**SHORT COMMUNICATIONS**

**Dithiourea-benzenetellurenylene nitrate,**

C\textsubscript{6}H\textsubscript{4}Te(tu)\textsubscript{2}NO\textsubscript{3}. M. p. 135\textdegree. (Found: S 15.49; Te 29.88. Calc. for C\textsubscript{6}H\textsubscript{4}TeN\textsubscript{2}O\textsubscript{3}Te: S 15.31; Te 30.46.) Monoclinic, \(a = 24.30\) \(\overline{A}\), \(b = 5.88\) \(\overline{A}\), \(c = 21.30\) \(\overline{A}\), \(\beta = 90\textdegree\). There are eight formula units per unit cell; density, calc. 1.83, found 1.83 g/cm\(^3\). On the basis of the systematic abscissae, \(h\ell\) when \(h + k\) is odd, \(h0l\) when \(h\) is odd or \(l\), the space group is either \(C\textsubscript{sh}^4\) or \(C\textsubscript{sh}^4\). 

**Dithiourea-benzenetellurenylene perchlorate,**

C\textsubscript{6}H\textsubscript{4}Te(tu)\textsubscript{2}ClO\textsubscript{4}. M. p. 136\textdegree. (Found: Te 27.97. Calc. for C\textsubscript{6}H\textsubscript{4}ClN\textsubscript{2}O\textsubscript{4}Te: Te 27.96.) Monoclinic prismatic, \(a = 12.24\) \(\overline{A}\), \(b = 5.86\) \(\overline{A}\), \(c = 22.76\) \(\overline{A}\), \(\beta = 96\textdegree\). There are four formula units per unit cell; density, calc. 1.87, found 1.87 g/cm\(^3\). The space group, from systematic absences, is \(C\textsubscript{sh}^4\). 

**Dithiourea-benzenetellurenylene thiocyanate,**

C\textsubscript{6}H\textsubscript{4}Te(tu)\textsubscript{2}SCN, was prepared via a compound, presumably C\textsubscript{6}H\textsubscript{4}Te(tu)SCN, obtained from phenylltellurium trichloride in the same way as C\textsubscript{6}H\textsubscript{4}Te(tu)Br, by use of 30 mmols 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\textdegree. (Found: S 22.98; Te 30.47. Calc. for C\textsubscript{6}H\textsubscript{4}TeN\textsubscript{2}S\textsubscript{2}Te: S 23.18; Te 30.75.)

The crystals occur as long, monoclinic prisms extended along the \(b\) axis. The unit cell dimensions are, \(a = 15.37\) \(\overline{A}\), \(b = 5.83\) \(\overline{A}\), \(c = 17.47\) \(\overline{A}\), \(\beta = 96\textdegree\), and the space group, from systematic absences, \(C\textsubscript{sh}^4\). There are four formula units per unit cell; density, calc. 1.77, found 1.78 g/cm\(^3\).

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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* This approximation will cause the \(K\) 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 \(K\) values. With phenyl-\(a\)-d-glucopyranoside as inhibitor the \(K\) 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.

**In previous papers the separation of hog intestinal maltase activity into three fractions (maltase I—III) with different enzymatic properties has been described 1–8. In the present paper the specificity of one of these fractions, maltase III, will be further discussed.**

**Maltase III preparations hydrolyze phenyl-\(a\)-d-glucopyranoside 9, isomaltose (6-\((a\)-d-glucopyranosyl)-d-glucose) 4, turanose (3-(d-glucopyranosyl)-d-fructose) 4, and melezitose (d-glucopyranosyl-(1→3)-\(b\)-d-fructofuranosyl-(Q→1)-d-glucopyranoside) 4. (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 1–8.**

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 8. 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 8.
Maltase III was obtained by mutual displacement chromatography. 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) the $K_s$ for maltase III was determined at $4.2 \times 10^{-2}$ in agreement with the value obtained earlier. The degree of hydrolysis was measured with acid copper reagent (Caputto's modification). 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 $K_1$ were calculated by Dixon's modification of the method of Lineweaver and Burk as described earlier. The calculations for phenyl-$\alpha$-D-glucopyranosidase are seen in Figs. 1 and 2.

The inhibition of maltase III activity was purely competitive in all cases (which is seen by the fact that $V_{\text{max}}$ was unaltered by the presence of inhibitor). The value for $K_1$ in the different cases are given below, with the concentration of inhibitor used given within parentheses:

- **Phenyl-$\alpha$-D-glucopyranosidase (0.0200 M)** $K_1 = 1.5 \times 10^{-3}$ (Fig. 2).
- **Phenyl-$\alpha$-D-glucopyranosidase activity** was determined to be $2.0 \times 10^{-1}$ (substrate concentration 0.0025–0.0200 M, and the degree of hydrolysis measured with Somogyi-Nelson's reagent, Fig. 1).
- **Isomaltose (0.0050 M or 0.0100 M)** $K_1 = 2 \times 10^{-4}$. The $K_s$ for isomaltose activity was measured to be $2 \times 10^{-4}$ (substrate concentration 0.0100–0.0250 M, and the degree of hydrolysis measured by quantitative paper chromatography with 25 µl of the reaction mixture being applied).
- **Turanose (0.0020 M)** $K_1 = 3.3 \times 10^{-2}$. The $K_s$ for turanase activity was determined to be $2.3 \times 10^{-2}$ (substrate concentration 0.0030–0.0200 M, and the degree of hydrolysis determined with glucose oxidase).
- **Melezitose (0.100 M or 0.200 M*)** $K_1 = 5.0 \times 10^{-2}$. The $K_s$ for melezitase activity was measured to be $4.5 \times 10^{-1}$ (substrate concentration 0.0500–0.1400 M, and the degree of hydrolysis determined with 3,5-dinitrosalicylic acid reagent).

Thus in all cases the $K_1$ 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-$\alpha$-D-glucopyranosidase, isomaltase, 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 shermanii

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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. Janicki, Pawlakiewicz et al. used Propionibacterium shermanii 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 as is also the case with the addition of 5,6-dimethyl benzimidazole (DMB). The semi-synthetic medium used in fermentations with Propionibacterium shermanii 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 I. Semi-continuous stationary fermentations with Propionibacterium shermanii in 2 l conical flasks.

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>semi-synth.</td>
<td>semi-synth. + DMB 1 μg/ml</td>
<td>semi-synth. + corn steep liquor. **</td>
</tr>
<tr>
<td>E. coli activity after 5 days μg/ml</td>
<td>1.8</td>
<td>0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>E. coli activity after 12 days μg/ml</td>
<td>3.6</td>
<td>0.5</td>
<td>2.6</td>
</tr>
</tbody>
</table>

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

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