Dithiourea-benzenetellurenyl nitrate, $C_6H_5Te(tu)_2NO_3$. M. p. 135°. (Found: S 15.49; Te 29.88. Calc. for $C_8H_{13}N_5O_3S_2Te$: S 15.31; Te 30.46.) Monoclinic, $a = 24.30$ Å, $b = 5.88$ Å, $c = 21.30$ Å, $\beta = 90\frac{1}{2}°$. There are eight formula units per unit cell; density, calc. 1.83, found 1.83 g/cm³. On the basis of the systematic absences, hkl when $h + k$ is odd, $h0l$ when h is odd or l is odd, the space group is either C_{2h}^6—$C2/c$ or C_s^4—Cc.

Dithiourea-benzenetellurenyl perchlorate, $C_6H_5Te(tu)_2ClO_4$. M. p. 136°. (Found: Te 27.97. Calc. for $C_8H_{13}ClN_4O_4S_2Te$: Te 27.96.) Monoclinic prismatic, $a = 12.24$ Å, $b = 5.86$ Å, $c = 22.76$ Å, $\beta = 96°$. There are four formula units per unit cell; density, calc. 1.87, found 1.87 g/cm³. The space group, from systematic absences, is C_{2h}^5—$P2_1/c$.

Dithiourea-benzenetellurenyl thiocyanate, $C_6H_5Te(tu)_2SCN$, was prepared *via* a compound, presumably $C_6H_5Te(tu)SCN$, obtained from phenyltellurium trichloride in the same way as $C_6H_5Te(tu)Br$, by use of 30 mmoles of potassium thiocyanate instead of potassium bromide. The micro-crystalline solid (3 g) which separated on standing at ice temperature, was filtered off and dissolved in 60 ml of warm 10 % aqueous thiourea. On filtering, a clear, orange yellow solution resulted which on standing deposited yellow crystals (2.4 g) of the thiocyanate. M. p. 109°. (Found: S 22.98; Te 30.47. Calc. for $C_9H_{13}N_5S_3Te$: S 23.18; Te 30.75.)

The crystals occur as long, monoclinic prisms extended along the b axis. The unit cell dimensions are, $a = 15.37$ Å, $b = 5.83$ Å, $c = 17.47$ Å, $\beta = 96°$, and the space group, from systematic absences, C_{2h}^5—$P2_1/n$. There are four formula units per unit cell; density, calc. 1.77, found 1.78 g/cm³.

The crystal structures of representatives of the compounds are being studied with a view towards a possible bearing on the transition state in nucleophilic displacements on divalent tellurium, selenium and sulphur.

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Specificity of a Purified Hog Intestinal Maltase Fraction

Competitive Inhibition of Maltase Activity by Other Substrates

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In previous papers the separation of hog intestinal maltase activity into three fractions (maltase I—III) with different enzymatic properties has been described [1-3]. In the present paper the specificity of one of these fractions, *maltase III*, will be further discussed.

Maltase III preparations hydrolyze *phenyl-α-D-glucopyranoside* [2], *isomaltose* (6-(α-D-glucopyranosyl)-D-glucose) [2], *turanose* (3-(α-D-glucopyranosyl)-D-fructose) [2], and *melezitose* (α-D-glucopyranosyl-(1→3)-β-D-fructofuranosyl-(2→1)-α-D-glucopyranoside) [2]. (Melezitose is hydrolyzed to glucose and sucrose). Heat inactivation experiments support the theory that these activities and the maltase activity of the purified maltase III preparation are caused by the same enzyme [2,3].

When two substrates are hydrolyzed by the same enzyme, they act as competitive inhibitors for the hydrolysis of each other. The study of this inhibition usually requires a method for the separate determination of the hydrolysis products of each substrate, when the substrates are present in a mixture [3]. With maltase III, however, the hydrolysis of the other substrates proceeds slowly compared with the hydrolysis of maltose (measured at 0.1388 M substrate concentration the rate for their hydrolysis is 1/20 or less of the rate for the hydrolysis of maltose). When maltose is present in a mixture with one of the other substrates, therefore, it may be assumed that all the glucose produced is derived from maltose, and thus the rate for the hydrolysis of maltose may be measured simply by measuring the increase in reducing power, as if no other substrate were present *.

* This approximation will cause the K_i value measured to be somewhat too high. The error to be expected was calculated from the relative rate of hydrolysis of the different substrates at 0.1388 M substrate concentration and their K_s values. With phenyl-α-D-glycopyranoside as inhibitor the K_i value measured should be about 15 % too high, with *iso*-maltose and turanose about 30 % too high, and with melezitose less than 10 % too high.

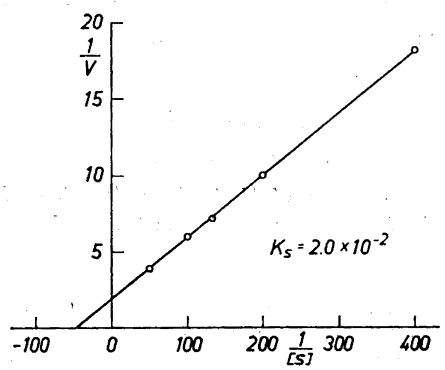

Fig. 1. Determination of K_s for phenyl-α-D-glucopyranosidase activity of purified hog intestinal maltase III. Reaction performed in 0.05 M maleate buffer pH 6.5 at 37°C.

