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AMPLIFICATION METHOD FOR THE TITRIMETRIC DETERMINATION OF L-RHAMNOSE BY PERIODATE OXIDATION

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The stoichiometry of the oxidation of rhamnose by periodate has been studied using a method based on the determination of iodate. Conditions have been found under which one mole of rhamnose stoichiometrically reduces six moles of periodate. An amplification method for the titrimetric determination of rhamnose is proposed in which one mole of rhamnose reacts with 6 moles of periodate with the formation of six moles of iodate, which is equivalent to 18 moles of triiodide, in the titration of which 36 gram-equivalents of sodium thiosulfate are consumed. The method is distinguished by high sensitivity and accuracy. The relative standard deviation in the determination of 2-3.5 mg of rhamnose does not exceed 0.5-1%.

L-Rhamnose (6-deoxy-D-mannose) is a methylpentose and is present as a component of numerous plant and bacterial polysaccharides and also of plant glycosides. In contrast to other aldoses and ketoses, in an aqueous solution rhamnose mutarotation is completed rapidly — in 10-60 min at 20°C — and a first-order kinetic equation is applicable to the rate of mutarotation of rhamnose in water [1]. The latter fact indicates that in a solution of rhamnose of appreciable concentration not more than two tautomeric forms of the substance are present. Consequently, it was to be expected that the periodate oxidation of rhamnose would take place more rapidly and more simply than in the case of other aldoses and ketoses [2, 3].

The information in the literature on the periodate oxidation of rhamnose is sparse and, at the same time, extremely contradictory, which can be explained by differences in the conditions of performing periodate oxidation and the methods of final determination. Formic acid is determined by visual titration [4, 5] — 1 mole of rhamnose yields 4 moles of HCOOH in 20 min at 100°C and in 45 h at room temperature. On the basis of a determination of the excess of periodate it has been established that in 3-24 h 3.56-3.64 moles of periodate [6] and at pH 3.6 and room temperature after 20 h 3.52 moles of periodate [7] is consumed per 1 mole of rhamnose. The excess of periodate is found by thermometric titration with a solution of hydrazine sulfate [8]. A method has been proposed for determining rhamnose which is based on the determination of acetaldehyde by the bisulfite method [9, 10].

It appeared of interest to ascertain the possibility of determining rhamnose by the amplification method [11] proposed for the determination of other aldoses and ketoses [2, 3]. In the amplification method, after the end of the periodate oxidation reaction the excess of periodate is masked by the addition of an excess of molybdate and the iodate is converted into the equivalent amount of triiodide.

$$IO_3^- + 8I^- + 6H^+ \rightarrow 3I_3^- + 3H_2O, \quad (1)$$

which is titrated with a solution of sodium thiosulfate [1].

The development of methods of determining monosaccharides and their derivatives based on the determination of iodate [2, 3] requires a careful study of the stoichiometry in order to choose the optimum conditions of oxidation.

We have been unable to find in the literature a stoichiometric equation for the oxidation of rhamnose by periodate. We have experimentally found linear sections on the curves

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TABLE 1. Stoichiometry of the Oxidation of Rhamnose by Periodate

Oxidant*	k†	t, °C	n‡ after oxidation for									Time of oxidation during which n** is observed
			30 min	1 h	2 h	3 h	4 h	5 h	24 h	48 h	72 h	96 h	
A	10	21	2,86	3,04	3,25	3,39	3,50	3,57	3,73	3,77	3,80	3,81	Not observed
A	10	37	3,35	3,62	3,72	3,76	3,77	3,78					"
A	10	60	3,76	3,78	3,79	3,83	3,85	3,87					"
B	10	21	2,36	2,52	2,63	2,75	2,81	2,84	3,33	3,52	3,56	3,68	"
B	10	37	2,66	2,86	3,06	3,26	3,30	3,38					"
B	10	60	3,10	3,40	3,60	3,72	3,75	3,82					"
C	20	100	4,20	4,57	5,98	6,02	5,98	5,99					2—5 h

*A — 0.025 M solution of KIO_4 in 0.1 N H_2SO_4; B — 0.025 M solution of $NaIO_4$ in water; C — 0.05 M solution of KIO_4 in 1 M H_2SO_4.
†k — Initial molar excess of periodate.
‡n — Found number of moles of iodate liberated in the oxidation of 1 mole of rhamnose.
**n — Equal to 6 for rhamnose.

