of the requirements for the Degree of Doctor of Philosophy, May, 1939. Paper XLV, *THIS JOURNAL*, **61**, 2204 (1939).

(2) Phillips and Goss, *THIS JOURNAL*, **55**, 3466 (1933).

(3) Bell, Hawkins, Wright and Hibbert, *ibid.*, **59**, 598 (1937).

(4) (a) Lief, Wright and Hibbert, *ibid.*, **61**, 1477 (1939); (b) A. Bell, Ph.D. Thesis, McGill University, 1937.

of ozone necessary to give a completely bisulfite soluble lignin. Accordingly, birch formic acid^{4a} and birch acetic acid^{4b} lignins were treated with ozone to the required degree and the sulfonic acids prepared by the customary "sulfite cook."

The lignin sulfonic acid prepared by cooking yellow birch wood meal with acid sodium sulfite yields, on alkaline cleavage, 2.7% vanillin and 2.8% syringaldehyde based on the weight of lignin.⁵ Acetosyringone, but not acetovanillone, also has been isolated from the same waste sulfite liquor although the yield was much smaller, being of the order of 0.8%.⁶ Accompanying this was a small quantity of pyrogallol 1,3-dimethyl ether amounting to 0.2%.⁷ In view of these results it was of interest to determine the action of alkali on the sulfonated, ozonized birch lignins. The sulfonated, ozonized, birch formic acid lignin yields, on alkaline cleavage, only guaiacyl derivatives, namely, vanillin and acetovanillone, and in approximately equal amounts (1%). The same results have been observed in the case of the corresponding ozonized acetic acid lignin, the yields being of the order of 2.7% also based on the weight of lignin. Presumably this is the first time that acetovanillone has been obtained from a hard wood waste sulfite liquor since Leger and Hibbert⁶ found only acetosyringone.

There are two possible explanations as to why no syringyl analogs were found in the present investigation. In the first place the formic and acetic acid lignins which were used had been subjected previously to a solvent fractionation and in both cases the vanillin and acetovanillone were obtained from fractions of the crude lignin having the lowest methoxyl content. It is also to be noted that the formic and acetic acid lignins, previous to sulfonation, were subjected to the action of ozone and there is thus the possibility that, if syringyl groups did exist, they might have been split off as part of the water-soluble fraction. This latter possibility is at present under investigation.

Experimental

The Loss in Methoxyl and the Increase in Solubility in Sulfite Liquor of Birch Formic Acid Lignin with Respect to the Degree of Ozonization.—A 2.0-g. sample of formic acid lignin^{4a} (18.6% OCH_3) was dissolved in 50 cc. of formic acid (95%) and at 0° ozone was passed into this solution for varying lengths of time. The amount of

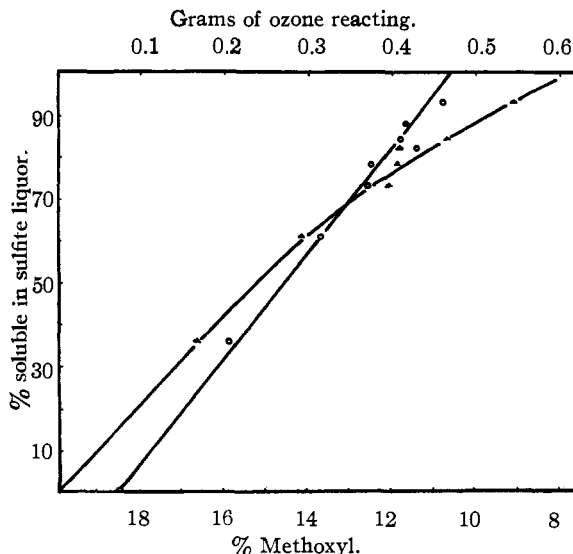


Fig. 1.—The action of ozone on birch formic acid lignin: ▲, increase in solubility of birch formic acid lignin in sulfite liquor with increasing ozonization; ○, increase in solubility of birch formic acid lignin in sulfite liquor with loss in methoxyl.

ozone delivered by the machine was determined at intervals by passing the gas through a neutral potassium iodide solution for a definite period of time and titrating with standardized thiosulfate the iodine liberated on acidification. The gas passing out of the lignin solution was also treated in a similar manner in order to determine the amount of ozone not absorbed by the solution. When the ozonization was complete the formic acid was removed by distillation under reduced pressure, 300 cc. of water added to the residue and the solution refluxed for thirty minutes. On cooling, the water-insoluble lignin was filtered off, washed with water, dried at 60° (20 mm.) and then weighed.

