Experimental

Determination of the Formaldehyde Liberated by the Action of Acids on Extracted Lignins and Related Substances.—This was carried out as described in the previous communication.[6]

Stability of Piperonal toward Boiling Formic Acid (95%).—Piperonal (2.000 g.) was refluxed with 95% formic acid (20 cc.) for sixteen hours following the method of Wright and Hibbert.[14] After removing the formic acid under reduced pressure, the residue was dissolved in ether and the ether solution extracted with cold 1% sodium hydroxide solution. The ether extract was dried over anhydrous calcium chloride, concentrated, and the residue distilled under reduced pressure. The distillate (1.696 g., equivalent to 84.8%) crystallized at room temperature, m. p. 36–38°. A mixed melting point with the original product showed no depression.

Stability of Piperonal to Hot Ethanol Containing 3% Hydrogen Chloride.—Piperonal (2.000 g.) was refluxed for twenty-four hours with absolute ethanol containing 3% hydrogen chloride. These conditions were somewhat more drastic than those used for the ethanolysis of maple and sassafras woods. The ethanol was removed under reduced pressure, the residue dissolved in ether, and the piperonal isolated as above: weight of recovered piperonal, 1.827 g. (91.3%).

Behavior of Lignins toward Boiling Formic Acid (95%).—The various ethanol lignins (3.0 g.) were each refluxed with 95% formic acid (30 cc.) for sixteen hours. The solution was then poured with stirring into 300 cc. of distilled water and the precipitated lignins washed free from acid. The precipitates were vacuum dried over calcium chloride at room temperature and then over phosphorus pentoxide at 56° (14 mm.). The products were analyzed for methoxyl and the formaldehyde yield determined. The results are shown in Table I.

Preparation of Sassafras Ethanol Lignin.—Sassafras woodmeal (450 g.) previously extracted with (i) alcohol–

benzene, (ii) benzene and (iii) water, after drying was refluxed for twenty-four hours with 3 liters of absolute ethanol containing 2% hydrogen chloride, as previously described.[9] This gave 52.0 g. of sassafras ethanol lignin and 36.3 g. of crude ether soluble oils, as compared with 44.6 g. of lignin and 36.7 g. of crude oils from a similar run with maple wood. From the sassafras crude oils there was obtained a neutral fraction of 2.2 g. Distillation of this latter with 28% sulfuric acid gave no formaldehyde.

Summary

The absence of the piperonyl group in the structure of lignin is indicated by (a) the inability of workers in this field to isolate from an extracted lignin a piperonyl-containing derivative; (b) the pronounced instability toward formic acid of the formaldehyde-producing constituent in lignin as compared with the very much greater stability of the piperonyl radical present in known compounds; (c) the liberation of formaldehyde from such derivatives as cinnamyl alcohol, and the probability of the presence of this type of grouping in extracted lignins; (d) the behavior of ethanol sassafras lignin and the water-soluble ethanolysis products from sassafras wood toward hot dilute acids, whereby yields of formaldehyde similar in amount to those found with other woods are obtained from the former, and none from the latter.

(14) Wright and Hibbert, This Journal, **59**, 125 (1937).

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Studies on Lignin and Related Compounds. XLIV. The Ethanolysis of Maple Wood; Separation and Identification of the Water-Soluble Aldehyde Constituents[1]

By James J. Pyle, Leo Brickman and Harold Hibbert

In two recent communications[2] of this series, a description was given of the isolation, in considerable amount, of water-soluble aromatic constituents formed in the ethanolysis of spruce and maple woods. The crude oils were shown to consist of a phenolic, aldehydic, acidic and neutral

(1) From a thesis submitted to the Faculty of Graduate Studies and Research, McGill University, by James J. Pyle in partial fulfilment of the requirements for the degree of Doctor of Philosophy, May, 1939.