TABLE 2. Results of the Titrimetric Determination of Rhamnose

Time of oxidation, h	k*	g, mg	n	$\bar{X}$, %	$S_{\bar{x}}$, %
2	22	3,430	12	99,0	0,9
2	32	2,214	15	99,2	0,5
3	25	2,800	10	99,1	0,4
3	30	2,240	8	99,1	0,4
4	30	2,270	10	99,2	0,5
4	25	2,800	12	99,2	0,6
5	31	2,230	15	99,3	0,8
5	22	3,430	10	99,3	0,7

*k — Initial molar excess of periodate.
1 ml of 0.0250 N sodium thiosulfate corresponds to 0.1265 mg of rhamnose. Here n is the number of parallel determinations.

of the formation of iodate as a function of the time of oxidation of rhamnose at room temperature and at elevated temperatures and with different compositions of the oxidation medium. The results obtained are shown in Table 1.

When rhamnose was oxidized with a solution of KIO_4 in 0.1 N H_2SO_4, the formation of iodate was nonstoichiometric both at room temperature and at elevated temperatures. Thus, at room temperature after 72-96 h 3.80 mole of iodate was formed; at 37°C after oxidation for 2-5 h, 3.7-3.8 moles; and at 60°C after 30 min to 5 h, 3.8-3.9 moles per 1 mole of rhamnose. Raising the temperature to 100°C and lengthening the time of oxidation to 5 h likewise did not lead to the stoichiometric formation of iodate.

When a solution of $NaIO_4$ in water was used as the oxidant [12], the rate of the oxidation of rhamnose fell somewhat and the formation of iodate likewise did not take place stoichiometrically under similar oxidation conditions.

We then used as oxidant a 0.05 M solution of KIO_4 in 1 M H_2SO_4. An increase in the concentration of H_2SO_4 permitted the preparation of a more concentrated solution of periodate and, consequently, the creation of a larger excess of the latter in oxidation. Under these conditions, an increase in the time of oxidation of rhamnose led to only a slight dis-

turbance of the stoichiometry of the reaction in the direction of increasing it. The linear section found on the curve of the formation of iodate as a function of the time of oxidation of rhamnose was made the basis of titrimetric determination of rhamnose by the amplification method.

To determine the mechanism and the stoichiometric equation of the periodate oxidation of rhamnose additional investigations were necessary since this cannot be written as a special case of the Malaprade reaction. It is known [13] that methyl aldohexofuranosides reduce 6 moles of periodate unlike the initial aldohexoses, which reduce 5 moles. The oxidation of rhamnose by periodate apparently takes place through the formation of malondialdehyde as an intermediate [14].

Titrimetric Method of Determining Rhamnose. For the titrimetric determination of rhamnose, oxidation is carried out with a 0.05 M solution of KIO_4 in 1 M H_2SO_4 at 100°C for 2-5 h. Under these conditions, 1 mole of rhamnose reacts with 6 moles of periodate to form 6 moles of iodate, which is equivalent to 18 moles of triodide, in the titration of which 26 g-eq of sodium thiosulfate is consumed:

$$1\ \text{Rhamnose} \equiv 6IO_4^- \equiv 6IO_3^- \equiv 18I_3^- \equiv 36S_2O_3^{2-}. \quad (2)$$

The results of the titrimetric determination of rhamnose treated statistically according to IUPAC recommendations are given in Table 2. In the determination of 16.67 μmole of rhamnose, in the final titration 24.0 ml (with deduction of the correction for the consumption of titrant in the blank experiment) of 0.0250 N sodium thiosulfate solution is consumed which brings the titration error to a minimum. The relative standard deviation does not exceed 0.5-1%.

