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A NEW COLOR REACTION FOR THE DETERMINATION OF ALDOPENTOSE IN PRESENCE OF OTHER SACCHARIDES*

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The occurrence of heptose- and hexose-phosphate esters as intermediates in the breakdown of ribose-5-phosphate in living cells may create considerable difficulty in the determination of pentose in extracts of living tissues. In the widely used Bial's orcinol reaction, the colored product from aldoheptoses[1] shows an absorption curve similar in its shape to that from pentose. Furthermore, heptoses, as well as large amounts of hexoses, influence to a considerable extent the absorption curve of pentoses, and this makes the quantitative determination of the latter sometimes rather difficult.

The phloroglucinol reaction of Tollens does not appear useful for quantitative determinations in its original from, because of the strong interference by other sugars and the instability of the color. The modification of this reaction by VON EULER AND HAHN[2] recommended for the determination of RNA appears insufficiently sensitive and its specificity has not been adequately investigated. The present report deals with another modification of the phloroglucinol reaction which permits the differentiation between aldo- and ketopentose and the determination of small amounts of pentoses and their nucleotides in presence of larger amounts of other sugars.

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EXPERIMENTAL

1. *Procedure*

To 0.4 ml of the unknown, containing 4 to 40 μg of pentose per ml, are added 5 ml of a freshly prepared mixture of 110 ml of glacial acetic acid C.P., 2 ml of HCl C.P. sp. gr. 1.19, 1 ml of 0.8% glucose and 5 ml of 5% phloroglucinol. The reaction mixture is shaken, immersed for 15 minutes in a vigorously and uniformly boiling waterbath and cooled in tap water. A blank containing water instead of pentose is run simultaneously.

2. *The reaction and absorption spectra with various sugars*

All 4 aldopentoses produce an intense red color with a sharp absorption maximum at 552 mμ. The phosphoric esters of ribose, AMP, yeast adenylic acid, coenzymes I, II, and ribose-5-phosphate yield the same color and absorption spectrum as ribose itself (Fig. 1). Aldohexoses yield at much higher concentrations (0.5 mg/ml) a faint brown color with a barely perceptible maximum at 555 mμ and a stronger maximum in the blue part of the spectrum. Aldoheptoses, on the other hand, yield a brownish red color with an absorption maximum at 550, and a very different absorption curve from that of pentoses. Ketopentoses and ketoheptoses produce a green color with two absorption maxima which for ketopentoses are at 610 and 450 mμ, and for heptuloses at 640 and 450 with a distinct minimum between these two wavelengths. Ketohexoses react in much the same way as aldohexoses. The sensitivity for the reaction of all these sugars is 2–10% of that of aldopentoses. 2-Deoxyribose produces a color similar to aldopentose, but more brownish with two absorption maxima, one at 552 and the other at 470 mμ. An appreciable color is developed only at a concentration of about 1 mg per ml of DNA. Hexuronic acids and menthol glucuronide show a violet-red color with one absorption maximum at 552 mμ, with an absorption curve which differs significantly from that of aldopentose. α-Keto, di- and tricarboxylic acids found in animal tissues give no color.

3. *Quantitative determinations of aldopentose and ribose phosphates*

The optical density at 552 mμ of the color produced by ribose or its phosphate esters is proportional to the concentrations of compounds as can be seen in Fig. 2. In presence of a large excess of other sugars, however, it would not be possible to determine pentoses accurately because of the absorption of the product from these other sugars at 552 mμ. The influence of keto sugars as well as of DNA, however, can be eliminated by reading at 552 mμ and a second wavelength at which the absorption from the respective keto sugars differs very little from that at 552 mμ, whereas the difference in the extinction coefficients at the two wavelengths is considerable for the aldopentose. For determinations of ribose-5-phosphate in presence of ketopentoses, fructose phosphate, and sedoheptulose phosphate it has proved most convenient to make the second reading at 510 mμ and to use D_{552}–D_{510} as a measure of the concentration of the aldopentose. This difference will be either negligible for the keto sugars and their esters, even when they are present in considerable excess (Table I), or so small that it can be accounted for without appreciable loss of accuracy in the determination of aldopentose. Ketoheptoses and ketohexoses can, when present in concentrations comparable to that of pentose, depress the color from the latter by a few %. In determinations

