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# THE METABOLISM OF 2-CAROBXY-4-KETOPENTITOL DIPHOSPHATE

V. Moses and M. Calvin

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# THE METABOLISM OF 2-CARBOXY-4-KETOPENTITOL DIPHOSPHATE*

V. Moses** and M. Calvin

Radiation Laboratory and Department of Chemistry
University of California, Berkeley 4, California

## ABSTRACT

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2-Carboxy-4-ketopentitol is converted enzymatically by a cell-free preparation from spinach leaves into a substance undergoing acid-lactone interconversion. This substance has no phosphate or ketone groups and is probably a dicarboxylic, six-carbon sugar acid or the saccharic or saccharinic acid type. The significance of these findings with regard to the metabolic role of 2-carboxy-4-ketopentitol diphosphate is discussed.

* The work described in this paper was sponsored by the U.S. Atomic Energy Commission.

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The tentative identification of 2-carboxy-4-ketopentitol diphosphate (CKPD) among compounds incorporating $^{14}C$ from $^{14}CO_2$ by suspensions of photosynthesizing Chlorella cells has recently been reported[1]. Little evidence was available to determine whether this substance played a significant role in metabolism, or whether it was formed nonenzymatically by manipulation of the cell extracts during analysis.

Subsequent studies on its relative rate of formation from $^{14}CO_2$ compared with other photosynthetic intermediates in Chlorella cells have shown that it did not appear labelled in noticeable amounts in the first minute after the introduction of $^{14}CO_2$. This would seem to suggest that it is not an artifact of manipulation formed from the primarily-produced 2-carboxy-3-ketopentitol diphosphate[2,3]. However, the fact that it is never seen in very large amounts and that it would increase by a factor of six due to the labelling of the presumed precursor of carboxylation, ribulose diphosphate, might lead to such an observed result. The question of its enzymatic or nonenzymatic formation thus remains equivocal.

Attempts were made to feed Chlorella cells in the light with CKPD. The cells were suspended in 0.001 M ammonium phosphate buffer, pH 3.55, and were incubated for five minutes in the light with CKPD isolated chromatographically[1]

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from photosynthesis experiments; unlabelled carbon dioxide was also present. Subsequent chromatographic analysis of the cells after killing with boiling ethanol[4] showed the presence of unchanged CKPD (55% of the total $^{14}C$), the monophosphate corresponding to CKPD (38%) and ribulose monophosphate (7%); the last of these was probably derived from contaminating ribulose diphosphate in the CKPD used as substrate. Most probably, CKPD was unable to enter the cells, and was acted upon only by a surface phosphatase. Other experiments with ribulose diphosphate-$^{14}C$ showed that the cells were also impermeable to this substance.

As studies of photosynthesis were being carried out in this laboratory with chloroplast and sap preparations from spinach leaves, the opportunity was taken of using such preparations to study the utilization of CKPD. Chloroplasts were prepared by grinding the leaves with isotonic sucrose containing phosphate buffer, pH 7.22. The whole chloroplasts were separated from fragments and other smaller subcellular particles by centrifugation, and were resuspended in the cell sap. The latter was obtained by blending deep-frozen spinach leaves in a chilled blender (with no addition of liquid) and subsequently thawing the mush and centrifuging it for fifteen minutes at 20,000 g. The clear supernatant (yellowish-green in colour) was adjusted with potassium hydroxide to pH 7.22. The mixture of chloroplasts and sap was fortified with the following cofactors; magnesium and manganese ions, di- and triphosphopyridine nucleotides, adenosine diphosphate, coenzyme A, thiamine pyrophosphate, and ascorbic acid. Full details of these preparative techniques will be published elsewhere[2].