(2) (a) Cramer, Hunter and Hibbert, This Journal, **61**, 509 (1939); (b) Hunter, Cramer and Hibbert, ibid., **61**, 516 (1939).

fraction. In a later paper,[3] a revised method for their isolation was given by which much improved yields were obtained. The phenol fraction from spruce wood[2a] has been shown to consist in large part of α-ethoxypropiovanillone and that from maple wood[2b] of a mixture, in approximately equal amounts, of this and α-ethoxypropiosyringone.

In the present communication an account is given of the separation and identification of the

(3) Brickman, Pyle, McCarthy and Hibbert, ibid., **61**, 868 (1939).

METHODS OF SEPARATION OF SYRINGYL AND GUAIACYL COMPONENTS IN THE WATER-SOLUBLE ALDEHYDE FRACTION
FROM MAPLE WOOD

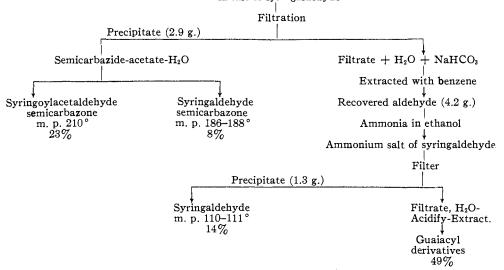Crude Ethanolysis Water-Soluble Aldehyde Fraction (9.1 g.)
|
Distillation; b. p. 160–170° (0.010 mm.)
↓
Clear yellow oil (7.25 g.; 80%)
|
Addition of KAc and KOH in EtOH
↓
K-salt of syringoylacetaldehyde
+
K-salt of syringaldehyde
|
Filtration

Precipitate (2.9 g.) ——————————————————————— Filtrate + H₂O + NaHCO₃

Semicarbazide-acetate-H₂O   Extracted with benzene
                                                              ↓
                                                       Recovered aldehyde (4.2 g.)
                                                              |
                                                       Ammonia in ethanol
                                                              ↓
                                                       Ammonium salt of syringaldehyde
                                                              |
                                                       Filter

Syringoylacetaldehyde   Syringaldehyde
semicarbazone   semicarbazone
m. p. 210°   m. p. 186–188°
23%   8%

Precipitate (1.3 g.)

Syringaldehyde   Filtrate, H₂O-
m. p. 110–111°   Acidify-Extract.
14%   ↓
                               Guaiacyl
                               derivatives
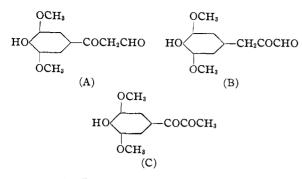   49%

aldehydic constituents present in the ethanolysis fraction from maple wood. It has been found that this consists of a mixture of syringoylacetaldehyde, syringaldehyde, vanilloylacetaldehyde and vanillin in approximately equal amounts, thus providing a further significant proof of this characteristic difference between soft and hard woods emphasized repeatedly by Hibbert and co-workers.[4] The total syringyl and guaiacyl fractions amount to 8.2% calculated on the weight of (Klason) lignin present in the wood.

The method used in separating these two types was that outlined in a previous communication,[4d] namely, precipitation of the syringyl, but not the guaiacyl, components by the addition of potassium acetate in anhydrous ethanol. Advantage was taken of an improvement on this method, found in the use of a solution of anhydrous ammonia in ethanol, whereby a quantitative precipitation of the syringyl derivatives is effected. Use of both reagents with the corresponding aldehydic fraction from spruce, and in which only the

guaiacyl derivatives (vanilloylacetaldehyde and vanillin in equal amounts) are present, gave no precipitate. The use of potassium acetate in ethanol brought about a practically complete precipitation of the syringoylacetaldehyde and a partial precipitation of the syringaldehyde. The remaining portion was recovered by treatment with anhydrous ammonia in ethanol as a yellow, crystalline precipitate. The filtrate from this latter treatment contained the whole of the guaiacyl components (vanilloylacetaldehyde and vanillin) the details of the separation and identification of which are to appear in a later communication.

