

2,3,4,5-Dicyclopentenobenzoic Acid (IX).—The above tetrahydro ester (18 g.) was heated at 300–305° for three hours in the presence of palladinized charcoal (1.0 g., 10%). The product was dissolved in ether, filtered, and fractionated. There was obtained an ester (10 g.); b. p. 110–115° (2–3 mm.). Saponification of a portion of this ester gave the desired acid; m. p. 200–202°.

Anal. Calcd. for $C_{13}H_{14}O_2$: C, 77.2; H, 6.9; neut. equiv., 202. Found: C, 77.14; H, 6.5; neut. equiv., 199.

Summary

1. Further evidence is presented to show that the steric effect of the methylene group in hydrindene is smaller than that in tetralin.

2. A new and convenient synthesis of 1,2-cyclopentenonaphthalene is described.

MINNEAPOLIS, MINNESOTA

RECEIVED JULY 26, 1946

[CONTRIBUTION FROM THE BIOCHEMICAL LABORATORY, DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY, NEW YORK STATE AGRICULTURAL EXPERIMENT STATION, CORNELL UNIVERSITY]

The "Browning (Maillard) Reaction" in Heated Solutions of Uronic Acids¹

BY JOAN L. SEAVER AND Z. I. KERTESZ

The increased production of dehydrated food during World War II intensified the interest in the formation of colored compounds from carbohydrates and amino acids. These reactions, first studied by Maillard,² are now believed to play important roles in the detrimental color changes which occur in many food products during manufacture and storage. During recent years research on this topic was greatly expanded and a number of excellent reviews dealing with the various aspects of the problem have been prepared.³ A perusal of the literature reveals a complete lack of information on the behavior of uronic acids under conditions which produce the "browning reactions" with other carbohydrates. We wish to report below some observations which indicate that uronic acids are even more active in forming dark colored compounds than are the common carbohydrates under similar conditions.

It is commonly known that uronic acids heated with strong acid will form furfural and dark colored "furanoid bodies."⁴ It is less appreciated that galacturonic acid in solution⁵ or even in the dry form and comparatively free from impurities may turn brown. This suggested that the instability of galacturonic acid may go hand in hand with a high degree of reactivity with amino acids to form the type of coloration described by Maillard.

Experimental Methods

Conditions of Heating.—The solutions (10 cc.) were 0.1 M with respect to all individual constituents when heated in loosely closed Pyrex test-tubes in a water-bath at 98°. All solutions were adjusted to pH 4.3 which was taken as representing an approximate average value of the pH of common fruits and fruit products. It is known, however, that the reaction proceeds faster at pH values approaching neutrality or below 1.5.

Determination of Color Formed.—Upon completion of heating, the solutions were prepared for a measurement of

the developed color by Anson's⁶ method. After cooling, 2.8 cc. of 20% metaphosphoric acid solution and 7 cc. of acetone were added and the mixture made up with water to 25 cc. and filtered. Since no precipitate was formed from these solutions upon further dilution with water, the filtrates were used directly for the determination of the color. In a few experiments the color developed even in a few hours was too dark for dependable measurement and in these cases diluted solutions were used. Tests indicated that Beer's law held in these diluted samples.

The per cent. light transmission was measured in a Lumetron Colorimeter using a 370 m μ filter. A standardization curve with potassium dichromate was prepared and the results read in "mg % potassium dichromate equivalent." The choice of the wave length of 370 m μ was made on the basis of the shapes of a series of transmission curves obtained for the visible spectrum for potassium dichromate solution, caramel solution and brown solutions formed by the heating with glycine of glucose, galacturonic acid or ascorbic acid. All these compounds showed the well-known typical transmission curves gradually rising from an optical density of about zero at 550 m μ to a maximum at 350 m μ , the end of the range measured.

Materials Used.—All carbohydrates used were commercial c. p. grade. The galactonic⁷ and mucic acids were prepared by the customary methods of oxidation with bromine and nitric acid, respectively. "Pectinic Acid I" was a sample of "Pectinum N.F.VII" obtained from the California Fruit Growers' Exchange; "Pectinic Acid II" an older unstandardized preparation from the same source. The hydrogen peroxide-degraded pectinic acid was prepared by keeping a mixture of 10 cc. of 3% pectinic acid plus 1 cc. of 30% hydrogen peroxide at room temperature for three days. The typical high viscosity of the pectinic acid solution disappeared completely during this period. The solution was then evaporated to dryness in vacuum at room temperature and the residue redissolved in water.

The α -methyl-D-galacturonic acid methyl ester was prepared by the method of Morell and Link.⁸

Results and Discussion

Tables I and II show the formation of color during the heating of D-galacturonic acid solutions. The extent of color formation both with and without glycine exceeds the coloration which will occur

(1) Journal Paper No. 681, New York State Agricultural Experiment Station, Geneva. Jean Griffin participated in the early phases of this work.