EXPERIMENTAL

A crystalline preparation of L-(+)-rhamnose monohydrate from Lachema (Czechoslovakia) with the composition $C_6H_{12}O_5 \cdot H_2O$ (mol. wt. 182.18) was used. Found, $[\alpha]_D^{20} \xrightarrow{1h} +8.2°$ (c 10; water); according to the specification, $[\alpha]_D^{20} \xrightarrow{1h} +8.2°$ (c 10; water). Found C 39.52%; calculated C 39.56%. Found H 6.60%; calculated H 6.64%.

Apparatus. Titration was performed with an ABU 11 autoburet (Denmark) having a capacity of 25 ml, which permitted the measurement of the volume of titrant consumed with an accuracy of ±0.01 ml. Samples of rhamnose were weighed out on a Mettler ME 22 microbalance with an accuracy of ±0.01 mg (100-mg samples) and ±0.001 mg (10-mg samples). Optical rotations were measured on a Perkin-Elmer 141 polarimeter in thermostated cells 1 dm long at 20.0°C.

Reagents and Their Preparation. A 0.025 M solution of KIO_4 in 0.1 N H_2SO_4 and a 0.05 M solution of KIO_4 in 1 M H_2SO_4 was prepared by dissolving weighed amounts of KIO_4 in a sulfuric acid solution of the required concentration with heating and they were stored in a dark glass vessel. A 0.025 M solution of $NaIO_4$ was prepared by dissolving a weighed amount of $NaIO_4$ in distilled water and this was stored in a dark glass vessel.

A solution of acetic acid (6 M) was prepared by diluting glacial acetic acid, kh.ch. ["chemically pure"] with distilled water.

A solution of sodium thiosulfate (0.0125 M, 0.025 N) was prepared from $Na_2S_2O_3 \cdot 5H_2O$, ch.d.a. ["pure for analysis"] in distilled water. Where necessary, the solution obtained was filtered through a paper filter.

A solution of potassium iodide (20%) was prepared by dissolving the ch.d.a. substance in distilled water and was stored in a dark glass bottle.

A 1% aqueous solution of soluble starch, kh.ch., was used as indicator.

A 2 M solution of NaOH was used to neutralize the H_2SO_4.

Performance of the Titrimetric Determinations. In the study of the stoichiometry of the periodate oxidation of rhamnose, 10.00-ml aliquots usually containing 16.67 mmole of rhamnose were each oxidized with 10 ml of periodate solution for the required time at the given temperature. The masking of the excess of periodate, the iodate-iodide synproportionation, and the performance of the final titration with sodium thiosulfate are described below. On using a 0.05 M solution of KIO_4 in 1 M H_2SO_4 as oxidant, before the masking of the excess of periodate 5 ml of a 2 M solution of NaOH was added to neutralize the sulfuric acid.

To determine rhamnose, an accurately weighed sample of 2-3.5 mg was oxidized with 10 ml of 0.05 M KIO_4 in 1 M H_2SO_4 at 100°C for 2-5 h. Then the mixture was cooled to room temperature, 5 ml of 2 M NaOH, 20 ml of 6 M acetic acid, and 5 ml of 1 M sodium (or ammonium) molybdate were added, the contents of the titration beaker were carefully stirred, 5 ml of a 20% solution of potassium iodide was added and, with stirring, titration was carried out with a standardized 0.025 N solution of sodium thiosulfate in the presence of starch as indicator, added towards the end of titration until the blue-pink coloration of the solution had disappeared. A blank experiment was performed in parallel.

The percentage of rhamnose X was calculated from the formula

$$X = \frac{(V_1 - V_2) \cdot M \cdot N}{6 \cdot n \cdot g} \cdot 100, \quad (3)$$

where V_1, V_2 are the volumes of the standardized solutions of sodium thiosulfate consumed in titration in the analysis and in the blank experiment, respectively, ml; M is the molecular mass of rhamnose, g/mole; N is the concentration of standardized sodium thiosulfate solution, g-eq/liter; n is the number of moles of periodate reacting with 1 mole of the substance being determined (n = 6 in the case of rhamnose); and g is the weight of rhamnose, mg.

SUMMARY

1. The stoichiometry of the oxidation of rhamnose by periodate has been studied using a method based on the determination of iodate.

2. For the determination of rhamnose is proposed stoichiometric oxidation by periodate followed by the titrimetric determination of iodate formed by an amplification method.

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