The method of separation is outlined in the flow-sheet.

The analysis of the syringoylacetaldehyde and the preparation and analysis of its mono- and disemicarbazones established its empirical formula as $C_{11}H_{12}O_5$, and showed the presence of two carbonyl groups. Oxidation with hydrogen peroxide yielded syringic acid, thus establishing definitely the presence of the syringyl nucleus. Its structure must, therefore, correspond to one of three products

(4) (a) Hawkins, Wright and Hibbert, THIS JOURNAL, 59, 2447 (1937); (b) Leger and Hibbert, Can. J. Research, B16, 151 (1938); (c) Leger and Hibbert, THIS JOURNAL, 60, 565 (1938); (d) Hunter and Hibbert, ibid., 61, 2190 (1939).

$$\underset{\text{(A)}}{\underset{\overset{|}{OCH_3}}{\overset{\overset{|}{OCH_3}}{HO-C_6H_3}}-COCH_2CHO} \qquad \underset{\text{(B)}}{\underset{\overset{|}{OCH_3}}{\overset{\overset{|}{OCH_3}}{HO-C_6H_3}}-CH_2COCHO}$$

$$\underset{\text{(C)}}{\underset{\overset{|}{OCH_3}}{\overset{\overset{|}{OCH_3}}{HO-C_6H_3}}-COCOCH_3}$$

Formula C may be eliminated on the basis of the following results:

(a) Syringoylacetaldehyde gave a negative iodoform test, indicating the absence of the (—CO—CH₃) group; (b) it reduces ammoniacal silver nitrate indicating presence of an aldehydic group; and (c) it reacts *almost immediately* with semicarbazide hydrochloride in 3 N hydrochloric acid. Michael[5] has shown that ketones do not react under these acidic conditions.

In view of these facts structure (C) was eliminated and a decision between (A) and (B) was reached on the basis of the following experimental facts.

(a) Syringoylacetaldehyde gave a negative test with *o*-phenylenediamine, indicating absence of a 1,2-diketone.

(b) On oxidation with hydrogen peroxide a 65% yield of syringic acid was obtained, insufficient hydrogen peroxide being added to effect complete degradative oxidation of the side-chain.

Mechanism of Formation of Syringic Acid. —This will vary in the case of structures (A) and (B) as shown

$$R—COCH_2CHO \longrightarrow R—C(OH)\!=\!CHCHO \xrightarrow{H_2O_2} R—COOH$$

$$R—CH_2COCHO \longrightarrow R—CH\!=\!C(OH)CHO \xrightarrow{H_2O_2}$$
$$R—CHO \xrightarrow{H_2O_2} R—COOH$$

where R = syringyl

It is evident that with formula (B) oxidation to the acid proceeds through the intermediate compound, syringaldehyde. Syringaldehyde was therefore treated with hydrogen peroxide under the same experimental conditions and was *recovered unchanged*, indicating that it could not be an intermediate in the formation of the syringic acid.

(c) The similarity between formula (A) and compounds of the acetoacetic ester type, that is, with an active methylene group between two car-

bonyls, is evident. Thus under the influence of strong alkali, syringoylacetaldehyde would be expected to cleave to give syringic acid in the same manner that acetoacetic ester yields acetic acid. Karrer and Widmer[6] heated syringidin with 20% sodium hydroxide in a stream of hydrogen, and obtained a 30% yield of syringic acid. Isolation of syringic acid from syringoylacetaldehyde, also in yields of approximately 30% of the theoretical, under conditions in which no oxidizing medium is present, definitely establishes the position of the second carbonyl as adjacent to the nucleus.

On the basis of these results, this new aldehyde isolated from the ethanolysis products of maple wood must have formula (A).