The ozonized lignin was analyzed for methoxyl and the solubility in sulfite liquor determined in the following manner. Exactly 0.100 g. of the lignin was sealed in a small glass bomb with 6 cc. of sodium bisulfite "cooking liquor" (6.0% total and 1.15% combined sulfur dioxide) and the bomb placed in a toluene vapor bath (110°) for twelve hours. On completion of the cook the bomb was cooled to 0°, opened, and the contents washed into a small, tared, centrifuge cup. The insoluble lignin was centrifuged off, washed with water, and dried overnight at 60° (20 mm.). The per cent. soluble in the sulfite liquor was then calculated by difference. These results have been summarized in Table I.

The Isolation of Vanillin and Acetovanillone from Ozonized Birch Formic Acid Lignin.—Ten grams of birch formic acid lignin (15.5% OCH_3)^{4a} was dissolved in 400 cc. of formic acid (95%) and at 0° this solution was treated with 2.56 g. of ozone over a period of eight hours, during which time 2.34 g. of ozone was absorbed by the solution. The solvent was then removed under reduced pressure and the residue refluxed for one hour with one liter of water. Upon cooling, the water-insoluble fraction was filtered off, washed with water and dried. This product

(5) Hawkins, Wright and Hibbert, *This Journal*, **59**, 2447 (1937).

(6) Leger and Hibbert, *ibid.*, **60**, 565 (1938).

(7) Leger and Hibbert, *Can. J. Research*, **16B**, 151 (1938).

was ground to a fine powder and again refluxed with one liter of water for forty-five minutes. The lignin was then filtered, washed and dried; yield 6.9 g.; 8.3% OCH_3 ; 95% soluble in bisulfite "cooking liquor" after heating at 110° for twelve hours.

Sodium bisulfite liquor (200 cc. containing 6.0% total and 1.15% combined SO_2) and 6.8 g. of the ozonized lignin were heated in a stainless steel bomb to 110° over a period of one and one-half hours. The bath was held at 110° for four hours and then raised to 130° for an additional eight hours. Upon cooling, the contents of the bomb were filtered and the volume of the filtrate made up to 500 cc. with water. The insoluble material after drying weighed 0.8 g.

A portion of the filtrate (100 cc.) was freed from inorganic material by dialysis against running distilled water for seven days. On evaporating the solution to dryness a relatively pure sample of the sulfonated lignin product was obtained; analysis, 3.3% OCH_3 , 6.6% sulfur.

The alkaline cleavage of the sulfonated product was carried out by adding 70 g. of sodium hydroxide to 290 cc. of the crude liquor and heating this at 135° for twelve hours under an atmosphere of oxygen-free nitrogen. The solution was cooled, just acidified with 50% sulfuric acid and extracted continuously with benzene for forty-eight hours. The benzene extract was shaken out ten times with 15-cc. portions of 5% sodium bisulfite; this latter solution was acidified and the sulfur dioxide removed under reduced pressure. The solution was then filtered and the volume made up to 200 cc. The total aldehydes present were determined by adding 10 cc. of an aqueous solution of 2,4-dinitrophenylhydrazine hydrochloride to a 20-cc. aliquot of the solution. After standing for six hours the precipitate was filtered on to a tared sintered glass funnel, washed and dried at 105° ; weight, 0.1005 g. or 1.3% calculated as vanillin based on the lignin in the liquor.

The remaining 180 cc. of solution was extracted continuously with ether. The residue after the removal of the ether was sublimed at 61° (2 mm.) yielding 0.040 g. of a crystalline product. Recrystallization from petroleum ether (80 – 90°) gave white, feathery crystals, m. p. 81 – 82° . A mixed m. p. with an authentic sample of vanillin showed no depression. The yield of the vanillin based on the lignin was 1.1%.

