Can. J. Chem. Downloaded from www.nrcresearchpress.com by 64.107.14.30 on 12/11/14 For personal use only.

### NOTES

vertically stacked boat molecules, on the other hand, adjacent molecules have only a small ( $\sim 1$  D) vertical component of moment in common.

## ACKNOWLEDGMENTS

We are indebted to R. Ironside and M. E. Bednas for Fischer, infrared, and gas chromatographic analyses, and to Mrs. G. Wheeler for technical assistance.

LE FÈVRE, C. G., LE FÈVRE, R. J. W., and SMITH, M. R. J. Chem. Soc. 16 (1958).
CLARKE, H. T. J. Chem. Soc. 103, 1788 (1912).
DAVIDSON, D. W. Can. J. Chem. 35, 458 (1957).
JACOBS, C. J. and PARKS, G. S. J. Am. Chem. Soc. 56, 1513 (1934).
GUGGENHEIM, E. A. Trans. Faraday Soc. 47, 573 (1951).
BÖTTCHER, C. J. F. Theory of electric polarization. Elsevier Pub. Co., Inc., Amsterdam, 1952. p. 323.
OTTO, M. M. J. Am. Chem. Soc. 59, 1590 (1937).
ALLEN, J. S. and HIBBERT, H. J. Am. Chem. Soc. 56, 1398 (1934).

RECEIVED OCTOBER 19, 1958. DIVISION OF APPLIED CHEMISTRY, NATIONAL RESEARCH COUNCIL OF CANADA, OTTAWA, CANADA.

# ON THE CONSTITUTION OF 2-KETO-L-GULONIC ACID

## N. J. GASPAR\* AND J. M. LOS

In the course of a polarographic investigation of 2-keto-L-gulonic acid (1) the following constitutional features were observed.

In aqueous solution the compound exists in the free acid lactol form as proposed by Reichstein and Grüssner (2), in equilibrium with an extremely small concentration of the free keto acid. The solid state can be either a free keto acid or the corresponding lactone. The latter crystallizes from a solution either of high viscosity or of high ionic strength. Sometimes mixtures of the two solid forms were obtained. Each form was invariably found to contain one molecule of water of crystallization.

COOH	СООН	0=C
C-OH	C=O	Č=O
носн	носн	носн о
р нсон	HCOH · H <sub>2</sub> O	HC
носн	HOCH	носн
LCH <sub>2</sub>	CH2OH	ĊH₂OH
free acid lactol in solution	free keto acid in solid state	lactone

The lactone is, of course, the keto tautomer of L-ascorbic acid.

The solid 2-keto-L-gulonic acid was made by the method of Reichstein and Grüssner (2): 20 g of diacetone-2-keto-L-gulonic acid were dissolved in 200 ml of distilled water. The solution was heated to boiling and was allowed to stand over a boiling water bath for 45 minutes. The water was then evaporated in vacuo till a sirup remained. We found

\*Holder of a National Research Council of Canada Studentship.

Can. J. Chem. Vol. 37 (1959)

495

that crystallization from a low-viscosity solution yielded a different substance from that crystallizing at high viscosity. At intermediate viscosity, mixtures of these two would form.

This conclusion was drawn primarily from the results of alkalimetric titration: the product from low-viscosity solution was found to be 99.9% pure if its molecular weight was assumed to be 212, i.e. that of the free acid plus one molecule of water of crystallization. The substance obtained from the high-viscosity solution showed the same degree of purity if its molecular weight was put at 194. This could mean either that the water of crystallization does not appear in the latter case, or that the lactone ring has closed during crystallization.

Heating of the higher molecular weight compound at  $50^{\circ}$  in vacuo for a few hours also produced the compound of molecular weight 194, the loss of weight corresponding to exactly one molecule of water.

The decision as to the nature of the lower molecular weight compound could be made on the basis of the infrared spectra of both solid compounds (Fig. 1). The spectra were taken from KBr pellets (about 1.5 mg of solid in 400 mg of KBr) with a Perkin-Elmer Model 21 spectrophotometer. The product of molecular weight 212 has a strong adsorption peak at 5.75  $\mu$ , characteristic of the carbonyl stretching band and much like the corresponding peak of pyruvic acid at 5.73  $\mu$ . The product of molecular weight 194, however, shows a peak at 5.67  $\mu$ , which is typical of a lactone. Therefore it is concluded that the water is lost in the closing of the lactone ring and not by giving up water of crystallization (the slight adsorption around 6.10  $\mu$  seems characteristic of free water).