(2) Maillard, *Compt. rend.*, **54**, 66 (1912).

(3) Joslyn, *Ind. Eng. Chem.*, **33**, 308 (1941); etc.

(4) Lefèvre, *Dissertation*, Göttingen, 1907.

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

(6) Anson, *Proc. Subs. Res. Development Lab. Conference*, Chicago No. 1, p. 45, 1945.

(7) We are indebted to R. J. McColloch of this Laboratory for the preparation of the galactonic acid. The free acid was prepared from the sodium salt by the use of IR-100 Amberlite ion exchange resin.

(8) Morell and Link, *J. Biol. Chem.*, **100**, 385 (1933).

under similar conditions in solutions of the common sugars. Glucose, maltose, sucrose and inositol showed no color even when heated with glycine. Fructose, galactose, xylose and arabinose showed no color when heated alone but some color with glycine.

TABLE I

PROGRESS OF COLOR DEVELOPMENT IN HEATED SOLUTIONS OF GALACTURONIC AND ASCORBIC ACIDS

All at pH 4.3, 0.1 M solutions held at 98°

No.	Material heated	Color as mg. % $K_2Cr_2O_7$ (measured at 370 m μ , dilution 1:2.5)			
		Heating time, hr.			
		1	2	3	4
1	D-Galacturonic acid	0.9	1.4	2.0	3.1
2	Same, plus glycine	2.0	4.9	10.5	19.5
3	L-Ascorbic acid	1.0	1.5	1.7	2.1
4	Same, plus glycine	4.1	2.8	3.4	5.3

TABLE II

COLOR FORMATION IN SOLUTIONS OF CARBOHYDRATES

(All at pH 4.3, 0.1 M solutions held at 98° for four hours.)

No.	Material heated	Color as mg. % $K_2Cr_2O_7$ (measured at 370 m μ dilution 1:2.5)	
		Alone	With 0.1 M glycine
1	D-Glucose, maltose, sucrose, inositol	0	0
2	D-Fructose	0	1.0
3	D-Galactose	0	1.3
4	D-Xylose	0	1.5
5	L-Arabinose	0	0.7
6	D-Glucuronic acid	1.2	4.2 ^a
7	D-Galacturonic acid	3.1	20.3
8	Dulcitol	0	0
9	Galactonic acid	0	1.0
10	Mucic acid	0	0.5
11	α -Methyl-D-galacturonic acid methyl ester	...	2.0
12	Pectinic acid I	0.4	0.8
13	Pectinic acid II	1.9	1.4
14	Pectinic acid degraded with H_2O_2	9.6	27.1
15	Pectinic acid heated for 8 hr. to 98°	1.3	1.7

^a Only two hours of heating. Corresponding value with D-galacturonic acid, 7.2.

The behavior of L-ascorbic acid with glycine was peculiar in that the depth of the yellow color at first increased rapidly, then decreased, and

after the third hour again increased through dark yellow, brown, and dark brown (Table I). Such a hump has been observed at times in curves for ascorbic acid heated alone but it was much more pronounced in solutions also containing glycine.

The substantial development of color is not brought about by the presence of the carboxylic acid groups in themselves since in the series dulcitol-galactose-galactonic acid-galacturonic acid-mucic acid, only galacturonic acid showed strong color formation (expts. 8-11, Table II). This indicates that the presence of free carbonyl groups in addition to the carboxyl groups is also necessary. That pectinic acids and α -methyl-D-galacturonic acid methyl ester did not give any substantial coloration seems to confirm this assumption. The little color which did develop with the ester may be attributable to impurities or partial hydrolysis during heating.

Pectinic acid degraded by heating showed no increased tendency to form color. On the other hand, under the conditions used in these tests, pectinic acids degraded in the cold by hydrogen peroxide developed more color than any of the other compounds investigated.

With increased galacturonic acid concentration, the color formation is enhanced. Addition of certain salts such as phosphates also facilitates color formation. Other amino acids gave similar results although different absolute rates of color formation.

Summary

1. When heated alone or with amino acids, D-galacturonic and D-glucuronic acids form colored compounds at a rate exceeding that found with most common sugars. L-Ascorbic acid forms colored compounds more quickly than common sugars but still at a lower rate than the uronic acids.

2. Pectinic acids and α -methyl-D-galacturonic acid methyl ester do not show coloration comparable to D-galacturonic acid. The presence of both carboxylic groups and free carbonyl groups seems to be required for this rapid formation of colored compounds.

3. Heat degraded pectinic acids do not show an increased tendency to form color, but pectinic acids degraded with hydrogen peroxide give products which show color formation exceeding that observed with D-galacturonic acid.

GENEVA, N. Y.

RECEIVED AUGUST 1, 1946