COMMUNICATIONS TO THE EDITOR

THE SYNTHESIS AND PROPERTIES OF D-ERYTHROSE-4-PHOSPHATE

Sir:

D-Erythrose-4-phosphate has been postulated as an intermediate in carbohydrate metabolism, arising from sedoheptulose phosphate or fructose phosphate by the action of transaldolase[1] and transketolase,[2] respectively. However, the failure of this substance to accumulate in these systems, and the failure to trap it as a derivative, have prevented the direct demonstration of the involvement of this 4-carbon sugar in the reactions studied and, furthermore, have prevented a study of its properties.

We wish to report the synthesis of D-erythrose-4-phosphate by an unequivocal method similar to that used recently in the new synthesis of D-glyceraldehyde-3-phosphate.[3] D-Erythrose diethyl mercaptal, formed by the action of ethyl mercaptan and concentrated hydrochloric acid on 2,3-*O*-isopropylidene-D-erythrose[4] or D-erythrose prepared from D-arabinose by the disulfone degradation,[5] was converted to the crystalline 4-*O*-trityl-2,3-di-*O*-acetyl-D-erythrose diethyl mercaptal, m.p. 105–106°, $[\alpha]_D$ +3.7° (*c* 4, chloroform). Calculated for $C_{31}H_{35}O_5S_2$ (552.7): C, 67.4; H, 6.6; S, 11.6. Found: C, 67.2; H, 6.7; S, 11.5. The mercaptal was deacetylated and then demercaptalated in methanol to give the dimethyl acetal isolated as 4-*O*-trityl-2,3-di-*O*-benzyl-D-erythrose dimethyl acetal, m.p. 122–124°, $[\alpha]_D$ +18.3° (*c* 3, chloroform). Calculated for $C_{39}H_{36}O_7$ (616): C, 76.0; H, 5.8; OCH_3, 10.1. Found: C, 75.8; H, 5.9; OCH_3, 10.5. Following debenzoylation, this product consumed one mole of periodate per mole, as required if the trityl group occupied position 4. Reductive detritylation gave 2,3-di-*O*-benzoyl-D-erythrose dimethyl acetal (not isolated), which was phosphorylated to 4-diphenylphosphoryl-2,3-di-*O*-benzoyl-D-erythrose dimethyl acetal. This substance was unblocked by reductive cleavage of the phenyl groups with hydrogen and platinum, followed by saponification of the ester groups to give 4-phosphoryl-D-erythrose dimethyl acetal, isolated as the crystalline cyclohexylammonium salt, m.p. 160–165°, $[\alpha]^{25}_D$ 0° ± 0.2° (*c* 5, water or 1 *N* hydrochloric acid). Calculated for $C_6H_{15}O_8P \cdot 1.5C_6H_{11}NH_2$ (395): C, 45.5; H, 8.7; N, 5.3; P, 7.9; OCH_3, 15.7. Found C, 45.4; H, 8.7; N, 5.6; P, 8.0; OCH_3, 16.1.

This compound consumed one mole of periodate per mole, consistent with the presence of two adjacent free hydroxyl groups. The acetal structure was readily hydrolyzed in aqueous solution at the *p*H of its own free acid (24 hours at 40°) to give D-erythrose-4-phosphate. The latter had no detectable rotation in acid or neutral solution. It is strongly reducing and gave the theoretical value by the Willstätter-Schudel alkaline iodine titration. The compound was decomposed in 1 *N* acid at 100°, and in 1 *N* alkali at room temperature with the elimination of inorganic phosphate at a rate similar to that for D-glyceraldehyde-3-phosphate.

This synthetic D-erythrose-4-phosphate couples readily with dihydroxyacetone phosphate in the presence of rabbit muscle aldolase to give a sedoheptulose phosphate ester that has the properties of the diphosphate described recently.[6] Enzymatic dephosphorylation yields sedoheptulose (characterized chromatographically), while acid hydrolysis results in the elimination of two moles of phosphate per mole at two distinctly different rates, comparable to the hydrolysis curve of fructose-1,6-diphosphate.

(1) B. L. Horecker and P. Z. Smyrniotis, THIS JOURNAL, **75**, 2021 (1953).

(2) E. Racker, G. de la Haba and I. G. Leder, *Arch. Biochem. Biophys.*, **48**, 238 (1954).

(3) C. E. Ballou and H. O. L. Fischer, *Federation Proc.*, **14**, in press (1955); THIS JOURNAL, in press.

(4) C. E. Ballou and H. O. L. Fischer, unpublished.

(5) D. L. MacDonald and H. O. L. Fischer, *Biochem. Biophys. Acta*, **12**, 203 (1953); L. Hough and T. J. Taylor, *Chem. and Ind.*, 575 (1954).

(6) B. L. Horecker and P. Z. Smyrniotis, H. H. Hiatt and P. A. Marks, *J. Biol. Chem.*, **212**, 827 (1955).

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RECEIVED MARCH 25, 1955

THE PERTECHNETATE ION AS AN INHIBITOR OF CORROSION[1]

Sir:

In a theoretical study of alternative mechanisms to account for the action of corrosion inhibitors of the type of the chromate ion it was concluded that the property responsible for inhibition must involve the internal polarity of the XO_4^{n-} particle. This hypothesis led to the prediction that, of all the elements in groups V to VIII of the periodic system, technetium, in the form of the pertechnetate ion, TcO_4^-, was most likely to possess the requisite properties. This expectation has been amply confirmed. For example, it has been found that mild carbon steels may be effectively protected by from 5 to 50 p.p.m. of technetium element (5×10^{-5} to 5×10^{-4} *f* $KTcO_4$) in aerated distilled water at temperatures up to at least 250°. Certain specimens have been observed at room temperature for over two years with no evidence whatever of attack.