Further evidence in support of this conclusion was obtained when the syringoylacetaldehyde was subjected to the action of aqueous bisulfite followed by alkaline cleavage. This experiment was one of a series[7] on the mechanism of sulfite pulp manufacture to determine whether any of the products isolated by ethanolysis would give rise to vanillin or syringaldehyde when heated with aqueous bisulfite followed by alkaline cleavage.

Instead of syringaldehyde the products isolated were acetosyringone and syringic acid. This result apparently is due to the incomplete removal of oxygen from the nitrogen used during the experiment to provide an inert atmosphere. Some of the syringoylacetaldehyde evidently was oxidized to syringoylacetic acid which latter, being a β-keto acid, underwent decarboxylation in the presence of the alkali, to give acetosyringone.

$$RCOCH_2CHO \xrightarrow{\text{[O]}} RCOCH_2COOH \xrightarrow{\text{[}-CO_2\text{]}} RCOCH_3$$
R = syringyl

The accompanying syringic acid probably is formed from some unoxidized syringoylacetaldehyde. Unfortunately it was not found possible to duplicate this experiment. ·

Recovery of Syringaldehyde.—As indicated in the flow-sheet, the monosemicarbazone of syringoylacetaldehyde was obtained directly from the potassium salt of the mixture of the two aldehydes. The filtrate from this contained the semicarbazone of syringaldehyde, which precipitated out from the solution on boiling and was identified as such.

The crystalline ammonium salt of the syringal-

(5) Michael, THIS JOURNAL, **41**, 393 (1919).

(6) Karrer and Widmer, *Helv. Chim. Acta*, **10**, 5 (1927).

(7) Under investigation in these Laboratories by Dr. Rodger Dorland.
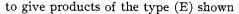
dehyde (see flow-sheet) gave with 2,4-dinitro-phenylhydrazine the hydrazone of syringaldehyde, the yield indicating that the ammonium salt was practically pure syringaldehyde.

On the basis of the yields of the semicarbazones and 2,4-dinitrophenylhydrazone and those obtained using known quantities of the pure aldehydes under the same experimental conditions, the syringoylacetaldehyde and syringaldehyde amount to 23 and 22%, respectively, of the total aldehyde fraction. Analysis of the guaiacyl fraction indicates that approximately the same ratio holds for the vanilloylacetaldehyde and vanillin.

The relationship of the various ethanolysis products to the structure of "protolignin" has been discussed by Hibbert[8] in a previous communication. He suggests that lignin is probably derived from the condensation of an aldehyde (methyl glyoxal) with a phenol (guaiacol or 1,3-pyrogallol dimethyl ether). Such a dimeric building unit (D) can, theoretically, undergo ring fission by hydrogen migration in a number of ways. Cleavage may thus give rise, as pointed out by him, to (a) two moles of a hydroxy ketone (α-hydroxypropiovanillone and α-hydroxypropiosyringone), or (b) to aldehydic constituents.

It is thus of considerable interest that the aldehydes obtained in the present investigation can be visualized as cleavage products formed by a specialized type of reversed aldol formation, from Hibbert's hypothetical dimer (D).

(D)

CH₂OH—CH₃

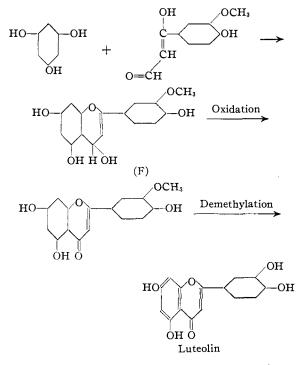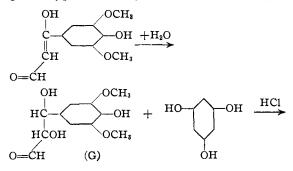to give products of the type (E) shown

(8) Hibbert, THIS JOURNAL, **61**, 725 (1939). Due to a printer's error, p. 729, the structure of the aldehyde isolated from maple wood was incorrectly given as corresponding to Formula IV instead of Formula VI.
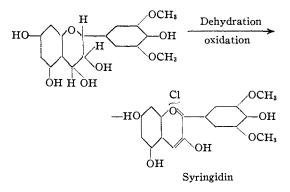

(E)

It is to be anticipated that vanillylacetaldehyde would undergo a similar condensation to give (F). The latter on oxidation and loss of methoxyl would yield a typical flavone, namely, luteolin.

