CONFIRMATION OF THE DEGRADATION PRODUCTS
OF THIAMINE BY THIAMINE-DECOMPOSING
THERMOSTABLE FACTOR AND THE MECHANISM
OF THIAMINE DEGRADATION

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Hasegawa et al. (1) have demonstrated that the natures of thiamine-decomposing thermostable factors are flavonoids. Subsequently, Matsukawa et al. (2) have showed that phenol compounds also have the same action and Hasegawa et al. (1) have determined the thiamine-decomposing activity of many flavonoids and phenols, and pointed out that rutin and fisetin among flavonoids, and o- and p-diphenols among phenols have especially a strong activity in degrading thiamine. Hasegawa et al. (3) have degraded thiamine with rutin and isolated a degradation product as pure crystals. They demonstrated the product to be a kind of thiamine disulfide, and designated it tentatively as Rutinothiamine (RT). Other degradation products of thiamine by phenols, however, have so far not been clarified. The authors have investigated the reaction between thiamine and pyrocatechol, which has the simplest structure among diphenols and at the same time, the strongest activity of thiamine degradation. The reaction product was isolated as pure crystals. The reaction product of thiamine with hydroquinone was also isolated similarly. The chemical structures of both substances were studied and were found to be identical.

EXPERIMENTAL

1. Isolation of the Reaction Product of Thiamine with Pyrocatechol

Fourteen g of thiamine hydrochloride and 4.0 g of pyrocatechol were dissolved in 18 l of water. Two l of M/15 phosphate buffer (pH 7) was then added and the pH of this solution was adjusted exactly to 7.0 using 1 N NaOH. After the reaction for 7 to 9 hours, the thiamine added has been decomposed up to 90 per cent. In the course of this reaction, the pH of the solution fell gradually inspite of the existence of buffer. Therefore, the pH of the solution was determined each 30 min and the solution was adjusted to pH 7.0 using 1 N NaOH. After the completion of the reaction, the solution was concentrat-
ed at 40—50° up to about 500 ml in vacuo, and extracted with butanol. The brown extract was further reconcentrated in vacuo up to about 200 ml and passed through an alumina column (20 × 3 cm), whereby colored contaminations in the extract were almost removed and the pale brown solution was obtained. It was concentrated and stored in a refrigerator. A large amount of white crystals were separated.

On recrystallization with butanol, long needles were obtained, mp 131° (micro), yield ca. 3 g. This crystal was tentatively named Catechothiamine (CT).

2. Isolation of the Reaction Product of Thiamine with Hydroquinone

Eight g of thiamine hydrochloride and 4.0 g of hydroquinone were dissolved in 18 l of water and 2 l of M/15 phosphate buffer (pH 7) was added. The pH of the solution was adjusted to 7.0 using 1 N NaOH. As hydroquinone was less active than pyrocatechol in thiamine degradation, the amount of thiamine degraded by hydroquinone was much less than that by the same amount of pyrocatechol, only 8 g of thiamine being degraded even at 70° for 9 hours by 4 g of hydroquinone. After the reaction the solution was extracted with butanol and was passed through an alumina column. In this case, however, the solution did not become colorless even after repeated alumina treatment. On concentration and cooling of this solution some pale pink needles appeared.

After 4 recrystallizations from butanol almost colorless needles were obtained, mp 177° (with decomp.), yield 0.5 g. This crystal was tentatively named Hydroquinothiamine (HT).

3. Difference among CT, HT and RT

CT and HT obtained as mentioned above bore a close resemblance to RT in their crystal form and melting-point. Hence, the authors synthesized pure RT crystals according to Hasegawa et al. (3) and subjected to following tests.

Ultraviolet Absorption Spectra—

Each sample was dissolved in absolute ethanol at the concentration of 40 μg/ml and the absorption spectra were determined using Beckman Spectrophotometer. As shown in Fig. 1, two absorption maxima lie at 223 and 277 mμ, with the values of $E_{1\%}^{1\text{cm}}$ 410 and 180, respectively.

Paper Chromatography—Each sample was dissolved in absolute alcohol, developed with n-butanol-acetic acid—water (4 : 1 : 2) up to about 25 cm in length and examined by spraying the Dragendorff reagent. The results are given in Table I, each Rf-value being 0.50—0.51 and no separation of spots was observed in mixed chromatography.
Mixed Melting Point — Samples recrystallized from n-butanol and air-dried for a day were subjected to the micro determination of melting point according to Hasegawa (4). All of them melted at 130—131°, colored at around 135° and decomposed completely at about 170°. No depression was observed when two or three samples were mixed together. In macro method the melting point of the thoroughly dried material was 177—8° with partial decomposition and the mixed melting point was also the same.