The chloroplast-sap mixture was incubated for thirty minutes in the light with CKPD The reaction was stopped by the addition of four volumes of ethanol at room temperature, and the soluble materials analyzed by chromatography and radioautography as before[4]. Fig. 1 shows the pattern of $^{14}C$ incorporation from CKPD by chloroplasts and sap. Apart from a few very weakly active substances,

19% of the $^{14}C$ in the original substrate was recovered as CKPD, 9% as the corresponding monophosphate ester, and 72% as a new compound, henceforth referred to as substance I (Fig. 1). Compound I had approximately the same chromatographic behaviour as citric acid in the solvent system used, but was not this substance. It was quantitatively converted to another substance (II) by treatment for a few minutes with 0.1 N hydrochloric acid at 100°. Mere elution of substance I from the paper chromatogram, followed by rechromatography, resulted in the formation of some II (35% of I and 65% of II: Fig. 2). I could be recovered from II by heating for a few minutes at 100° with 0.1 N sodium hydroxide. This behaviour of I and II on treatment with acid and alkali suggested that substance I was an acid, and II the corresponding lactone. I was not dephosphorylated CKPD: the latter does not lactonize, and its chromatographic relation to hamamelonic acid and lactone is quite different from I (Fig. 2)[1]. Heating I with 0.1 N hydrochloric acid or 0.1 N sodium hydroxide for thirty minutes at 100° had no effect other than the formation of II in the presence of acid. Treatment of I with 2:4-dinitrophenylhydrazine, followed by extraction of the hydrazones and chromatography[8], produced no hydrazone of I, and no change in the chromatographic behaviour of I was obtained by treatment of it with potassium borohydride; I therefore possessed no ketone function. Human seminal acid phosphatase was without effect on I, thus indicating the absence of a phosphate ester.

Substance I was compared with a number of authentic markers by electrophoresis at 600 V. for three hours in 0.1 M ammonium acetate buffer, pH 9.1; the mobility was equal to that of glyceric acid, and greater than that of ribonic and hamamelonic acids. However, I was not glyceric acid (from chromatographic and lactonization data), and the possibility arose that it was a dicarboxylic sugar acid (a saccharid acid) which would have the same charge/mass ratio as glyceric acid, and hence have similar electrophoretic properties at pH 9.1. Electrophoretically, gluco-saccharic acid moved a little faster than glyceric

acid and I. Chromatographically, gluco-saccharic acid produced a double spot, both of which moved approximately together with hamamelonic acid. The gluco-saccharic lactone, formed on heating the acid with hydrochloric acid, also produced two spots; one of these was chromatographically very close to II, while the other moved an equal distance in phenol-water and a little more slowly in butanol-propionic acid-water. There is no doubt that I is not identical with gluco-saccharic acid itself: the electrophoretic mobilities are a little different; I lactonizes more readily and forms only a single lactone spot, and I also moves further in butanol-propionic acid-water than does gluco-saccharic acid.

Two of the possible structures which might fulfill the requirements of the existing evidence are a saccharic acid and a saccharinic acid derived from CKPD:

| | |
|---|---|
| $CH_2OH$ | $CH_2OH$ |
| HOOC-C-OH | HOOC-C-OH |
| H-C-OH | H- C-H |
| H-C-OH | H-C-OH |
| COOH | COOH |
| a saccharic acid | a saccharinic acid |

Further studies[5] on the feeding of the chloroplast-sap preparation with labelled ribulose diphosphate or labelled phosphoglyceric acid have shown that the same preparation used for CKPD studies converted both these substrates to other intermediates of the carbon reduction cycle[4]. In the presence of unlabelled carbon dioxide, $^{14}C$ from ribulose diphosphate appeared mainly in phosphoglyceric acid, the sugar monophosphates and phosphoenolpyruvate. Labelled phosphoglyceric acid was converted predominantly into the sugar monophosphates and phosphoenol-pyruvate, with a small amount of activity in the diphosphates. The fact that CKPD was not converted into any of these cycle intermediates (Fig. 1) argues against its direct participation in the photosynthetic carbon cycle.

Although the utilization of CKPD by a cell-free preparation from spinach has been demonstrated, its formation in this plant has not yet been investigated.

The fact, also, that CKPD was converted to only one other substance by the spinach preparation (apart from the monophosphate ester) allows no definite conclusions to be reached of its role in metabolism. The conversion of CKPD to I may be a nonspecific enzymatic reaction, and the formation of I may have no physiological significance.