The benzene extract of the acidified alkaline cleavage product, left after the extraction with bisulfite, was evaporated to dryness under reduced pressure. The residue was sublimed at 150° (2 mm.). Resublimation of this product at 90° (2 mm.) yielded 0.055 g. of crystalline material. Recrystallization from petroleum ether (80 – 90°) gave white crystals, m. p. 111 – 112° . A mixed melting point with an authentic sample of acetovanillone showed no depression. The yield of acetovanillone based on the lignin was 1.2%.

The Isolation of Vanillin and Acetovanillone from Ozonized Birch Acetic Acid Lignin.—Twelve grams of birch acetic acid lignin (18.8% OCH_3)^{4b} was dissolved in 300 cc. of formic acid and at 0° the solution then treated with 2.16 g. of ozone over a period of five and one-half hours, during which time 1.84 g. of ozone was absorbed by the solution. The solvent was removed under reduced pressure and the residue refluxed with one liter of water for

forty-five minutes. Upon cooling, the water-insoluble fraction was filtered off, washed with water and dried. This product was ground to a fine powder and again refluxed with one liter of water for forty-five minutes. The ozonized lignin was then filtered, washed and dried; yield 5.4 g.; 13.3% OCH_3 ; 95% soluble in sulfite liquor at 110° for twelve hours.

Five grams of the ozonized lignin and 200 cc. of the above sodium bisulfite "cooking liquor" were heated in a stainless steel bomb to 110° over a period of one and one-half hours and the bath then maintained at that temperature for twelve hours. The bomb was cooled, the contents filtered and the volume of the filtrate made up to 250 cc. with water. The insoluble material after drying weighed 0.02 g.

A portion of the filtrate (50 cc.) was purified by dialysis against running distilled water for seven days. The sulfonated product was obtained by evaporating the solution to dryness; 6.4% OCH_3 ; 6.15% sulfur.

The alkaline cleavage of this sulfonated product was carried out by adding 33.6 g. of sodium hydroxide to 140 cc. of the crude, filtered liquor and heating the mixture at 135° for twelve hours under an atmosphere of oxygen-free nitrogen. The cooled reaction product was just acidified with 50% sulfuric acid and extracted continuously with benzene for forty-eight hours. This benzene extract was then shaken out with bisulfite solution, as previously described, the bisulfite solution acidified, the sulfur dioxide removed, solution filtered and made up to a volume of 250 cc. The total aldehydes present were determined as the 2,4-dinitrophenylhydrazones on a 20-cc. aliquot of this solution; yield 0.0208 g. or 4% calculated as vanillin based on the lignin in the liquor.

The subsequent ether extract of the remaining acidified bisulfite solution was worked up in the same manner as described previously. The residue on sublimation and recrystallization yielded 0.075 g. of white crystals, m. p. 82 – 83° . The mixed m. p. with an authentic sample of vanillin showed no depression. The yield of vanillin based on the lignin was 2.7%.

The benzene extract of the acidified alkaline cleavage solution, after extraction with the bisulfite, was worked up in a similar manner and yielded 0.075 g. of crystalline material, m. p. 112 – 113° . The mixed m. p. with an authentic sample of acetovanillone showed no depression. The yield of acetovanillone based on the lignin was 2.7%.

Acknowledgments.—The authors wish to thank the Canadian National Research Council for the grant of a Bursary to one of them (R. M. D.).

Summary

Isolated lignins, obtained by extraction with formic and acetic acids, and which are normally insoluble in aqueous bisulfite solution, have been shown to yield soluble sulfonic acids after a prior treatment of the lignin with ozone.

Sulfonic acids obtained in this manner from birch formic and acetic acid lignins have been shown to yield vanillin and acetovanillone on alkaline cleavage. Presumably this is the first

time that acetovanilone 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 Acid²

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 significance⁶ 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 acid⁷ 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 fulfillment 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 Guttman, *Biochem. Z.*, **259**, 100 (1933).

(5) Ehrlich, Guttman 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*, **52**, 2474 (1930).

Experimental Part

Preparation of Polygalacturonic Acid.—One hundred grams of powdered apple or citrus pectin⁹ 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 chloride¹⁰ 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 pH 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.