Treatment of **22** with methyl iodide (room temperature, THF) led to the thioimino ether **23**, which was not isolated but immediately hydrolyzed in dilute acid to give the crystalline hydrochloride of **24** (82%) contaminated with a small amount of an anhydro compound. This material was immediately alkylated (dimethyl sulfate, Hünig's base, THF). After purification by chromatography on polyamide, Terramycin (**1**) was isolated (33%) as light yellow crystals (mp 200° dec) containing 0.8 mol of acetone after thorough drying. Terramycin obtained by fermentation[13] also contained about 1 mol of acetone when recrystallized from acetone. Synthetic and authentic Terramycin samples were then compared. The nmr spectra in pyridine-d_5 were identical provided that comparison was made at the same concentration. Ultraviolet spectra were superimposable. The identity was further established by mass spectral data and chromatography on polyamide in different solvent systems.[14] A bacteriological assay[13] showed synthetic Terramycin to be 50% as active as Terramycin from *Streptomyces rimosus*. Elemental analyses of synthetic Terramycin (plus 0.8 mol of acetone as measured from the nmr spectrum) and of all crystalline intermediates are in agreement with the structures.

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(13) We are grateful to Dr. I. A. Solomons and Dr. L. H. Conover of Chas. Pfizer Medical Research Laboratories for supplying us with authentic Terramycin. We also thank Mr. Roland Plude for running the bioassay of synthetic Terramycin

(14) Systems applied distinguished clearly between Terramycin, N-demethylterramycin, 4-epiterramycin, and **24**.

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On the Structure of Ribulose 5-Phosphate as an Intermediate of the Photosynthetic Pentose Phosphate Cycle[1]

Sir:

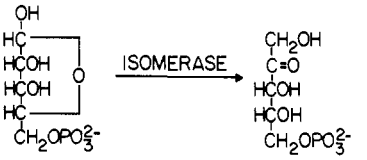
The product of the oxidative decarboxylation of 6-phosphogluconate by the specific dehydrogenase was shown to be ribulose 5-phosphate (**2**). The same report[2] also described the discovery of ribose phosphate isomerase (isomerase) which was shown to catalyze the interconversion of **2** and ribose 5-phosphate (**1**).

(1) This work was supported in part by National Science Foundation Grant GB-6795 and the Cancer Research Funds of the University of California.

(2) B. L. Horecker, P. Z. Smyrniotis, and J. E. Seegmiller, *J. Biol. Chem.*, **193**, 383 (1951).

This communciation describes the results of absorption, rotatory dispersion, and circular dichroism studies of the spinach leaf chloroplast isomerase catalyzed reaction using **1** as the substrate. The results of these studies are not in accordance with the predicted spectral properties of **2** which, therefore, leads to the conclusion that structure **2** does not hold for the reductive pentose phosphate cycle.

Solutions of **1** in 0.037 *M* potassium phosphate (pH 7.38) are transparent above 220 mμ. The ultraviolet absorption spectrum of the isomerase-catalyzed reaction showed an absorption band with λ_{max} 280 mμ and a minimum at 242 mμ. The initial rate of the isomerase-catalyzed reaction, measured at a fixed wavelength of 280 mμ, varied linearly with isomerase concentration. The time course of the reaction was determined by direct spectrophotometric measurement at 280 mμ and by the colorimetric keto sugar assay[3] which is the conventional technique for the measurement of isomerase activity. The time course curves coincided when they were plotted as per cent reaction, 100% reaction being the optical density end points for



1 *2*

the spectrophotometric and colorimetric assays, 0.0231 at 280 mμ and 0.729 at 540 mμ, respectively.[4]