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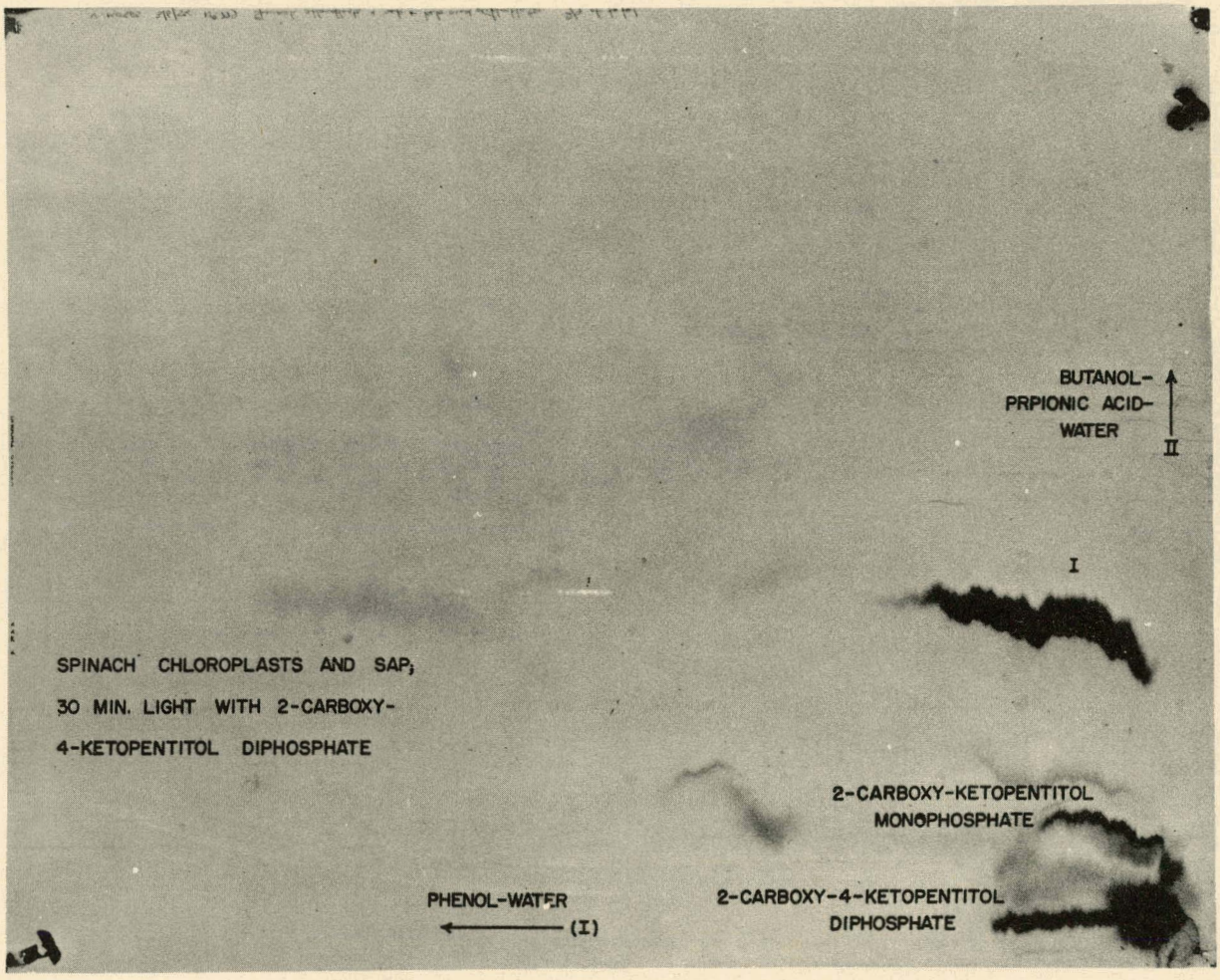


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Fig. 1. Radioautogram of extract of spinach chloroplasts and sap incubated in the light with 2-carboxy-4-ketopentitol diphosphate for 30 min.