(F)

Luteolin

On the other hand, hydration of syringoylacetaldehyde could give rise to formation of (G) which on condensation with phloroglucinol followed by loss of water and accompanying oxidation would give a typical anthocyanidin, namely, syringidin

(G)

Syringidin

Experiments with a view to effecting such typical syntheses are to be carried out.

It is thus of considerable interest that such apparently unrelated plant constituents as lignin and plant pigments may be visualized as originating from the same type of building unit.

Experimental

Ethanolysis of Maple Wood.—A complete and detailed account of the ethanolysis procedure and fractionation of water soluble oils is given in a recent communication,[3] so that only a brief summary is necessary.

A solvent-extracted maple wood meal was refluxed with ethanol containing anhydrous hydrogen chloride in an inert atmosphere (carbon dioxide) and the reaction mixture filtered. The ethanol filtrate was brought almost to neutrality by addition of a solution of sodium ethylate in ethanol, then concentrated, and precipitated into water. The filtrate was concentrated, and continuously extracted with benzene.

The benzene extract was shaken with aqueous bisulfite solution, the solution acidified and back-extracted with benzene. The benzene solution of the crude aldehyde fraction was used for the following investigation.

Separation of Guaiacyl and Syringyl Components.—A portion of the benzene solution was concentrated under reduced pressure, leaving 9.1 g. of a brownish-colored, very viscous residue. This on distillation gave two fractions (88% yield): (1) b. p. 160–170° (0.010 mm.), 7.25 g., clear yellow oil; (2) b. p. 250–350° (0.025 mm.), 0.73 g., red resin.

To a solution of the distilled aldehyde (Fraction 1, 7.25 g.) dissolved in ethanol (60 cc.) was added a saturated ethanolic solution of potassium acetate (60 cc.) followed by 18 cc. of 2% potassium hydroxide in ethanol to neutralize the acetic acid formed, and the mixture shaken well. The red crystalline precipitate was filtered, washed with ethanol and dried; yield, 2.9 g., or 34.6% on the basis of free aldehyde.

The filtrate was diluted with 2–3 volumes of water, made slightly alkaline with sodium bicarbonate, and continuously extracted with benzene in an atmosphere of carbon dioxide. The amount of recovered aldehyde was 4.2 g. or 90% of the theoretical. This recovered aldehyde gave no precipitate with potassium acetate in ethanol solution.

The recovered aldehyde mixture (4.2 g.) was now dissolved in 25 cc. of anhydrous ethanol and 0.7 g. of anhy-

drous ammonia dissolved in 10 cc. of ethanol added. A yellow crystalline precipitate formed immediately. This was filtered and dried; wt. 1.3 g., or 16.8% calculated as the free aldehyde.

The guaiacyl derivatives were recovered from the filtrate by diluting again with several volumes of water, acidifying with sulfuric acid and continuously extracting with benzene in an atmosphere of carbon dioxide.

Isolation of Syringoylacetaldehyde.—The red crystalline potassium salt (11.3 g.) obtained above was dissolved in 100 cc. of water and 1 cc. of concentrated sulfuric acid added. The solution was extracted with benzene leaving after removal of the solvent 9.3 g. of a yellow oil, which crystallized almost completely on standing. Recrystallization first from low-boiling (60–70°), and then from high-boiling petroleum ether (100–110°) gave 4.0 g. of crude syringoylacetaldehyde, m. p. 64–68°. After several recrystallizations a constant melting point, 74.0–74.5°, was reached.