The addition of 0.10 ml of 1.0 *N* NaOH to 0.90 ml of the isomerase-generated chromophore caused a bathochromic shift in λ_{max} of 28.5 mμ.[5] Neutralization of the NaOH led to a reversal of the bathochromic shift, a hypsochromic shift of 28.5 mμ which regenerated the original chromophore. The addition of an excess of HCl, however, led to a hypsochromic shift of 33.5 mμ, from 308.5 to 275 mμ. The absorption band with λ_{max} 275 mμ was not stable, a spontaneous bathochromic shift of 5 mμ occurring in the acidic solution which regenerated the chromophore formed from **1** by isomerase. This 5-mμ bathochromic shift was characterized by an isoabsorption point at 296 mμ which showed that only two chromophores were involved in the spectral shift. The addition of 0.10 ml of 1.0 *N* HCl to 0.90 ml of the isomerase-generated chromophore left its absorption spectrum unchanged. A kinetic analysis of the NaOH-induced bathochromic shift showed the reaction to be first order with a rate constant, k, of approximately 1.9×10^{-3} sec^{-1}. The rate equation was $\log [1/(C - A_{308.5})] = kt$, where C is the ratio of the initial concentration of the isomerase-generated chromophore to the molar extinction co-

(3) B. Axelrod and R. Jang, *ibid.*, **209**, 847 (1954). This determination is a modification of the procedure originally described by Z. Dische and E. Borenfreund, *ibid.*, **192**, 583 (1951).

(4) The initial concentration of **1** was 2.0×10^{-3} *M* for the correlation of the spectrophotometric and colorimetric assay procedures. The isomerase activity in the substrate solution was approximately 0.014 μmol of **2**/min as assayed by the colorimetric assay.

(5) Axelrod and Jang observed that **2** in 0.1 *M* Na_2CO_3 gave rise to an absorption band with λ_{max} 310 mμ. Their observation was made in an effort to explain the anomalous reactivity of **2** under the conditions of the Willstätter–Schudel alkaline NaOI test. According to our scheme, **5** would consume 2 equiv of NaOI by substitution of the methylene hydrogens.

efficient of the product of the bathochromic shift. This value was obtained indirectly by simultaneous solution of the rate equation at $t = 70$ and 140 sec to give a value of 1.247. The rate constant was obtained as the slope of log $[1/(1.247 - A_{308.5})]$ *vs.* t, which varied linearly from $t = 40$ to $t = 150$ sec. The rates of both hypsochromic shifts were too fast to be measured. Although the rate of the 5-mμ bathochromic shift was slow, its rate constant was not obtained. The ratios[6] of the molar extinction coefficients at λ_{max} were approximately 22 for $A_{308.5}/A_{280}$ and 13 for A_{275}/A_{280}.

Solutions of **1** in 0.037 *M* potassium phosphate (pH 7.38) showed a plain positive rotatory dispersion curve and were transparent to circularly polarized light above 220 mμ. Rotatory dispersion spectra of mixtures of isomerase and **1** showed a negative Cotton effect with a trough at 298 mμ and a peak at 255 mμ. The Cotton effect had inverted sign at 280 mμ. The same solution showed a negative dichroic absorption band with a wavelength of maximum dichroic absorption at 280 mμ. The position of the Cotton effect was not affected by those conditions which led to the spectral shifts in the absorption spectrum of the isomerase-generated chromophore, although the amplitude of the Cotton effect was decreased. Dialysis of isomerase against **1** showed that both the Cotton effect and the dichroic absorption band were associated only with solution containing isomerase, **1**, and the absorption chromophore (the retentate). The isomerase-generated chromophore could be detected in the diffusate, but the diffusate showed only a plain positive rotatory dispersion curve and no dichroic absorption.

The reversible NaOH-induced bathochromic shift of 28.5 mμ in the absorption spectrum of the isomerase-generated chromophore suggests that it is a weak acid. The first-order rate constant of 1.9×10^{-3} sec^{-1} is of the same order of magnitude as many carbon acids.[7] The two hypsochromic shifts of 28.5 and 33.5 mμ in the absorption spectrum of the conjugate base of the isomerase-generated chromophore lead to the conclusion that the acid can exist in two tautomeric forms. Since the Cotton effect and the dichroic absorption band do not undergo pH-dependent shifts, whereas the absorption spectrum does, it can be concluded that the absorption chromophore is either optically inactive or that its asymmetry is destroyed by production of its conjugate base. The dialysis experiment indicates that the isomerase-generated chromophore is optically inactive, however, and it appears that the Cotton effect and dichroic absorption band are due either to the association of the absorption chromophore with isomerase or the production of the *aldehydo* form of **1** (**3**).