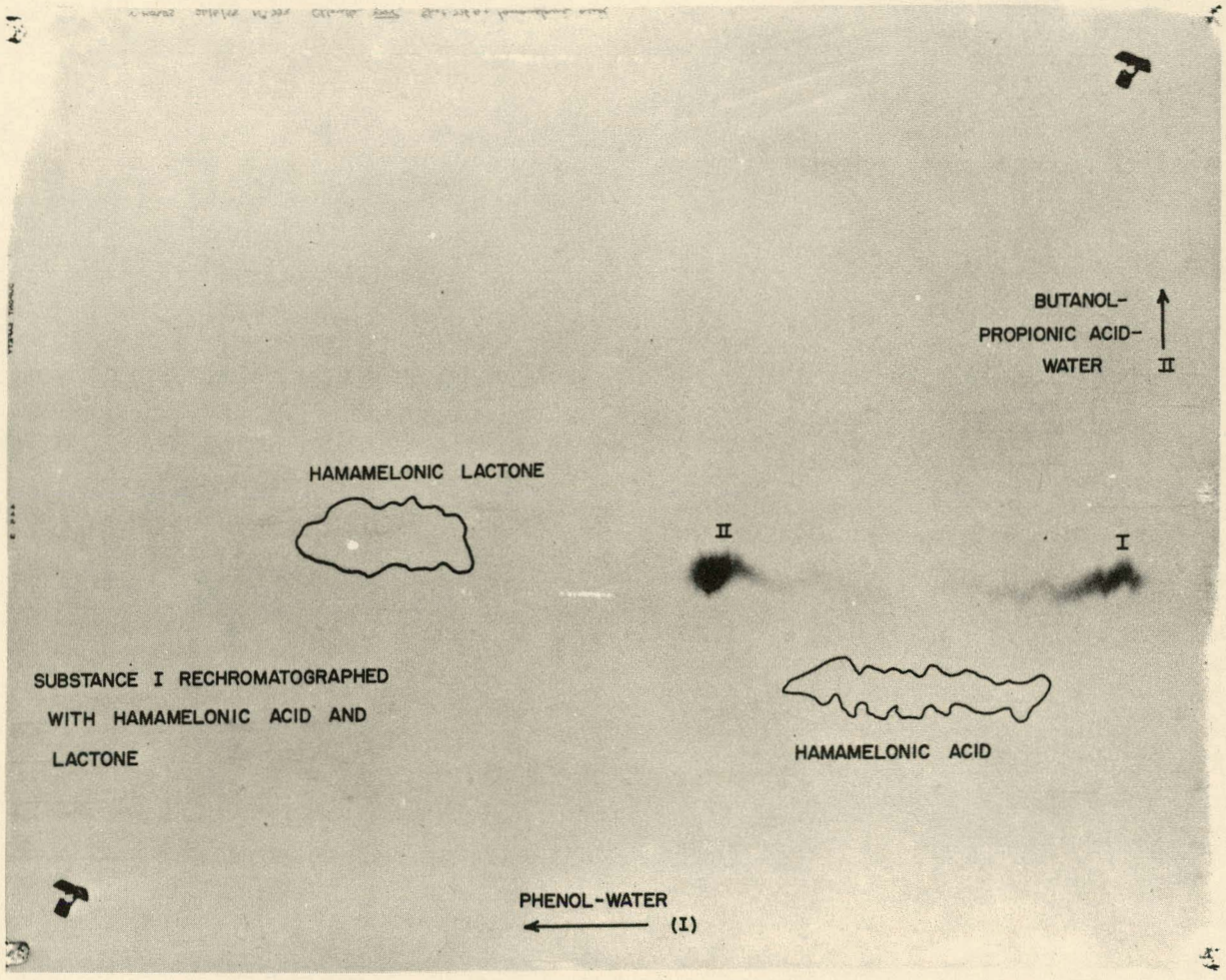


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Fig. 2. Radioautogram of substance I after elution and rechromatography showing presumed acid and lacone forms (I and II). The outlines of the marker spots of hamamelonic acid and lactone are included for comparison.