Biochemical Studies on Carbohydrates

CLCVIII. Spectrophotometric Determination of Glucuronic Acid in Co-presence of Galacturonic Acid

By

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In 1947, Dische informed of a specific color reaction of glucuronic acid. The writers made up the reaction into a quantitative-analytical method after careful study of the conditions for its occurrence.

The Analytical Procedure

0.2 cc. of 0.2% mannose is pipetted into a 18×180 mm. test-tube and 0.8 cc. of a 5–12.5 mg% solution of the unknown material in terms of glucuronic acid is added. The mixture is placed on ice, slowly added to along the tube wall with 4.5 cc. of H₂SO₄-H₂O mixture in the volume proportion of 6:1 and shaken under continuous cooling for 2–3 minutes. It is stood in water at 20–25°C for about 10 minutes and then heated in a vigorously boiling water-bath for accurately 2 minutes, immediately followed by placing in iced water for 2 minutes. Thereupon it is warmed to room temperature again, and after addition of 0.2 cc. of 2.5% thioglycolic acid in water, shaken. The mixture here is placed vertically in an incubator (60×90×60 cm.) at 22±2°C for 50 hours to measure extinctions at the wave-lengths of 530 and 600 mμ against the blank by the aid of a spectrophotometer. The blank and control are carried out, replacing water and standard solutions, which contain various amounts of glucuronic acid, respectively for the solution of the unknown.

Calculation. The density increments $E_{530} - E_{600}$ are a linear function of the concentration of glucuronic acid within the range 5–12.5 mg%, and so the concentration of glucuronic acid in the solution analyzed is read from the standard curve plotted from the increments in the control.

Explanatory Experiments and Remarks

1) Fig. 1 shows the absorption spectra after the reaction of 10 mg% solutions of glucuronic and of galacturonic acid. The extinctions at the wave-lengths of 530 and 600 mμ for galacturonic acid nearly equal each

other. The spectra are plotted from the figures in Table I.

2) As shown in Fig. 2, E_{530} and $E_{530} - E_{600}$ values for glucuronic acid reach maximum at the 50th hour and remain constant for 20 hours, when final mixtures are stood at 20–22°C.

Fig. 1

Fig. 2

Fig. 1. Absorption spectra for 10 mg% glucuronic (a) and galacturonic (b) acids. Cell 1 cm. thick.

Fig. 2. E_{530} (a) and E_{600} (b) and the increment between them (a-b) for 10 mg% glucuronic acid and increment $E_{530} - E_{600}$ (c) for 10 mg% galacturonic acid as functions of time of standing the final reaction mixtures at 20–22°C.

TABLE I
Spectral Data for 10 mg% Glucuronic Acid and 10 mg% Galacturonic Acid

Wave-length mμ	-log T	
	Glucuronic acid	Galacturonic acid
440	0.075	0.210
460	0.120	0.145
480	0.175	0.085
500	0.290	0.040
520	0.464	0.030
530	0.610	0.030
540	0.575	0.050
560	0.340	0.075
580	0.195	0.075
600	0.125	0.028
620	0.100	0.005
640	0.075	0.005

3) Relation between extinctions for glucuronic acid and time of heating at 100°C of the unknown with sulfuric acid. Heating for 2 to

2½ minutes gives rise to the strongest absorption at the 530 mμ wave-length and the weakest at the 600 mμ wave-length. Heating for more than 2½ minutes weakens the former absorption and strengthens the latter absorption. See Fig. 3.

4) When mixtures after addition of thionalide are stood at 20°C or a lower temperature, E_{530} and E_{600} values for glucuronic and galacturonic acids are independent upon temperature. On increasing the temperature above 20°C, E_{530} for glucuronic acid lowers gradually and the other three extinctions rise suddenly.

Fig. 3

Fig. 3. E_{530} (a) and E_{600} (b) for 10 mg% glucuronic acid and $E_{530} - E_{600}$ (c) for 10 mg% galacturonic acid as functions of time of heating at 100°C of the hexuronic acids with sulfuric acid.

Fig. 4

Fig. 4. E_{530} (a) and E_{600} (b) for 10 mg% glucuronic acid and $E_{530} - E_{600}$ (c) for 10 mg% galacturonic acid as functions of temperature of standing the reaction mixtures after addition of thionalide.

5) Concentration of the sulfuric acid reagent. Sulfuric acid (sp. gr. 1.84) was diluted with 1/5, 1/6, 1/7, 1/8, 1/10 and 1/15 the volume of water, and 0.8 cc. of the sulfuric acid solutions were mixed together with 0.2 cc. of 10 mg% glucuronic acid or 10 mg% galacturonic acid to submit to the subsequent operations in the analytical procedure. As shown in Fig. 5, E_{530} for glucuronic acid decreases swiftly with increase of the concentration of the sulfuric acid reagent, contrary to E_{600} for the same hexuronic acid which scarcely alters within the range of the proportions of H_2SO_4 to H_2O from 6 : 1 to 10 : 1. When the reagent of 6 : 1 proportion is applied, E_{600} and E_{530} for galacturonic acid show the same and lowest values. Since E_{530} for glucuronic acid varies considerably even by small change of the sulfuric acid concentration, the reagent must be prepared very carefully and preserved avoiding strictly the contact with moisture. This

is one of the main reasons why the control with standard glucuronic acid solutions has to be processed always in parallel with the main run.

