Short Communication

[Agr. Biol. Chem., Vol. 30, No. 11, p. 1173~1174, 1966]

The Determination of 5-Keto-D-Fructose

Sir:

In this communication, as a prelude to studies of 5-keto-D-fructose formation during the oxidative fermentation of sorbitol by acetobacters, we present a new procedure for the absorption spectrophotometric determination of the ketose. Avigad and Englard¹⁾ reported on a method for the colorimetric determination of the ketose by using *o*-amino-biphenyl thiourea reagent.

The authors employed *o*-phenylenediamine dihydrochloride as a reagent for the quantitative assay, which reacted with 5-keto-D-fructose to give a stable maximum absorbance at 350 m μ . The mechanisms of this reaction have not been investigated yet. 0.5 μ mole of 5-keto-D-fructose were treated with 2 ml of

2.5% *o*-phenylenediamine dihydrochloride in a total volume of 5 ml at 100°C for 30 minutes. After cooling, ultraviolet absorption spectrum of the resulting solution was determined. The absorption spectra are shown in Fig. 1, with that of this amine. A high molar ratio of the reagent to the ketose was necessary to give a good result. Since measurement of the absorption at 350 m μ was possible in the presence of excess of *o*-phenylenediamine, the isolation of the reaction product was unnecessary for its estimation.

Such a condition as mentioned above was preferable for determination of 5-keto-D-fructose alone in amount of 10 to 100 μ g. However it was not suitable for the determination of 5-keto-D-fructose in the fermentation broth containing sorbitol, fructose or sorbose, especially the latter two.

By varying the concentration of the reagent, the temperature and time of the reaction, convenient quantitative assay procedures for the ketose in the presence of them were examined, and the following procedure was found to be most simple and accurate.

The reagent was a freshly prepared 1.0% aqueous solution of *o*-phenylenediamine dihydrochloride. To 1 ml of an aqueous solution containing 10 to 100 μ g of the ketose in a reaction tube were added 2 ml of the reagent and 2 ml of distilled water. The reaction tube was immersed in a boiling water bath for 10 minutes and then cooled to room temperature. Total volume of the reaction mixture was adjusted to 5 ml by distilled water. Optical density was measured at 350 m μ . Optical density-concentration re-

FIG. 1. Absorption Spectra.

Curve A: Aqueous solution of *o*-phenylenediamine dihydrochloride (1×10^{-3} M).

Curve B: Reaction mixture of 5-KF (0.5 μ mole) with *o*-phenylenediamine dihydrochloride (276 μ mole) in a total volume of 5 ml at 100°C for 30 minutes.

1) G. Avigad and S. Englard, *J. Biol. Chem.*, **240**, 2290 (1965).

FIG. 2. Relationship between Quantity of the 5-Keto-D-Fructose and Maximum Absorbance at 350 m μ .

relationship was linear as shown in Fig. 2.

Experimental results of this procedure in the presence of large excess of sorbitol, fructose or sorbose are shown in Table I.

Therefore this procedure can be used for the specific estimation of 5-keto-D-fructose in solution with them, although it may be interfered by the presence of α -keto acid such as

TABLE I. 5-KF RECOVERY IN SOLUTION WITH OTHER SUGARS OR SORBITOL

Composition of Sample (ml)				5-Keto-Fructose	
(μ g) 5-KF	(mg) Sorbitol	(mg) Sorbose	(mg) Fructose	(μ g/ml) Found	(%) Recovery
50	—	2	—	50.2	100.4
50	—	1	—	49.6	99.2
50	—	0.5	—	51.5	103.0
50	2	—	—	51.5	103.0
50	1	—	—	50.4	100.8
50	0.5	—	—	48.5	97.0
50	—	—	2	49.0	98.0
50	—	—	1	49.6	99.2
50	—	—	0.5	49.6	99.2

$$\text{Recovery \%} = \left(\frac{\text{5-KF found}}{\text{5 KF added}} \right) \times 100$$

2-keto hexonic acid,²⁾ or compounds of ascorbate type.³⁾

Katsumi IMADA
Kiyoshi SATO
Shunichiro ŌGA
Kazuo ASANO

*Fermentation Research Laboratory,
Daiichi Pharmaceutical Co., Ltd.,
Aketacho, Takatsuki, Osaka, Japan*

Received August 2, 1966

2) M. C. Lanning and S. S. Cohen, *J. Biol. Chem.*, **189**, 509 (1951).

3) H. Ohle and E. Erlbach, *Ber. Chem. Ges.*, **67**, 55 (1934).