Summary

It is proposed to abandon the use of the symbols α and β as parts of the actual names of the higher carbon sugars and to designate these by the abbreviated names of two hexoses, one of which indicates the hexose that is used in the synthesis and the other of which shows the hexose that the new sugar resembles in its ring configurations. Examples are shown for the proposed names and for the use of the symbols α and β in designating the stereostructures of the pyranoid, furanoid and septanoid ring forms.

WASHINGTON, D. C. RECEIVED MARCH 3, 1938

[CONTRIBUTION NO. 239, DEPARTMENT OF CHEMISTRY, KANSAS STATE COLLEGE]

The Reaction of Kojic Acid with Aldehydes[1,2]

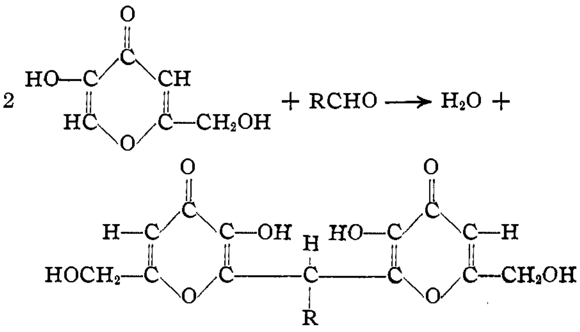
BY H. N. BARHAM AND G. NATHAN REED

Kojic acid, 2-hydroxymethyl-5-hydroxy-γ-pyrone, was first obtained by Saito[3] in a culture of *Aspergillus oryzae* grown upon steamed rice. Since then it has been found to be produced when different species of *Aspergilli* are grown upon a variety of organic substances.[4] The acid used in this research was produced in the laboratories of Kansas State College by the growth of *Aspergillus flavus* upon a glucose medium.

While investigating the properties of kojic acid it was observed that a solid reaction product was obtained readily with formaldehyde. This reaction was extended to other aldehydes until a series of solid derivatives was obtained which included derivatives from all of the normal, saturated, aliphatic aldehydes up to heptaldehyde, as well as acrolein, benzaldehyde, hydrocinnamaldehyde, cinnamaldehyde, and α-furfuraldehyde.

An investigation of these derivatives was made in order to establish their constitution. The molar quantities involved in their preparation, together with other quantitative data, indicated that the products were formed by the elimination of one molecule of water from two molecules of kojic acid and one molecule of aldehyde. The aldehyde products gave red colorations with ferric chloride, could be titrated with standard alkali and formed insoluble copper salts which contained the calculated per cent. of copper. Upon treating with thionyl chloride, chloro compounds were formed which could be produced also by the reaction of the corresponding aldehydes with 2-chloromethyl-5-hydroxy-γ-pyrone (obtained from kojic acid with thionyl chloride). Acetate esters were prepared and when hydrolyzed gave acetyl values corresponding to four hydroxyl groups per molecule. Attempts to hydrolyze the aldehyde products with dilute acid or alkali were unsuccessful. When the aldehyde products were treated with phenyldiazonium chloride and the resulting mixture neutralized with alkali, colored compounds were produced which appeared to be unstable diazonium salts of the phenolic hydroxyls. Under similar conditions, kojic acid coupled with phenyldiazonium chloride to form a stable dye.

It was concluded, therefore, that these aldehyde products were formed according to the equation



Experimental Data

Reagents.—The crude kojic acid obtained by concentrating the fermentation liquor was recrystallized twice from water, using Norite in the first recrystallization to decolorize the solution. The final purification was accomplished by chilling hot alcoholic solutions with rapid stirring. Prepared in this manner, the acid was obtained as fine, light cream-colored needles of m. p. 152–153°.

The formaldehyde used was a U. S. P. formalin solution. The other aldehydes were Eastman preparations.

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(1) Abstract of a thesis presented by G. Nathan Reed to the Graduate Council of Kansas State College in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

(2) Presented in part before the Division of Organic Chemistry of the American Chemical Society at the Rochester meeting, September, 1937.

(3) K. Saito, *Bot. Mag. Tokyo*, **21**, No. 249 (1907).

