Biosynthesis of Vitamin B_6 and Pyridoxal Phosphate by *Streptococcus thermophilus*

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In two previous papers from this laboratory [1,2] the biosynthesis of vitamin B_6 in *Escherichia coli* cells was reported to take place at a significant rate already during the lag phase of growth and to rise to a maximum during the acceleration phase. Excretion of vitamin B_6 into the medium was observed only when the organisms were grown in a medium rich in amino acids.

We have now found that the formation of vitamin B_6 in *Streptococcus thermophilus* (strain KQ) cells is very similar to that in *E. coli*. Fig. 1 shows the results of an

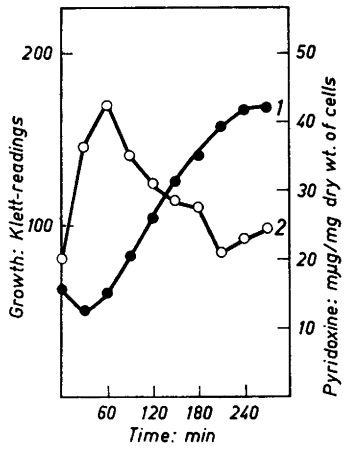

Fig. 1. Formation of vitamin B_6 (calculated as pyridoxine) in *Streptococcus thermophilus* (strain KQ) cells during the growth. 1, Growth curve (left-hand scale); 2, vitamin B_6 content of the cells.

experiment in which the biosynthesis of vitamin B_6 in *Str. thermophilus* cells was followed using *Saccharomyces carlsbergensis* (ATCC 9080) as test organism in the microbiological assay. The level of vitamin B_6 was maximal at the very beginning of the exponential growth phase.

We also followed the production of pyridoxal-5-phosphate during the growth of *Str. thermophilus* using a specific assay method.[3] It appears from the results in Fig. 2 and Table 1 that the formation curve for pyridoxal phosphate is very similar to the corresponding curve for vitamin B_6. Of special interest is the fact that the levels of both vitamin B_6 and pyridoxal phosphate reached a maximum almost at the same point at the very beginning of the exponential phase.

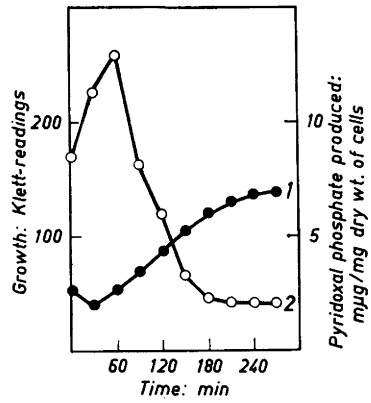

Fig. 2. Formation of pyridoxal phosphate in *Streptococcus thermophilus* (strain KQ) cells during the growth. 1, Growth curve (left-hand scale); 2, pyridoxal phosphate content of the cells.

Table 1. Points at which the formation of vitamin B_6 and pyridoxal phosphate reached a maximum level, expressed as a percentage of the growth time of *Streptococcus thermophilus* with the time between the moment of of inoculation and the end of the retardation phase taken as 100.

Experiment No.	Vitamin B_6	Pyridoxal phosphate
1	22.2 %	16.7 %
2	16.7 »	16.7 »
3	19.9 »	16.7 »
4	16.7 »	20.8 »
5	15.4 »	19.1 »
Average	18.2 »	18.0 »

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1. Nurmikko, V. and Laaksonen, S. *Suomen Kemistilehti* **A 34** (1961) 7.
2. Nurmikko, V. and Raunio, R. *Acta Chem. Scand.* **15** (1961) 856.
3. Gunsalus, I. C. and Smith, R. A. in *Methods in Enzymology* **3** (1957) 963. Eds. Colowick, S. P. and Kaplan, N. O., Academic Press Inc., N.Y.

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On the Synthesis and Properties of 1-Thiacyclobutane-3-carboxylic Acid 1-Oxide

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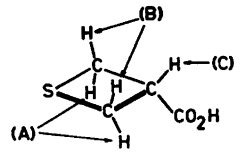
Heretofore, compounds having a carboxyl group directly attached to a thiacyclobutane ring have not been reported. This paper will describe the synthesis of 1-thiacyclobutane-3-carboxylic acid 1-oxide and some of its chemical properties. The compound, however, was obtained as a mixture of its geometrical isomers which have not yet been separated.

For the synthesis of the thiacyclobutanecarboxylic acid, both ethyl β,β'-dibromoisobutyrate and β,β'-dibromoisobutyric acid were chosen as suitable starting materials. Treatment of the ester with sodium sulfide in absolute ethanol only led to the formation of polymers in spite of the fact that the reaction was carried out in very dilute solution by using the method of simultaneously adding the reaction components dropwise to the solvent over a period of several hours. However, when β,β'-dibromoisobutyric acid, neutralized with sodium carbonate, and sodium sulfide were allowed to react in water solution no polymers were formed but instead a small amount of the desired compound was obtained. The larger fraction of the reaction mixture probably consisted of thiodimethacrylic acid, $(CH_2=C(COOH)CH_2)_2S$. The synthesis was then modified in such a way that the neutralized β,β'-dibromoisobutyric acid was first treated with sodium hydrogen sulfide and a few drops of piperidine in water solution and later an equivalent amount of piperidine was added. In this way a 20 % yield of 1-thiacyclobutane-3-carboxylic acid could be obtained. The acid could be purified by distillation *in vacuo* yielding an oil which immediately solidified in the receiver to a white crystalline mass having a m.p. of 64.0–65.5°. The substance was very soluble in ether and acetone but only moderately soluble in carbon tetrachloride and water. The NMR-spectrum of the compound was complex and not easily interpreted, but it suggested an A_2B_2C-type in accordance with the non-equivalent methylene hydrogens in the ring:



On titration of the compound with bromide-bromate in acid solution only slightly more than two equivalents of bromine were consumed, corresponding to an oxidation of the sulfide group to sulfoxide. This is somewhat unusual, because in most cases the oxidation by this method readily gives the sulfone, corresponding to a consumption of four equivalents of bromine. Therefore, such titrations have been used as an excellent method for determining equivalent weights of sulfide-acids.[1,2] The oxidation of a thiacyclobutane ring, however, is likely to be accompanied by an increase of ring strain, so the result in this case is not quite unexpected.

The 1-thiacyclobutane-3-carboxylic acid 1-oxide was prepared by oxidation of the sulfide-acid with hydrogen peroxide in acetone. The compound obtained was a white solid which was almost insoluble in ether and acetone but extremely soluble in water. Its m.p. was not sharp but most of the substance melted in the region 95–100°. Its infra-red spectrum (in KBr) showed a strong double peak for the S = O stretching vibration frequency: ν_{SO} = 1043 and 1027 cm^{-1}, probably corresponding to the two geometrical isomers obtained.