The scheme shown in Figure 1 interprets these results in terms of the absorption chromophore produced from **1** by isomerase as being a β-diketone phosphate **5**. The three ultraviolet absorption chromophores with λ_{max} 275, 280, and 308.5 mμ then correspond to the β-hydroxy enone **6**, the β-diketone **5**, and the enolate anion **4**, respectively. The spectral shifts observed for the isomerase-generated chromophore are close to those observed for 2,4-pentanedione, as are the ratios of

(6) We have been unable to assign a molar extinction coefficient to the isomerase-generated chromophore. An ε of approximately 800 is indicated from the colorimetric assay, however.

(7) D. J. Cram, "Fundamentals of Carbanion Chemistry," Academic Press, New York, N. Y., 1965, p 10.

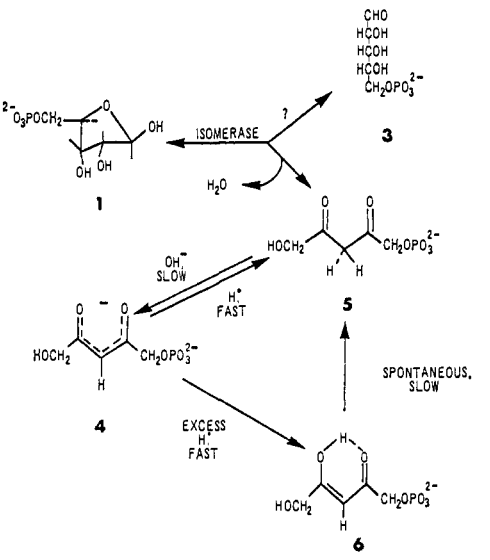


Figure 1. Scheme which proposes the structures associated with the spectral displays of the product of the isomerase-catalyzed transformation of **1**.

molar extinction coefficients at λ_{max}.[8] Furthermore, the preferred tautomeric form of β-diketones in polar solutions is known to be the diketo form.[8] The first-order rate constant for the production of **4** from **5** is less than that of 2,4-pentanedione (1.7×10^{-2} sec^{-1}),[7] but the phosphate group in the 5 position would be expected to lower the rate constant by repelling the approach of hydroxide ions to the methylene hydrogens in the 3 position.

The β-diketone phosphate **5** differs from **2** by the elements of water. The rehydration of **5** to generate **2** could be accomplished by another enzyme, although no such enzyme was detected. The possibility that **2** is also generated from **1** by isomerase exists, although the dialysis experiment indicates that this is not the case. The combination of two asymmetric centers and a carbonyl group in **2** should give rise to a dichroic absorption band and a Cotton effect, both of which should be centered in the region from 270 to 290 mμ. If **2** were not stable to dialysis or if the molecular ellipticity of **2** was very low, then it would not have been detected.

(8) A. I. Scott, "Interpretation of the Ultraviolet Spectra of Natural Products," Pergamon Press, New York, N. Y., 1964, pp 69 and 267.

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Concerning the σ_R^0 Scale of Substituent π Delocalization Parameters. Theory and Experiment[1]

Sir:

We report herein accord between the empirical σ_R^0 scale of substituent π delocalization power[2] and a theoretically calculated scale of such effects. The latter scale has been obtained by use of Pople's CNDO/2 theory.[3] The comparison is timely both with regard

(1) This work was supported in part by the National Science Foundation.

(2) R. W. Taft, S. Ehrenson, I. C. Lewis, and R. E. Glick, *J. Amer. Chem. Soc.*, **81**, 535 (1959).