(4) A critical review of the literature upon kojic acid was made by Barham and Smits, *Trans. Kansas Acad. Sci.*, **37**, 91–113 (1934).

Preparation of the Aldehyde Products.—Although in the preliminary study slightly better yields were obtained from the use of two mols of kojic acid for each mol of aldehyde, in the actual preparation equimolar proportions were used because the products obtained in this way were easier to purify. A typical reaction mixture contained 10 g. of kojic acid and an equivalent amount of aldehyde dissolved in 100 cc. of ethyl alcohol. One cc. of concentrated ammonium hydroxide was added as a catalyst. The reaction mixture was refluxed one hour and then placed in an electric air-oven at 60–65° for from two to forty-eight hours depending upon the aldehyde used. By attaching a long glass tube to the reaction flask and passing this through the vent in the top of the oven, evaporation was prevented and the heating could be continued as long as required. Following this period in the oven, the reaction mixture was poured into a crystallizing dish and concentrated by surface evaporation. The solid material which formed was removed by filtration, washed with a little alcohol, and dried in a desiccator under reduced pressure.

The reaction of kojic acid and the aldehydes was always accompanied by the formation of resinous material. The quantity produced was influenced decidedly by the solvent, the catalyst, and the temperatures employed. Formaldehyde and kojic acid gave good yields with water as the solvent if no ammonium hydroxide was added, but in the presence of this catalyst resin formation was dominant. The remaining aldehydes did not react in water solutions. Likewise, the crystalline products were not formed when alcoholic solutions were used without ammonium hydroxide. On the other hand, using alcoholic solutions and ammonium hydroxide, the presence of any appreciable concentration of water favored the formation of these resins. Furthermore, the quantity of this resinous material was increased by lengthening the period of heating, especially at the boiling point of the alcoholic solutions. The particular conditions described above were selected in order to reduce to a minimum the amount of these resinous substances in the crude products.

The lower aldehydes reacted readily. Among the higher aliphatic aldehydes, the rate of reaction was influenced by the length and character of the carbon chain—those with an even number of carbon atoms reacted more easily than those with an odd number. The yields were good in most instances. Benzaldehyde reacted readily, giving good yields. Hydrocinnamaldehyde was somewhat slower in action while cinnamaldehyde reacted very slowly. Furfuraldehyde reacted readily but gave only fair yields. Acrolein reacted with extreme difficulty and the yields were very poor.

Purification of the Products.—The crude reaction products were purified by extraction with boiling chloroform or by recrystallization from alcohol. In the latter case, the alcoholic solutions were chilled in a freezing bath of salt and ice while stirring vigorously to prevent supersaturation. Repeated extractions or recrystallizations were necessary to eliminate the greater portion of the contaminating resinous substances but it is believed that traces still remained in spite of continued effort to remove them.

Physical Properties of the Products.—The purified products are light cream-colored, powdery solids. They are practically insoluble in cold water, while their solubility in hot water decreases rapidly as the molecular weight increases. All are somewhat soluble in alcohol with a pronounced tendency to give supersaturated solutions. They are slightly soluble in methyl alcohol and acetone, much less soluble in chloroform, and apparently insoluble in ether, ligroin, benzene, toluene, and carbon tetrachloride. They are soluble in alkali but insoluble in acids. The melting points of the products are given in Table I.

TABLE I

MELTING POINTS OF PRODUCTS

Aldehyde used	M. p., °C. (corr.)
Formaldehyde	248.3–249
Acetaldehyde	211.2–212
Propionaldehyde	217.5–218
Butyraldehyde	192.4–193
Valeraldehyde	185.6–187.2
Capraldehyde	144–147
Heptaldehyde	152.6–153.6
Benzaldehyde	242.4, dec.
Hydrocinnamaldehyde	182–183.5
Cinnamaldehyde	175–176
α-Furfuraldehyde	210–211
Acrolein	About 250[a]

[a] Yield too small to purify and determine m. p. carefully.

Combustion Analysis.—The combustion analyses of the aldehyde products are given in Table II. The calculated values are for a product formed by two mols of kojic acid and one mol of aldehyde with the loss of one mol of water.