Concentration of the mother liquors yielded an oil which on treatment with semicarbazide acetate gave, as shown later, a mixture of the semicarbazones of syringoylacetaldehyde and syringaldehyde.

Analysis of Syringoylacetaldehyde.—Calcd. for $C_{11}H_{12}O_5$: C, 58.9; H, 5.4; OCH$_3$, 27.9. Found: C, 58.8; H, 5.6; OCH$_3$, 27.9.

Syringoylacetaldehyde is soluble in almost all organic solvents and also relatively soluble in water. When crystallized very slowly from petroleum ether (b. p. 100–110°), it separates in the form of colorless, hexagonal crystals.

Proof of Presence of the Syringyl Nucleus.—The aldehyde (0.050 g.) was dissolved in 5 cc. of water and 0.2 cc. of 3% hydrogen peroxide added. The reaction mixture was allowed to stand at room temperature for four hours, and then at 0° for a further two hours. Long colorless needles crystallized from the aqueous solution, m. p. (crude) 198–200°. After recrystallization from water the melting point rose to 204–205° and a mixed melting point with an authentic sample of syringic acid showed no depression.

Proof of the Dicarbonyl Structure by Preparation of a Mono- and Disemicarbazone. (a) Monosemicarbazone. —The isolated syringoylacetaldehyde (0.050 g.), semicarbazide hydrochloride (0.030 g.) and potassium acetate (0.035 g.) were dissolved in 5 cc. of water. Long, colorless needles crystallized from the solution on standing for several hours and were recrystallized from hot water; yield, 0.045 g., 71.5%; m. p. 210–210.5°.

This derivative was also prepared in 1 and 3 N hydrochloric acid solution. It could be obtained directly from the crystalline mixture of the potassium salt of syringoylacetaldehyde and syringaldehyde (see flow-sheet) by dissolving the mixture in an aqueous solution of semicarbazide acetate and removing the crystalline monosemicarbazone after a period of two hours in order to avoid admixture with the semicarbazone of syringaldehyde.

Analysis of Monosemicarbazone of Syringoylacetaldehyde.—Calcd. for $C_{12}H_{15}O_5N_3$: C, 51.2; H, 5.59; N, 14.9; OCH$_3$, 22.0. Found: C, 51.6; H, 5.61; N, 14.8; OCH$_3$, 22.0.

(b) Preparation of the Disemicarbazone.—The monosemicarbazone (0.090 g.) was mixed with a solution of 0.050 g. of freshly-prepared, free semicarbazide in 10 cc. of eth-

anol and refluxed for five hours. The hot ethanol solution containing the insoluble disemicarbazone was filtered and the product recrystallized from hot water; short, colorless needles, insoluble in all common organic solvents; yield, 0.080 g., 75%; m. p. 239.0°.

Anal. Calcd. for $C_{13}H_{18}O_5N_6$: C, 46.2; H, 5.3; N, 24.9; OCH_3, 18.4. Found: C, 46.5; H, 5.6; N, 24.9; OCH_3, 18.4.

Alkaline Cleavage of Syringoylacetaldehyde.—Syringoylacetaldehyde (0.100 g.) dissolved in 5 cc. of water was placed in a small distilling flask, fitted with a reflux condenser ("cold finger") and then a stream of nitrogen, from which all traces of oxygen had been removed, was passed into the flask through the side-arm. When all of the oxygen had been removed, 1.2 g. of sodium hydroxide was added and the reaction mixture heated to 130–140° for twelve hours, a nitrogen atmosphere being maintained throughout. The solution was cooled, acidified with dilute sulfuric acid and extracted continuously with benzene. The benzene was removed and the residue sublimed at 117–125° (2 mm.). The white, crystalline sublimate was recrystallized from water; melting point 203–205°, a mixed melting point with authentic syringic acid showed no depression; yield, 0.026 g., 30%.