The favorable nuclear and chemical properties of technetium have made it possible to investigate several questions bearing upon the mechanism of the inhibitory process. Thus, it has been found possible to achieve inhibition under very corrosive conditions without depositing more than 3×10^{12} atoms of technetium per sq. cm. It also was shown that the inhibition depends upon the maintenance of some minimum concentration of inhibitor, though there appears to be no continuous reduction of it over long periods of time.

(1) This work was performed for the U. S. Atomic Energy Commission.

From the standpoint of theory, the important observation has been made that the perrhenate ion does not inhibit, although its gross properties are otherwise very similar to those of the pertechnetate ion. Results of several studies will be presented soon in detail.

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RECEIVED MARCH 21, 1955

THE METABOLISM OF THIOCTIC ACID IN ALGAE

Sir:

Chromatographic separation of extracts from various photosynthetic organisms have shown several compounds that have the biological activity of thioctic acid (6T). The criterion for biological activity of these compounds was the response of propionate-inhibited *S. faecalis* grown on an acetate-free medium.[1] Chromatography in a mixture of butanol–ethanol–water gave the major biologically-active compounds at R_f's of 0.4, 0.7 and 0.9. The compounds at R_f = 0.4 and 0.7 were identified as thioctic acid sulfoxide and thioctic acid, respectively. The very lipid soluble compound at the front was not identified.

The synthesis of S^{35}-labeled 6T[2] has made possible a further investigation of thiotic acid metabolism in photosynthetic organisms.

0.5 g. of *Scenedesmus obliquus* suspended in 25 cc. 0.01 *M* phosphate buffer, *p*H 6.75, and containing 0.25 mg. of S^{35} 6T, was incubated in the dark aerobically. The distribution of the thioctic acid between algae and medium changed with time as follows: after 1 min., 16% in the cells; 10 min., 24%; 30 min., 42%; and 1 hour, 47%.

Such cells treated for at least one hour were extracted with ethanol and water and these extracts chromatographed in butanol saturated with 0.5 *N* ammonia. Five radioactive compounds were observed in the butanol–ammonia solvent at the R_f values: 0.98, 0.51, 0.33, 0.17, 0.1. The majority of activity was observed at R_f 0.98, 0.51 and 0.17. The latter two spots were identified as 6T and 6T sulfoxide. In a butanol–ethanol–water solvent system at least seven radioactive components could be observed. In long-term experiments the high R_f thioctic acid compound appeared to be a major component of the cells. To demonstrate that this compound was truly a metabolic product and not an artifact of the killing of the cells, extraction procedure, or chromatography, the S^{35} 6T was added to living algae which were then killed immediately and chromatographed. The results shown in Table I indicate clearly that this compound is a major metabolic constituent of the cell. An important observation is that it is not formed in any significant degree by cells under anaerobic conditions.

It has been shown by Bradley and Calvin[3] that thioctic acid must be metabolized aerobically by

(1) M. W. Bullock, John A. Brockman Jr., E. L. Patterson, J. V. Pierce, M. H. von Saltza, F. Sanders and E. L. R. Stokstad, THIS JOURNAL, **76**, 1828 (1954).

(2) P. Adams, Univ. Cal. Rad. Lab. Rep. U.C.R.L.-2949.

(3) D. F. Bradley and M. Calvin, *Arch. Biochem. Biophys.*, **53**, 99 (1954).

TABLE I

Compound	S^{35} 6T	S^{35} 6T added and cells killed at once	5.25 hr. uptake in dark, aerobic
6T	91.4[a]	85	6
6T sulfoxide	5.4	5	14
Front	0.85	2.2	36
Origin	2.3	5.3	41

[a] Percentage distribution of total activity on chromatogram.

Scenedesmus cells before any stimulation of the Hill reaction could be observed. It was therefore of interest to see if there was any localization of this lipid compound in the cell in relation to the photochemical apparatus. S^{35} 6T-fed Chlorella (we have not yet succeeded in obtaining good plastid preparations from *Scenedesmus* but the total thioctic acid distribution in the two organisms is very similar) were ruptured by ultrasonication and chloroplast fragments isolated and washed. Both an extract of the plastids and the plastid-free cellular supernatant material were chromatographed. The results in Table II show clearly that, of the material in the chloroplast fragments, a large amount is aerobically-formed thioctic lipid.

Table II

DISTRIBUTION OF COMPOUNDS IN CELL FRACTIONS

	Chloroplast fragments	Plastid-free supernatant
6T	7[a]	15
6T sulfoxide	5	8
Front	45	18

[a] Percentage distribution of total activity on chromatogram.

The very high R_f values and the behavior on alumina-column chromatography indicate that this conjugated thioctic acid is closely associated with the most lipid-soluble (hydrophobic) compounds in the cell and is readily converted back to thioctic acid by 4.0 *N* HCl, 1 hr., 120°. Further studies are underway to determine the structure of this thioctic-containing lipid and its possible relationship to photosynthesis.

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

THE METABOLISM OF PROPIONATE BY RAT LIVER SLICES AND THE FORMATION OF ISOSUCCINIC ACID[1]

Sirs:

The pathways of propionate metabolism in mammalian tissues have not as yet been definitely established. According to one view, propionate is metabolized by conversion to acrylate and lactate,[2]

(1) Aided by a grant from the American Cancer Society.

(2) H. R. Mahler and F. M. Huennekens, *Biochim. Biophys. Acta*, **11**, 575 (1953).