TABLE II

ANALYTICAL RESULTS BY COMBUSTION

	Carbon, %			Hydrogen, %		
Aldehyde product	Calcd.	Found		Calcd.	Found	
Formaldehyde	52.70	52.78	52.85	4.05	4.42	4.22
Acetaldehyde	54.19	54.28	54.16	4.51	4.80	4.67
Propionaldehyde	55.55	55.71	55.28	4.94	5.11	5.02
Butyraldehyde	56.81	56.99	56.92	5.33	5.61	5.61
Valeraldehyde	57.95	58.01	57.98	5.68	5.81	5.94
Heptaldehyde	59.99	60.12	60.82	6.32	6.58	6.69
Benzaldehyde	61.31	61.96	61.35	4.30	4.52	4.43
Hydrocinnamaldehyde	63.00	62.50	62.31	5.00	5.06	5.05
α-Furfuraldehyde	56.35	55.99	56.01	3.87	4.08	4.04

Chemical Properties of the Products.—The aldehyde products gave a red coloration with ferric chloride. The intensity of the color produced decreased as the molecular weight increased, as would be expected from the solubilities. The products reacted readily with alkali and it is believed that they could be titrated with 0.1 *N* sodium hydroxide if an indicator were used which changed color at a *p*H of about 12. When the products were dissolved in standard alkali, back titrated with acid until neutral to phenolphthalein, and treated with an excess of copper acetate, insoluble copper salts were precipitated. These salts were analyzed for copper by igniting to copper oxide. The results given in Table III are for 1-g. samples of the aldehyde products and the calculated values are for products containing two phenolic hydroxyls and resulting from the reaction of two mols of kojic acid with one mol of aldehyde through the loss of one mol of water.

TABLE III

COPPER CONTENT

Product	Copper, % Calcd.	Found
Formaldehyde	17.785	17.690
Acetaldehyde	17.115	17.084
Propionaldehyde	16.494	16.675
Butyraldehyde	15.916	16.090
Heptaldehyde	14.403	14.370
Benzaldehyde[a]	14.668	16.550

[a] Copper precipitate brownish in color and obviously contained some reduced copper.

The kojic acid–aldehyde products reacted readily with thionyl chloride. The unchanged thionyl chloride was removed from the reaction mixture with petroleum ether and the derivatives recrystallized from water. These derivatives did not show the presence of ionizable halogen but a sodium fusion revealed the presence of organic halogen. The chloro compound prepared in this manner from the butyraldehyde product melted at 180–184°. When 2-chloromethyl-5-hydroxy-γ-pyrone (prepared from kojic acid through the action of thionyl chloride) was treated with butyraldehyde, using the procedure outlined in the preparation of the aldehyde products, a product was obtained which melted at 183–185°. Apparently the same compound was prepared by these two reactions.

The acetates of the aldehyde products were prepared by treating with an excess of acetic anhydride and a few drops of concentrated sulfuric acid. If these mixtures were allowed to stand for twelve hours at room temperature, dark red solutions were formed. Upon decomposing the excess of acetic anhydride with water and evaporating the solutions before a fan, resinous products were obtained. The repeated extraction of these resinous products with water gave white solids which were purified by recrystallizing from ethyl alcohol or ethyl acetate. If the original reaction mixtures were treated with a large excess of ether, the acetates were precipitated immediately as crystalline solids. The melting points of the acetates were: from formaldehyde product, 105–107°; from acetaldehyde product, 134–136°; and from benzaldehyde product, 166–168°.

The acetates of the aldehyde products were hydrolyzed quantitatively by dissolving a weighed sample in a known quantity of standard alkali (an excess was always used), brought to colorless toward phenolphthalein with standard hydrochloric acid, and back titrated to full red with standard alkali. The net quantity of alkali used was determined by difference. The amount of alkali required for the liberated aldehyde product was determined by a blank. When this value was subtracted from the net quantity of alkali used, the difference was taken as a measure of the acetic acid produced upon hydrolysis. The values obtained for 1-g. samples of the acetates are given in Table IV and compared with the theoretical amounts of acetic acid calculated for diacetate and tetraacetate derivatives.