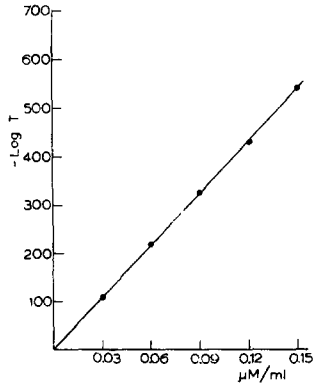

Fig. 1. Proportionality between concentration of adenosine-5-phosphate and optical densities.

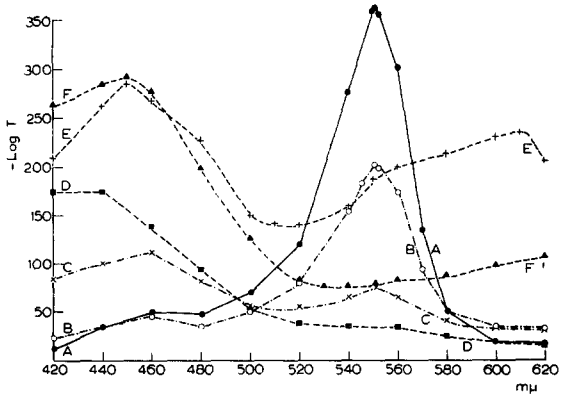

Fig. 2. Absorption spectra of various saccharides in the phloroglucinol reaction. A = ribose 5 mg %. B = galacturonic acid 10 mg %. C = deoxyribonucleic acid 100 mg %. D = fructose 10 mg %. E = ribulose 20 mg %. F = sedoheptulose monophosphate 25 mg %.

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TABLE I

OPTICAL DENSITIES AT 552 AND 510 mμ AND THEIR DIFFERENCES IN THE PHLOROGLUCINOL REACTION OF VARIOUS SUGARS AND SUGAR ESTERS

Expt. No.	*Substance*	*Concentration in moles/ml*	$D_{552} \times 1000$	$D_{510} \times 1000$	$D_{552}-D_{510} \times 1000$
I	Ribose	0.33	369	93	276
	Arabinose	0.33	385	96	289
	Xylose	0.33	372	92	280
	Lyxose	0.33	378	96	282
II	Ribose	0.15	154	44	110
	Adenosine	0.15	134	35	99
	Adenosine-5-phosphate	0.15	315	76	239
	TPN	0.15	184	46	138
	DPN	0.15	134	36	98
III	Adenosine-5-phosphate	0.15	290	72	218
	Ribose-5-phosphate	0.20	380	96	284
	Ribose-5-phosphate	0.15	283	70	213
	Ribose-5-phosphate	0.10	188	48	140
	Ribose-5-phosphate	0.05	95	22	73
IV	Ribulose	1.00	410	313	97
	Glucuronic acid	0.5	39	8	31
	Galacturonic acid	0.5	202	62	140
	Fructose	0.5	31	38	−7
	Glucoheptulose	0.5	18	19	−1
	Sedoheptulose-7-phosphate	0.25	67	81	−14
	Dihydroxyacetone	1.00	4	4	0
	Glyceraldehyde	1.00	3	3	0
	Fucose	0.6	3	5	−2
	Glucose	5.00	30	30	0
	Gluco-ido-heptose	5.00	375	252	123
	Gluco-gala-octose	4.00	101	162	−61
	7-Deoxymanno-gala-heptose	5.00	62	80	−18
	DNA	0.75	75	57	18
	Mentholglucuronide	1.5	10	30	−20

on tissue extracts it is, therefore, necessary to use internal standards. If the tissue extracts are prepared with TCA or $HClO_4$ it is necessary to have in the standard solution and in the blank approximately the same amount of the deproteinizing agent as is present in the experimental sample. $D_{552}-D_{510}$ is proportional to the concentration of aldopentose (Table I).