6) Concentration of the thioglycolic acid reagent. Increase of the concentration of the glycolic acid reagent increases extinctions E_{530} and E_{600} for glucuronic acid almost in parallel. $E_{530} - E_{600}$ values for galacturonic acid take negative values at concentrations of the reagent higher as well as lower than 2.5%. See Fig. 6.

Fig. 5

Fig. 5. E_{530} (a) and E_{600} (b) for 10 mg% glucuronic acid and E_{530} (c) and E_{600} (d) for 10 mg% galacturonic acid as functions of concentration of the sulfuric acid reagent with which the hexuronic acid solutions are heated at 100°C.

Fig. 6

Fig. 6. E_{530} (a) and E_{600} (b) for 10 mg% glucuronic acid and $E_{530} - E_{600}$ for 10 mg% galacturonic acid (c) as functions of concentration of the thioglycolic acid reagent.

7) Concentration of the mannose solution. E_{530} for glucuronic acid increases abruptly and E_{600} for glucuronic acid less abruptly with the increase of the concentration of mannose, whereas increment $E_{530} - E_{600}$ for galacturonic acid is neglectable when 0.1 to 0.2% mannose solutions are applied. The concentration of the mannose reagent is another influential factor comparable with that of the sulfuric acid reagent in the color development. Fig. 7.

8) Effect of N-acetylhexosamine upon color development of the hexuronic acids. N-Acetylglucosamine does not influence the intensity of absorption given rise to by the hexuronic acids at all in so far as its amount is twice that of glucuronic or galacturonic acid or less (Fig. 8).

9) Effect of NaCl. As shown in Fig. 9, the presence of sodium

Fig. 7

Fig. 7. E_{530} (a) and E_{600} (b) for 10 mg% glucuronic acid and $E_{530} - E_{600}$ (c) for 10 mg% galacturonic acid as functions of concentration of the mannose reagent.

Fig. 8. E_{530} (a) and E_{600} (b) for 10 mg% glucuronic acid and $E_{530} - E_{600}$ for 10 mg% galacturonic acid (c) in the co-presence of N-acetylglucosamine.

Fig. 9. Effect of sodium chloride and sodium acetate upon E_{530} and E_{600} for 10 mg% glucuronic acid and $E_{530} - E_{600}$ for 10 mg% galacturonic acid. Sodium acetate was used as an anhydrous salt. a and d: E_{530} values for glucuronic acid in the presence of sodium chloride and of sodium acetate respectively. b and e: E_{600} values for glucuronic acid in the presence of sodium chloride and of sodium acetate respectively. c and f: $E_{530} - E_{600}$ for galacturonic acid in the presence of sodium chloride and of sodium acetate respectively.

chloride raises E_{530} for glucuronic acid more than E_{600} for the same hexuronic acid, but sodium acetate is of no influence upon those absorptions.

TABLE II
 E_{530} and E_{600} Values for Various Amounts of Glucuronic Acid
in the Presence and Absence of Galacturonic Acid and
Those for Various Amounts of Galacturonic Acid

Concentration of hexuronic acid solutions (mg%)		E_{530}	E_{600}	$E_{530}-E_{600}$
Glucuronic acid	2.5	0.130	0.030	0.100
	5.0	0.302	0.060	0.242
	7.5	0.440	0.088	0.352
	10.0	0.610	0.125	0.485
	12.5	0.754	0.150	0.600
	15.0	0.898	0.213	0.685
Mixture	Glucuronic acid 5.0, galacturonic acid 5.0	0.330	0.080	0.250
	Glucuronic acid 5.0, galacturonic acid 10.0	0.345	0.095	0.250
	Glucuronic acid 10.0, galacturonic acid 10.0	0.640	0.150	0.490
	Glucuronic acid 10.0, galacturonic acid 15.0	0.662	0.185	0.477
	Glucuronic acid 10.0, galacturonic acid 20.0	0.700	0.210	0.490
	Glucuronic acid 12.5, galacturonic acid 12.5	0.820	0.205	0.615
	Glucuronic acid 15.0, galacturonic acid 15.0	0.960	0.280	0.680
Galacturonic acid	5.0	0.010	0.010	0.0
	7.5	0.020	0.022	-0.002
	10.0	0.030	0.028	0.002
	12.5	0.050	0.050	0.0
	15.0	0.060	0.060	0.0

Fig. 10. $E_{530}-E_{600}$ values as a function of concentration of glucuronic acid solutions.

Both the salts are almost ineffective upon $E_{530} - E_{600}$ for galacturonic acid. In short, chloride ion is detrimental to the analysis.

10) Table II and Fig. 10 show that increments between E_{530} and E_{600} for glucuronic acid solutions follow Lambert-Beer law between the limits 5 and 12.5 mg% concentrations, whether galacturonic acid is co-present or not.

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References

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