TABLE IV

HYDROLYSIS OF ACETATE ESTERS

Acetate prepared from	Total titration	No. of acetate groups	Required for nuclear OH	CH_3COOH by difference	Calcd.
Formaldehyde product	123.8	2	46.1	77.7	56.62
		4	37.7	86.1	86.70
Acetaldehyde product	123.2	2	41.0	82.2	50.76
		4	33.8	89.4	83.70
Benzaldehyde product	117.0	2	41.2	75.8	43.86
		4	34.8	82.2	74.1

A sample of the acetaldehyde product was dissolved in glacial acetic acid. The addition of an excess of ether to this solution precipitated a solid which, after recrystallizing from alcohol, required 49.50 cc. of 0.1 *N* sodium hydroxide to titrate a 1-g. sample to the full color of phenolphthalein. Since 50.80 cc. of 0.1 *N* sodium hydroxide was required to titrate a 1-g. sample of the original acetaldehyde product, it is evident that an oxonium salt was not formed by the aldehyde product.

The aldehyde products were not hydrolyzed either by refluxing for nineteen hours with dilute sulfuric acid or by stirring for forty-eight hours with 0.1 *N* sodium hydroxide.

Kojic acid did not react with benzal chloride in alcoholic solution. However, when the method of Friedel and Crafts was employed, using freshly distilled nitrobenzene as a solvent, a free evolution of hydrogen chloride was observed. After heating for two hours at 100–150° the reaction mixture was poured into an excess of water and the solid which precipitated removed by filtration. This solid was extracted repeatedly with ether and finally with alcohol. Evaporation of the alcohol gave a small amount of material which melted at 250–256° with decomposition; this compares favorably with the melting point of the crude benzaldehyde product.

When kojic acid was dissolved in alkali, cooled to 0–5°, treated with phenyldiazonium chloride, and the mixture made alkaline, a dark red solid was precipitated. This substance was stable. It could be dried and kept exposed to the air for an indefinite period of time. No apparent decomposition occurred when it was treated with moderately strong acid. When the same procedure was used with the aldehyde products, red and brown solids were obtained. However, these solids were unstable and turned dark upon drying in air. Apparently a gas was liberated during this decomposition. A freshly prepared sample of the colored compound obtained from the acetaldehyde product was suspended in cold, dilute (2:1) hydrochloric acid. After standing for a half hour at 0–5° the solution was filtered and the filtrate placed in a solution of "H-acid" (1-amino-8-naphthol-3,6-disulfonic acid). When this mixture was made alkaline an intense red color was produced which appeared to be identical with the color formed when "H-acid" was treated with phenyldiazonium chloride. The residue from the acid treatment melted at 188–198° and when mixed with an equal amount of acetaldehyde product the melting point rose to 201–210°. From these results, the colored compounds were assumed to be diazonium salts of the phenolic hydroxyls and not the products of true coupling reactions.

Discussion of Results

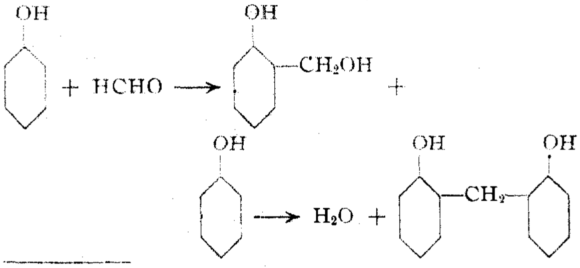
The qualitative analysis of the products consistently gave negative results for nitrogen. The production of color with ferric chloride, the reaction with alkali, and the precipitation of copper salts all indicate that the phenolic hydroxyls in position 5 are also present in the aldehyde products. Since these products do not hydrolyze readily they probably are not acetals. Further, the reaction with thionyl chloride points to the presence of the hydroxymethyl groups in the aldehyde products. These conclusions are all fully confirmed by the formation of tetraacetate esters.

This leaves position 6 as the most probable point of attack. It is known that when the Friedel and Crafts reaction is used with aromatic phenols, the hydrogen in the para position is replaced. However, when the para position is blocked, the reaction may occur at the ortho positions. If it is assumed that the hydroxymethyl group of kojic acid is para to the phenolic hydroxyl, then the only available point of attack is at position 6.