Elementary Analyses — The samples five times recrystallized from n-butanol and dried in a silica dessicator with reduced pressure were analyzed and the results are given in Table II. Practically no difference was observed among the three samples, indicating that CT, HT and RT are identical compounds.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$R_f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>0.50</td>
</tr>
<tr>
<td>HT</td>
<td>0.50</td>
</tr>
<tr>
<td>RT</td>
<td>0.50</td>
</tr>
<tr>
<td>CT + RT</td>
<td>0.51</td>
</tr>
<tr>
<td>CT + HT + RT</td>
<td>0.51</td>
</tr>
<tr>
<td>Thiamine</td>
<td>0.45</td>
</tr>
</tbody>
</table>

### Table II

**Elementary Analyses of CT, HT and RT**

<table>
<thead>
<tr>
<th>Sample</th>
<th>C (per cent)</th>
<th>H (per cent)</th>
<th>N (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>53.15</td>
<td>7.00</td>
<td>17.51</td>
</tr>
<tr>
<td></td>
<td>52.68</td>
<td>7.17</td>
<td>17.15</td>
</tr>
<tr>
<td>HT</td>
<td>52.92</td>
<td>7.12</td>
<td>17.26</td>
</tr>
<tr>
<td></td>
<td>53.01</td>
<td>7.13</td>
<td>17.21</td>
</tr>
<tr>
<td>RT</td>
<td>52.65</td>
<td>6.91</td>
<td>16.85</td>
</tr>
<tr>
<td>Thiamine disulfide</td>
<td>53.02</td>
<td>7.01</td>
<td>16.66</td>
</tr>
</tbody>
</table>

4. Elucidation of Structure

Hasegawa *et al.* (3) isolated 2-methyl-4-amino-5-aminomethylpyrimidinone from the hydrolysate of RT and proposed a disulfide form of thiamine based upon the findings that RT was reduced *in vivo* to thiamine and was roughly equally active biologically to thiamine and that the infrared spectrum was similar to thiamine disulfide.

It was confirmed in the present experiment that the infrared spectra of these three compounds were identical and similar to that of thiamine disulfide which had been thoroughly dried (Fig. 2). In elementary analyses, the values of the sample agreed fairly well with those of thiamine disulfide containing 1 mole of crystal butanol as shown in Table II.

The sample of thiamine disulfide has been recrystallized from butanol.
After standing in air for a long time it was converted to an amorphous substance. The substance recrystallized from butanol has already been shown to have a solvent in the crystal by Zima et al. (5). These facts necessarily lead to the presumption that the sample had crystal butanol. A mixture of the dried sample and thiamine disulfide showed a melting point of 176–8° with decomposition without showing any depression.

From the above results it has been elucidated that CT, HT and RT are identical one another and they are all thiamine disulfide of Zima.

**DISCUSSION**

The problem whether the thiamine degradation product by phenol is identical with that by flavonoid is very interesting concerning their degradation mechanism. Our experiments have however elucidated that the products produced by phenol and by flavonoids were identical, i.e., thiamine disulfide. Therefore, thiamine is oxidized to thiamine disulfide by diphenols or their derivatives, flavonoids. But both pyrocatechol and hydroquinone are strong reductants, which are not considered to oxidize directly thiamine to thiamine disulfide. On the other hand, benzoquinone, an oxidation product of hydroquinone, has been found to have a very strong activity for thiamine degradation, Q value being 325,000 at pH 7 and 70° for 1 hr, about ten times larger than that of hydroquinone under the same condition. Furthermore, it has been observed by Fujita et al. (6) that the degrading activity of thermostable factors increase in the presence of oxygen and decrease in the presence of nitrogen or hydrogen. From these results it is reasonable to consider that diphenol is first oxidized to a quinone in the presence of oxygen and the quinone thus formed in turn oxidizes thiamine to thiamine disulfide as follows:
According to Katsui et al. (7, 8) o- or p-diphenols acts as strong antioxidants against vitamin A. This action is considered to be due to the reduced diphenol. In the case of thiamine-degrading thermostable factors on the contrary, the oxidation of the vitamin takes place by the oxidized diphenols.

**SUMMARY**

1. "Catechothiamine” and “Hydroquinothiamine”, thiamine degradation products by pyrocatechol and hydroquinone respectively, were isolated in a crystalline form. They were proved to be identical with “Rutinothiamine”. It was finally demonstrated that they were all identical with thiamine disulfide of Zima by ultraviolet and infrared absorption spectra, paper chromatography, elementary analyses, and mixed melting-point determination.

2. The mechanism of thiamine degradation by thermostable factors is presented.

**ACKNOWLEDGEMENT**

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**REFERENCES**