Maltase III was obtained by mutual displacement chromatography [4]. By measuring the rate of hydrolysis (at 37°C in 0.05 M maleate buffer pH 6.5) in a series of maltose solutions containing 0.0020—0.0050 M maltose (chromatographically pure [4]) the K_s for maltase III was determined at 4.2×10^{-3} in agreement with the value obtained earlier [4]. The degree of hydrolysis was measured with acid copper reagent (Caputto's modification) [4,5]. It was not allowed to exceed 20 %. Then the inhibition of maltase III activity was studied, using the same series of maltose solutions in the presence of a suitable amount of the other substrates. The values of K_s and K_i were calculated by Dixon's [8,9] modification of the method of Lineweaver and Burk [10] as described earlier [3]. The calculations for phenyl-α-D-glucopyranoside are seen in Figs. 1 and 2.

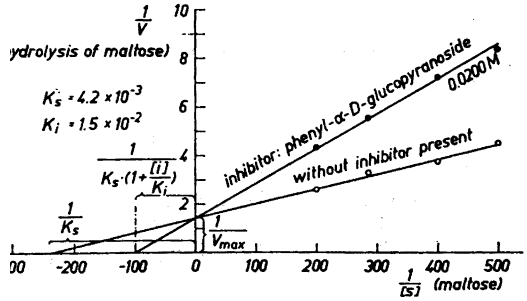

Fig. 2. Inhibition of hog intestinal maltase III activity by phenyl-α-D-glucopyranoside. Reaction performed in 0.05 M maleate buffer pH 6.5 at 37°C. The inhibition is *competitive* (the V_{max} is not altered by the presence of inhibitor). K_s (for maltase III activity) and K_i (for the inhibition by phenyl-α-D-glucopyranoside) are calculated from the intercept with the abscissa as seen in the figure.

The inhibition of maltase III activity was *purely competitive* in all cases (which is seen by the fact that V_{max} was unaltered by the presence of inhibitor). The value for K_i in the different cases are given below, with the concentration of inhibitor used given within parentheses:

Phenyl-α-D-glucopyranoside (0.0200 M) $K_i = 1.5 \times 10^{-2}$ (Fig. 2). The K_s for phenyl-α-D-glucopyranosidase activity was determined to be 2.0×10^{-2} (substrate concentration 0.0025—0.0200 M, and the degree of hydrolysis measured with Somogyi-Nelson's reagents [3], Fig. 1).

Isomaltose (0.0050 M or 0.0100 M) $K_i = 2 \times 10^{-2}$. The K_s for *iso*maltase activity was measured to be 2×10^{-2} (substrate concentration 0.0100—0.0250 M, and the degree of hydrolysis measured by quantitative paper chromatography [6] with 25 μl of the reaction mixture being applied).

Turanose (0.0020 M) $K_i = 3.3 \times 10^{-3}$. The K_s for turanase activity was determined to be 2.2×10^{-3} (substrate concentration 0.0030—0.0200 M, and the degree of hydrolysis determined with glucose oxidase [7]).

Melezitose (0.100 M or 0.200 M*) $K_i = 5.0 \times 10^{-1}$. The K_s for melezitase activity was measured to be 4.5×10^{-1} (substrate concentration 0.0500—0.1400 M, and the degree of hydrolysis determined with 3,5-dinitrosalicylic acid reagent [4]).

Thus in all cases the K_i for the inhibition of the maltase III activity by the other substrates tested was of the same order of magnitude as the K_s for the same substance as substrate. This strongly supports the theory that the phenyl-α-D-glucopyranosidase, *iso*maltase, turanase, and melezitase activities of the purified maltase III preparation are caused by the maltase *per se*, and not by contaminating enzymes.

* In the experiments where 0.200 M melezitose was used as inhibitor, the inhibitor concentration was 100 times the lowest substrate (maltose) concentration. Even during these circumstances, however, it was calculated (from the relative rate of hydrolysis at 0.1388 M substrate concentration and the K_s values) that the amount of glucose produced from melezitose was less than 1 % of the amount produced from maltose.

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Sewage as a Substitute for Casein Hydrolyzates in Fermentations with *Propionibacterium shermannii*

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The bacteria belonging to the genus *Propionibacterium* are characterized by an outstanding ability to produce vitamin B_{12} as reported by several authors [1,2]. Janicki, Pawelkiewicz *et al.*[3-5] used *Propionibacterium shermannii* in their comprehensive studies on vitamin B_{12} production by propionic acid bacteria and isolated several vitamin B_{12} factors from cultures of this organism. These authors found that the addition of small amounts of Aureomycin or sulfathiazole stimulated the synthesis of cyanocobalamin at the expense of other vitamin B_{12} factors [6,7] as is also the case with the addition of 5,6-dimethyl benzimidazole (DMB)[6]. The semi-synthetic medium used in fermentations with *Propionibacterium shermannii* is composed of casein hydrolyzates, glucose, phosphates, certain mineral salts and the vitamins, calcium pantothenate and biotin. Since the most expensive constituents of the medium are the casein hydrolyzates, it was considered

Table 1. Semi-continuous stationary fermentations with *Propionibacterium shermannii* in 2 l conical flasks.

Expt. No.	1	2	3
Medium	semi-synth.	semi-synth. + DMB 1 μg/ml	semi-synth. + corn steep liquor, **
E. coli activity after 5 days μg/ml *	1.8	0.7	0.9
E. coli activity after 12 days μg/ml *	3.6	0.5	2.6

* calculated as cyanocobalamin in cup plate assay.
** 4 % calculated on the basis of dry product containing 4 % N.