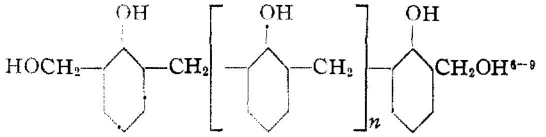
Further confirmation is given by the formation of the diazonium salts. Sidgwick[5] explains the coupling reaction of diazonium salts with phenols as forming a diazonium salt of the phenol which later rearranges by the diazo group passing to the para or ortho position. These diazonium salts of the phenol group are unstable, decomposing easily to give nitrogen, and when treated with dilute acids the original compounds are formed. Since both of these properties were observed in the products which were formed by the aldehyde products reacting with phenyldiazonium chloride, it may be assumed that diazonium salts of the phenolic hydroxyl groups were formed. If the aldehyde has attacked position 6, then all possible positions for the rearrangement of these phenolic salts are blocked and the true coupling reactions cannot proceed beyond this initial step.

It is concluded, therefore, that the aldehyde products may be represented by the general formula previously given.

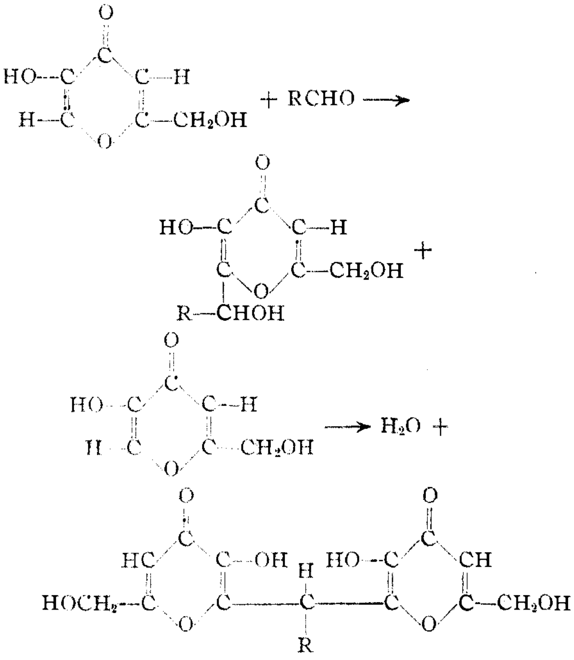
A study of the reaction of aromatic phenols with aldehydes to form resins of the Bakelite type reveals that the mechanism of reaction is probably



and that this reaction continues until a compound is formed which may be represented by



In the case of para substituted phenols, *i. e.*, *p*-cresol, the reaction occurs only at the ortho positions. Assuming that kojic acid reacts as a para substituted phenol, the analogous reaction would be



From this point of view, these aldehyde products may correspond to the compounds which appear in the early stages of phenolic resin formation. The reaction, however, stops at this stage because the ortho and para positions in both rings are blocked. That resins may be formed under certain conditions has already been mentioned under the preparation and purification. We believe that these resinous substances are produced either by molecules of kojic acid condensing from the hydroxymethyl group of one molecule to position 6 of a second molecule and so on, or by the aldehyde products condensing with unchanged kojic acid by a similar mechanism. Further investigation of this field is planned.

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(5) Nevil V. Sidgwick, "Organic Chemistry of Nitrogen," Oxford University Press, Oxford, 1910, p. 287.

(6) Baekeland and Bender, *J. Ind. Eng. Chem.*, **17**, 225–237 (1925).

(7) Morgan, *J. Soc. Chem. Ind. Trans.*, **49**, 245–251 (1930).

(8) Megson and Drummond, *ibid.*, **49**, 251–257 (1930).

(9) Wanscheidt, Itenberg and Andrejewa, *Ber.*, **69**, 1900–1907 (1936).

Conclusions

1. We have shown that kojic acid reacts with aldehydes to form solid derivatives. A series of these products has been prepared using all of the normal, saturated, aliphatic aldehydes up to heptaldehyde, in addition to acrolein, benzaldehyde, hydrocinnamaldehyde, cinnamaldehyde, and α-furfuraldehyde.
2. The probable formula for these products is given.
3. A possible mechanism of reaction is suggested based upon the present theory of resin formation by aromatic phenols and aldehydes.

