*On the Occurrence of a New Sulphur Compound
in Yeast.*

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Ten years ago, one of the authors (U. Suzuki) isolated a basic compound from the alcoholic extract of yeast and gave the empirical formula $C_9H_{14}N_4O_4$ to it. It was a colourless prism, melting at 208° (not corr). The aqueous solution was nearly neutral to litmus, and possessed a slightly bitter taste. It was precipitated with phosphotungstic or phosphomolybdic acid, but not with basic lead acetate. It formed also the crystalline picrate, melting at 183° . (not corr.) (Jour. Tokio Chem. Soc., Vol. 34, p.p. 1134-1135. June. 1914)

Further studies on this substance were interrupted for a long time; however, quite recently it was found by the authors that it contains sulphur, consequently the proposed formula must be abandoned. For such a reason the authors prepared this compound again in a large quantity and carefully investigated its chemical nature. The analysis of the free base as well as that of its picrate have shown that the empirical formula should be $C_{11}H_{15}N_5SO_3$.

The isolation of this compound was carried out as follows:—

The alcoholic extract of yeast was evaporated in vacuum. The resulting syrup was dissolved in a little water, and a concentrated tannin solution was added to it. The precipitate thus formed was rubbed with baryta water and filtered. The filtrate was freed from an excess of baryta, and evaporated to a syrup again, and treated with 50 % aceton, whereby a portion remained undissolved. The dissolved portion was evaporated to a thick brown syrup which possessed strong antineuritic properties. After long standing, the sulphur compound separated out from this syrup in colourless crystals, which were recrystallised from the water solution.

When this base is boiled with dilute acid it is easily hydrolysed, thereby adenine ($C_5H_5N_6$) and a thio-sugar being formed. Although the authors have not yet succeeded to isolate the free thio-sugar in crystalline state, but it was isolated as a nice crystalline phenylosazon, having the melting point of $158-159^\circ$, and the analysis of the latter compound agreed with the formula $C_{13}H_{22}N_4SO_2$, that is, phenylosazon of a thio-sugar having the formula $C_6H_{12}SO_4$.

The free base gives the pentose reaction with Bial's reagent, and it gives also furfural reaction when it is boiled with hydrochloric or sulphuric acid. By evaporating the base with strong nitric acid, the crystals of hypoxanthin nitrate were obtained, which was formed from adenine; the oxidation proceeded more energetically when a trace of vanadium oxide was added to the nitric acid solution, and besides hypoxanthin, crystals of xanthin nitrate were obtained. By shaking the mother liquid of hypoxanthin or xanthin with ether, and after evaporating the ethereal solution, colourless prisms of oxalic acid were obtained. Furthermore, in the remaining aqueous solution the presence of some sulphuric acid could be detected, this shows that a part of sulphur present in the base was oxidized to sulphuric acid.

When it is boiled with dilute hydrochloric acid, and barium chloride is added to it, no precipitate is formed, this indicates that the sulphur present does not exist in the sulphate form. In a cold dilute alkaline solution it gives no reaction with sodium nitroprusside, but it gives a dark red colour when it is previously boiled with concentrated alkali before adding sodium nitroprusside.

When this base is boiled with dilute hydrochloric acid, adding a piece of metallic zinc, there develops a gas having a strong pungent odour, which gives a white turbidity when it is introduced into an aqueous solution of mercuric nitrate or mercuric chloride. As neither the presence of hydrogen sulphide nor mustard oil could be detected in this case, it is most probable that this gas chiefly consists of mercaptane or dimethyl sulphide.

From these observations, the authors concluded that the basic substance is a compound of adenine and a thio-sugar (probably thio-methyl pentose) having one of the following formulae:—

Of the formulae above given, the formula (I) seems to agree best with various facts. Further studies will be required to decide whether this assumption is correct. Not only the occurrence of thio-sugar in organisms is quite new, but its combination with adenine is of special interest from the biochemical point of view, because it has a great resemblance with adenosine, a compound of adenine with ribose, which forms an essential part of nucleic acid.

Among the sulphur compounds in animal organisms cystin, cystein, taurin, and taurocholic acids are most popular ones. Further, chondroitic acid forms an essential part of cartilages. The etherial sulphates of urine are to be considered as the end product of sulphur metabolism. Mustard oil groups are widely distributed in plant. Recently, a sulphur derivative of histidine betaine, i. e. ergothionin was isolated by Tanret from ergot. J. H. Müller has reported a new amino acid containing sulphur, which is probably ethyl ester of cystein. $C_2H_5S-CH_2CH-NH_2-COOH$. (J. H. Müller: J. Biol. Chem. **56**. No. 1, pp. 157-169). The brain substance of animals contains also not inconsiderable sulphur, but nothing is known about its chemical nature, though it is generally believed that it plays just so important rôle like phosphoric acid in the nervous tissues. The authors suppose that the sulphur compound discovered by them has also a certain function in the yeast cells.

It may also be mentioned here that the alcoholic extract of rice polishings after long standing, always produces an amorphous brown precipitate. B. Suzuki has recently investigated this precipitate, and found that it has a strong stimulating action upon the growth of yeast, though it has no antineuritic action on pigeons (J. Chem. Soc. Japan, 1924). Now it was found by the authors that this precipitate, after being carefully purified, contains a little S. The crude oryzanin prepared from the alcoholic extract of yeast by the phosphotungstic acid method contained also more than 1 % of sulphur. Though it has no direct relation with the antineuritic properties, yet it is very interesting that it is always present in oryzanin preparation of yeast and rice polishings. The question that whether the sulphur compound of rice polishings is same with that of yeast or not still remains to be settled.