It is not likely that either of the two solid forms contains a lactol ring. Molecular models may show that the lactone cannot have a cyclohemiacetal ring as well. The most favorable case is a very strained double-ring structure obtained when the pyranose lactol has the boat conformation and the OH groups on  $C_2$  and  $C_3$  are *cis* to one another. Furthermore, it is not likely that a lactol ring exists in the solid free acid either: (a) the C=O adsorption peak at 5.75  $\mu$  is very large, (b) the above-mentioned transition of this free acid into the lactone form would probably require a total rearrangement within the crystal.

Both solid forms dissolve in water as the free acid, predominantly with a cyclohemiacetal ring. However, equilibrium with the free keto acid must exist, as borne out

Can. J. Chem. Downloaded from www.nrcresearchpress.com by 64.107.14.30 on 12/11/14 For personal use only.

NOTES

by the completely rate-controlled current-voltage wave at the dropping mercury electrode (1). The ionization constant  $(K = f \pm (c_{\rm H} + c_{\rm A} - / c_{\rm HA}))$  is  $2.86 \times 10^{-3}$  at 25° C and ionic strength 0.7 (1).

When KBr pellets were made not by mixing of the solid with KBr but by quickly freezing a solution of the free acid containing a high concentration of KBr, and then removing the water by freeze-drying, the lactone form was invariably produced.

In order to decide whether this is due to the high KBr concentration or to the lower temperature, the following experiment was carried out.

Two identical samples of the lactone (15 mg each) were dissolved in 10 ml of water. To one of these, 4 g of KBr was added. Each solution was then rapidly frozen in a dry ice – acetone mixture and the ice was sublimed *in vacuo*. The sample to which no KBr had been added was then mixed with 4 g of the latter. Identical pellets were made. The spectra showed clearly that the sample containing the KBr in solution had crystallized in the lactone form, the other as the free keto acid (in spite of the fact that it had its starting point in the solid lactone). We may therefore conclude that in the case of the first sample most of the water had been tied up as water of hydration of the K<sup>+</sup> and Br<sup>-</sup> ions, lowering the activity of the water to such an extent that only the lactone could crystallize, just as if the crystallization had taken place from a very viscous solution.

Our thanks go to the National Research Council for financial assistance and to Merck and Company, Montreal, for providing the diacetone 2-keto-L-gulonic acid.

1. LOS, J. M. and GASPAR, N. J. To be published.

2. REICHSTEIN, T. and GRÜSSNER, A. Helv. Chim. Acta, 17, 311 (1934).

Received August 25, 1958. Department of Chemistry, University of New Brunswick, Fredericton, New Brunswick.

Can. J. Chem. Downloaded from www.nrcresearchpress.com by 64.107.14.30 on 12/11/14 For personal use only.

### ISOLATION OF OLIGOSACCHARIDES IN IMPROVED YIELD BY THE ENZYMATIC DEGRADATION OF POLYSACCHARIDES

# T. J. PAINTER<sup>1</sup>

Theoretical considerations originating with Kuhn (1) predict that, for random degradation of a uniform linear macromolecule, the yield of the *n*-membered fragment is given by  $n\alpha^2(1-\alpha)^{n-1}$ , where  $\alpha$  is the degree of scission. The maximum possible yield of the fragment is then  $n[2/(n+1)]^2[(n-1)/(n+1)]^{n-1}$ , and occurs when  $\alpha = 2/(n+1)$ . Further calculation shows that the maximum possible yield of dimer plus trimer occurs when  $\alpha = 0.59$ , and amounts to 46%.

In the hydrolysis or acetolysis of polysaccharides, lack of randomness in the degradation usually results in considerably lower yields, and study of the literature shows that the combined yield of di- and tri-saccharides has seldom exceeded 35% by weight of the total hydrolyzate. Apart from the wastage of material, examination of the oligosaccharides obtained in these cases would reveal only the dominant structural features of the parent polysaccharide. In addition, misleading artifacts may arise from the recombination (reversion) of simple sugars, although in the case of acetolysis this effect appears to be minimized (2).

<sup>1</sup>Harold Hibbert Memorial Fellow, McGill University, Montreal, Quebec, 1957-58. Can. J. Chem. Vol. 37 (1959)