

β -Mercaptopyruvate as a Substrate for Rhodanese

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Wood and Fiedler¹ recently reported that liver extract catalyzed the formation of thiocyanate from β -mercaptopyruvate and cyanide. This was taken as evidence for β -mercaptopyruvate being a substrate for rhodanese, an enzyme catalyzing the formation of thiocyanate from thiosulfate and cyanide². As this enzyme recently has been purified and crystallized³, it was thought desirable to investigate the effect of a purified enzyme preparation on a mixture of β -mercaptopyruvate and cyanide. When this experiment was carried out no formation of thiocyanate was observed and β -mercaptopyruvate is consequently not a substrate for rhodanese. A rhodanese preparation, about 50% pure, was used in the previously described² test system for rhodanese assay, but sodium thiosulfate was replaced by ammonium β -mercaptopyruvate. When instead a rat liver homogenate was used as the enzyme source, a small but significant formation of thiocyanate was obtained, which could be increased 3-fold by preincubating the homogenate with β -mercaptopyruvate before the cyanide was added. From these results (shown in Table 1) it is evident that liver contains an enzyme system, which catalyzes the formation of thiocyanate from β -mercaptopyruvate and cyanide, but which is different from rhodanese. When the same liver homogenate was examined under the conditions of the test systems used by Wood and Fiedler (Table 2), the yield of thiocyanate obtained from β -mercaptopyruvate (as compared with the yield from thiosulfate) was considerably increased. A further increase was observed when the liver homogenate was replaced by an extract of acetone dried liver, which was the enzyme source used by Wood and Fiedler. Even in this case less thiocyanate was obtained from β -mercaptopyruvate than from thiosulfate, although Wood and Fiedler reported that β -mercaptopyruvate and thiosulfate were equally active as sulfur donors for the formation of thiocyanate. This discrepancy

can easily be explained since different liver extracts can be expected to differ in their relative content of rhodanese and the enzyme system responsible for the formation of thiocyanate from β -mercaptopyruvate. This enzyme system is probably identical with a recently described⁴ enzyme, which catalyzes the formation of free sulfur from β -mercaptopyruvate. The reaction between free sulfur and cyanide is then catalyzed by sulfhydryl and disulfide groups⁵, abundantly present in the liver proteins. The claim² that this reaction is catalyzed by rhodanese has previously⁶ been disproved.

Table 1. Thiocyanate formation of rhodanese preparations.

The test system contained 0.05 M sulfur donor and cyanide in a final volume of 2.5 ml. The pH was 8.6 and the system was incubated 5 min. at 20° C.

Enzyme	Sulfur donor	CNS ⁻ formed
		μ -eq.
Purified rhodanese	Thiosulfate	4.30
"	β -Mercaptopyruvate	0
Liver homogenate	Thiosulfate	5.53
"	β -Mercaptopyruvate	0.23
* "	"	0.84

* The homogenate was preincubated 30 min at 38° C with the β -mercaptopyruvate.

Table 2. Enzymatic formation of thiocyanate.

The test system contained 0.017 M sulfur donor and 0.0056 M cyanide in a final volume of 2.5 ml. The pH was 9.1 and the system was incubated 30 min. at 38° C.

Enzyme	Sulfur donor	CNS ⁻ formed
		μ -eq.
Liver homogenate	Thiosulfate	14.8
"	β -Mercaptopyruvate	6.75
Acetone liver extract	Thiosulfate	14.9
"	β -Mercaptopyruvate	9.32

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Use of Thiophene in Lengthening Carbon Chains of Aliphatic Acids by five Carbon Atoms

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In connection with other works, lengthening of aliphatic acids with several carbon atoms by a simple method was of interest. Since thiophene compounds are readily available and easily desulphurated to saturated aliphatic compounds, a method using thiophene as a starting material seems to be convenient.

Starting from an aliphatic acid RCOOH, the corresponding acylthiophene $\text{RCOC}_4\text{H}_9\text{S}$ can be prepared by one of the general methods given by Hartough¹⁻³. This acylthiophene is reduced to the corresponding alkylthiophene $\text{RCH}_2\text{C}_4\text{H}_9\text{S}$ by a modified Wolff-Kishner method⁴. From this alkylthiophene the 5-alkyl-2-acetylthiophene $\text{RCH}_2\text{C}_4\text{H}_7\text{SCOCH}_3$ is prepared and oxidized to the 5-alkyl-2-thiophenecarboxylic acid $\text{RCH}_2\text{C}_4\text{H}_7\text{SCOOH}$ ⁵. From thiophenecarboxylic acids, sulphur has been removed by means of Raney nickel. This operation requires great amounts of Raney nickel in order to give the hydrogen required. Since much hydrogen is produced in the preparation of Raney nickel from the alloy, it might be advantageous to use the Raney alloy directly. In this way the amount of the alloy can be greatly reduced compared with the amount necessary if the Raney nickel is first prepared. Another advantage is that neither the alloy nor the nickel residue obtained by the reduction is pyrophoric, if the alloy is used.

The method is exemplified by the preparation of *n*-heptanoic acid from acetic acid.

Experimental. 2-Acethiophene was prepared according to *Organic Syntheses*⁵, cf. Hartough and Conley².

2-Ethylthiophene. In a 1-l round-bottomed flask fitted with a condenser set for distillation, 79.5 g of potassium hydroxide were dissolved in 560 ml of diethylene glycol, 200 ml of hydrazine hydrate (85 %) and 103 g of 2-acethiophene were added and the mixture heated until no more 2-ethylthiophene distilled over. After washing with water, drying over sodium sulphate and distilling, the yield was 78 g, b.p. 135–140°.

5-Ethyl-2-acethiophene was prepared by the same method as 2-acethiophene.

5-Ethyl-2-thiophenecarboxylic acid was prepared by a modified procedure of that given for the preparation of 2-naphthoic acid from 2-acetylnaphthalene in *Organic Syntheses*⁶, cf. Hartough and Conley².

Removal of sulphur from 5-ethyl-2-thiophenecarboxylic acid. 75 g of the finely ground nickel-aluminium alloy (the minimum quantity necessary) was added slowly to a solution of 75 g of sodium hydroxide in 300 ml of distilled water and 8.8 g of 5-ethyl-2-thiophenecarboxylic acid contained in a 1-l three-necked round-bottomed flask fitted with a mechanical stirrer, a reflux condenser and a stopper, surrounded by ice. The mixture was refluxed for 5 hours, filtered while warm and the catalyst washed with a small quantity of water. The combined filtrate was acidified with concentrated hydrochloric acid and extracted with benzene. The benzene layer was washed with water, dried and distilled. The yield was 5.8 g of *n*-heptanoic acid, b.p. 122–124°/14 mm. A quantity of the acid was converted into the amide, m.p. 97 (from water) and this showed no melting point depression when mixed with an authentic sample of *n*-heptamid.

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