Action of Bisulfite Followed by Alkaline Cleavage.—A solution of 0.050 g. of syringoylacetaldehyde in 2 cc. of "sulfite cooking liquor" (containing 6% free sulfur dioxide and 1.1% combined sulfur dioxide) was placed in a small sealed tube and heated to 110° for twelve hours.

The sealed tube was cooled, opened, and the contents transferred by use of 3 cc. of water into a small distilling flask, fitted as in the preceding experiment. Sodium hydroxide (1.2 g.) was added as before, but inadvertently the oxygen was incompletely removed from the stream of nitrogen. The alkaline solution was heated to 135° for twelve hours, cooled, acidified and continuously extracted with benzene. The benzene solution was shaken with aqueous bisulfite (5%) and the bisulfite solution acidified and extracted with ether. The benzene solution left after the bisulfite treatment was evaporated to dryness and the residual dark brown solid sublimed at 115–120° (2 mm.). The sublimate was washed with cold petroleum ether (30–50°) and resublimed, m. p. 117–118°; a mixed melting point with authentic acetosyringone showed no depression; yield, 0.015 g., 34%.

The ether solution was treated in the same manner and yielded 0.008 g. of a white, crystalline sublimate which was shown by melting point (202–204°) and mixed melting point to be syringic acid.

Isolation and Identification of Syringaldehyde

(1) From the Crystalline Potassium Salt.—The mixture of the reddish-colored crystalline potassium salts (0.45 g.) was dissolved in 15 cc. of water and semicarbazide hydrochloride (0.30 g.) and potassium acetate (0.40 g.) added. The homogeneous reaction mixture was allowed to stand at room temperature for an hour, cooled to 0° and filtered. The crystalline precipitate was the monosemicarbazone of syringoylacetaldehyde obtained above; yield 0.22 g.

The filtrate was heated to 100° and then kept at 0° for several days. Clusters of short, colorless needles slowly formed throughout the solution. These were filtered off and dried. Recrystallization from water and methanol raised the melting point to 186–188° and a mixed melting point with an authentic sample of syringaldehyde semicarbazone showed no depression.

Anal. Calcd. for $C_{10}H_{13}O_4N_3$: OCH_3, 25.9. Found: OCH_3, 26.1.

(2) From the Crystalline Ammonium Salt.—To a solution of 0.10 g. of the ammonium salt, dissolved in 7 cc. of ethanol containing a few drops of concentrated hydrochloric acid, was added 0.085 g. of 2,4-dinitrophenylhydrazine, also dissolved in 5 cc. of ethanol–hydrochloric acid. The solution was allowed to stand at room temperature for four to six hours, during which period a voluminous red crystalline precipitate formed. The product was filtered off and recrystallized from ethyl acetate; yield, 0.165 g., 82%; m. p. 235–235.5°.

The 2,4-dinitrophenylhydrazone of an authentic sample of syringaldehyde was prepared in the same manner, m. p. 235.5–236.5°. A mixed melting point showed no depression.

Anal. Calcd. for $C_{15}H_{15}O_7N_4$: OCH_3, 17.2. Found: OCH_3, 17.3.

Summary

1. The water soluble "aldehyde fraction" obtained in the ethanolysis of maple wood has been shown to consist of a mixture of syringyl and guaiacyl derivatives, present in approximately equal amounts.

2. A method has been devised for the complete separation of syringyl constituents by precipitation of their insoluble potassium and ammonium salts.

3. The syringyl fraction has been shown to consist of syringoylacetaldehyde and syringaldehyde, present in approximately equal amounts.

4. Syringoylacetaldehyde has been isolated and completely characterized by analysis, preparation of derivatives, and degradation to known compounds.

5. The significance of the presence of syringaldehyde and syringoylacetaldehyde among the ethanolysis products from maple wood with regard to the structure of "protolignin" and their relation to the synthesis of plant pigments has been pointed out.