4. *Comparison of the intensity of the phloroglucinol reaction of various aldopentoses and various ribose phosphate esters*

The compounds used for the comparison of the extinction coefficients in the reaction were commercial preparations. The adenosine-5-phosphate (Nutritional Biochemical) was found on the basis of N and P analysis and ultra-violet absorption to be 95% pure. The TPN and DPN (Pabst Laboratories) were chromatographically pure and contained on the basis of enzymic analysis about 95% of coenzyme. The ribose-5-phosphate (Schwartz Laboratories) was standardized against the adenosine-5-phosphate preparation by the orcinol reaction.

As can be seen from Table I the four aldopentoses show practically identical extinction coefficients. The glycosidic link between ribose and adenosine influences very little, if at all, the reaction of ribose. The esterification with phosphate, on the other hand, may strongly influence the reactivity of ribose. The substitution at carbon 5 doubles the extinction coefficient, whereas substitution in position 3 is without significant effect. It should be noted, furthermore, that the intensity of the reaction depends also largely on whether the adenosine-5-phosphate is linked by its phosphate group to another nucleotide, as shown by the behavior of TPN and DPN. These two coenzymes show much lower extinction coefficients than adenosine-5-phosphate. These great differences in the behavior of various mono- and polynucleotides make it necessary to use

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as standard a nucleotide which is present in the experimental sample and if the latter contains a mixture of nucleotides it is necessary to use the phloroglucinol reaction in combination with other reactions so as to determine quantitatively all the nucleotides in the mixture. This difference in the color intensity between nucleotides is due, as in the case with Bial's orcinol reaction, to a different speed in the development of the color which is much greater in the esters substituted at carbon 5 than in the free sugar and other esters. The difference between adenosine-5 and adenosine-3-phosphate, therefore, is much greater after 5 minutes heating and tends to disappear when the heating is prolonged beyond 15 minutes. However, by longer heating the stability of the color is unfavorably affected.

DISCUSSION

The phloroglucinol reaction as compared with Bial's orcinol reaction appears about one half as sensitive in so far as the minimum absolute amount of material necessary for the determination is concerned. The minimum concentration of ribose or its esters which can be accurately determined is about five times as high in the phloroglucinol reaction as in the orcinol reaction. On the other hand, the phloroglucinol reaction appears much more specific and much less influenced by the presence of other sugars. Aldohexoses and aldoheptoses show only a negligible reaction with phloroglucinol, while the corresponding keto sugars show a negative value for $D_{552}-D_{510}$ which amounts to only about 1 to 2% of the value of an equivalent amount of ribose-5-phosphate. In mixtures, therefore, of the latter with phosphates of ketohexoses and ketoheptoses ribose-5-phosphate can be accurately determined when the relatively small corrections for the other sugar esters are applied. In this respect the phloroglucinol reaction appears to be superior to Bial's orcinol reaction.

This reaction, finally, can be used with advantage for the quantitative determination of aldopentoses in presence of ketopentoses, as the extinction coefficients of the latter are only a few % of those for aldopentoses. Although the reactivity of ribulose- and xylulose-5-phosphate in the phloroglucinol reaction has not yet been determined directly with sufficient accuracy, owing to the lack of sufficiently pure preparations of these compounds, determinations on mixtures of ketopentose- and ribose-5-phosphate, which will be reported later, indicate that the reactivity of these ketopentose esters can be assumed to be negligible as compared with that of equivalent amounts of ribose-5-phosphate.

SUMMARY

1. A new modification of the phloroglucinol reaction of pentoses is described.
2. The application of this reaction for the quantitative determination of aldopentoses in presence of other sugars is discussed.

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