MANHATTAN, KAN. RECEIVED MARCH 14, 1938

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

On the Mechanism of the Catechol-Tyrosinase Reaction

BY HARRY WAGREICH AND J. M. NELSON

Catechol has been used widely as a substrate in the study of oxidases, partly because the aerobic oxidation of this substance is catalyzed by a great many enzymes of this class. Robinson and McCance[1] showed that two atoms of oxygen are consumed in the complete oxidation of catechol, although only one atom is required, stoichiometrically, for the formation of *o*-benzoquinone, the normal product of primary oxidation. The reaction therefore is not a simple one, and a further complication arises from the fact that *o*-benzoquinone is very fugitive in aqueous solutions, especially in the presence of unoxidized catechol, as shown by Dawson and Nelson.[2]

Regarding the mechanism of the oxidation, Onslow,[3] Richter,[4] Platt and Wormall[5] and others favor the idea that hydrogen peroxide is one product of the reaction, which would account for the oxygen consumption

$$C_6H_4(OH)_2 + O_2 \longrightarrow C_6H_4O_2 + H_2O_2$$

On the other hand, Raper[6] suggests that one atom of oxygen converts catechol into *o*-benzoquinone and that this is oxidized further by the second atom consumed. To gain further insight into the problem, it seemed of interest to extend the observations of Dawson and Nelson by following the amount of oxygen consumed.

The apparatus shown in Fig. 1 was used to measure the amount of quinone formed when one atom of oxygen per mole of catechol had been consumed. The vessel was charged with 1250 cc. of 0.02 *M* phosphate-citrate buffer (*p*H 6.2) containing 100 mg. of catechol, and 500 units of tyrosinase preparation[7] (about 15 cc.) was placed in one of the side bulbs (F). The stirrer was started and, when the solutions in the vessel and

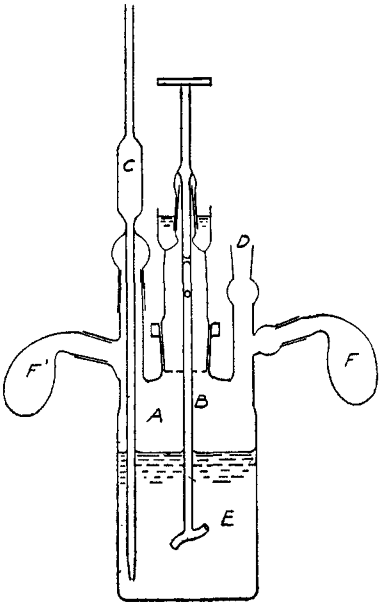

Fig. 1.—A modified form of the apparatus described by Reinders and Vles [*Rec. trav. chim.*, **44**, 1 (1925)]. A represents a heavy 2-liter glass reaction vessel, into which a pump-stirrer B was inserted. At the upper end of the stirrer was a water seal such as described by Rosenthal, Lorch and Hammett [THIS JOURNAL, **59**, 1795 (1937)]. The pipet C permitted the withdrawal of samples from the reaction solution. Tube D was connected to a manometer similar to that described by Reinders and Vles. Bulbs F and F' were used for introducing the enzyme. The vessel A was fitted into a metal frame so as to be held in position in the thermostat.

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[1] M. E. Robinson and R. A. McCance, *Biochem. J.*, **19**, 251 (1925).

[2] Charles R. Dawson and J. M. Nelson, THIS JOURNAL, **60**, 245 (1938).

[3] M. Wheldale Onslow, "Principles of Plant Biochemistry," University Press, Cambridge, 1931, p. 135.

[4] D. Richter, *Biochem. J.*, **28**, 901 (1934).

[5] B. S. Platt and A. Wormall, *ibid.*, **21**, 29 (1927).

[6] H. S. Raper, *Physiol. Rev.*, **8**, 245 (1928).

[7] Prepared from the common mushroom, *Psalliota campestris*, by the method of Graubard and Nelson, *J. Biol. Chem.*, **112**, 135 (1935).