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Studies on Biological Active Component in Garlic (*Allium Scorodoprasm L.* or *Allium Sativum*). I. Thioglycoside

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Since Schneider and Weltheim reported about the essential oils in garlic in 1892, many researchers have studied on its essential oils. Recently Stoll isolated odorless alliin from garlic and found this compound changed to odory allicin with the garlic enzyme. In this paper, isolation method of one kind of thioglycoside from garlic was reported. At first, garlic bulbs were treated with boiling water, crushed and extracted with methanol at a room temperature, and then thioglycoside was adsorbed on active carbon to exclude the concomitants. According to the color reactions and analyses, this compound was proved as R–CH:CH–CH_2–S–$C_6H_8O_6 \cdot \frac{1}{2}Ca$, in which R was a peptide containing a new amino acid, and carbohydrate part was calcium fructuronate. Its aglycon showed some sterilization effects for *E. coli*.

Since 1892, studies on the components of garlic has been continued, and Schemmler[2] and Weltheim[3] first extracted odory essential oils and described that these were active components of garilic. Stoll succeeded in isolating odorless alliin (sulfoxide of S-allyl ether of cystein) from garlic and found that this compound changed to odory and antibacterial allicin (S-allyl ester of allylthiosulfinic acid) by the action of garlic enzyme.[4] The author also started the research on garlic in 1922 and found many kinds of biological active compounds in garlic. Schemmler reported that active components in garlic were essential oils having special odor and that they were allyl mercaptan, diallyl sulfide or diallyl disulfide. The author found that some of these oils had sterilizing effect, but these were not seemed as main components of garlic, and we thought there must be some other active components. The most important meaning of garlic has been considered as nutrient and has been believed that main components of garlic has special odor, but from the results of our research it was proved that an active component of garlic was odorless, and was contained in a form of thioglycoside.

In sterilization tests, emulsified essential oils of garlic were used for *S. Typhi*. Bacteriostatic action was observed, but its antibacterial action was a little. In the nutrition tests using Albino rats, remarkable growth promoting effect could not be observed. Thereafter,

1) Location: *No. 48, Fukakusa-Mukaigawara, Fushimi-ku, Kyoto.*
2) F.W. Schemmler, *Arch. Pharm.*, **230**, 434 (1892).
3) T. Weltheim, *Arch. Pharm.*, **51**, 289 (1844).
4) A. Stoll, E. Seebeck, *Sci. Pharm.* **18**, 61 (1950); *Experientia*, **6**, 330 (1950).

many kinds of garlic harvested in Japan were tested and presumed that an active component may be an odorless compound isolated by an enzyme-inactivating procedure. This fact showed an active component itself was odorless such as glycoside of allyl mustard linkage.

The author speculated at first that the glycoside must be a homologue of sinigrine, sinalbin or related compounds which had been shown by Gadamer[5] and Schneider,[6] but later that it was not a compound of isocyanate type. From the results of experiments, it has been recognized that this compound was consisted from allyl mercaptan and carbohydrate, that is, one kind of allyl thioglycoside. It will be thought that allyl mercaptan was immediately oxidized in the presence of oxygen to diallyl disulfide which has no nutritive effect for rats, but this glycoside has some growth promoting effect for Albino rats. The author presumed that the greater parts of active components in garlic were in debt to glycoside descrived in this paper.

Experimental

Fractionation of Garlic Essential Oils——Thirty kg of raw garlic, after peeling, was crushed and stood at a room temperature for several hours. The material was mixed with 1 liter of water and carried out the steam distillation. The distillate, 3 liter, pH 3.0—3.2, was extracted with 1 liter of ether, and the ether layer was dried over dehyd. sodium sulfate, and evaporated to dryness to obtain about 10 g of a mixture of essential oils. The mixture was fractionated by distillation and four fractions were obtained. Properties of each fraction were shown in Table I. Fraction 1 and 2 had mercaptan-like, pungent odor, and stimulative. By the addition of sodium nitroprusside and sodium hydroxide solution to Fr. 1 or 2, changed to reddish violet. They easily absorbed bromine and decolorized potassium permanganate solution. Elemental analysis of Fr. 1 and 2: *Anal.* Calcd. for C_3H_6S: C, 48.59; H, 8.16; S, 43.25. Found: C, 48.57; H, 8.19; S, 43.45. Fr. 1 and 2 were proved to be allyl mercaptan. Fr. 4 was transparent liquid, had the same specific gravity as Fr. 1, and strong garlic oder. *Anal.* Calcd. for $C_6H_{10}S_2$: C, 49.32; H, 6.85; S, 43.84. Found: C, 49.18; H, 6.88; S, 43.90. Fr. 4 was proved to be diallyl disulfide.

Table I. Fractional Distillation of Garlic Essential Oils

Fraction	bp (°C)	Yield (g)	Property
1	40— 60	0.8	weak acidic, stimulate eye, mercaptan odor
2	60— 75	0.8	
3	75— 90	2.2	Acidic, mercaptan odor
4	90—120	3.4	neutral, not stimulative
Residue		3.3	dark brown odorless resin

Garlic Carbohydrates——Five kg of raw garlic was treated with 5 liter of boiling water for 15 min without peeling. After peeling the garlic was homogenized in a blender, and the slurry was freeze-dried. The powder obtained was assigned as odorless garlic powder. An aqueous solution, 2%, of the odorless garlic powder was positive for Seliwanoff reaction. With resorcinol and HCl, the solution changed to red. To 1 liter of 10% aqueous solution of the powder, 100 ml of 10% lead acetate was added. After filtration, lead ion was removed by passing of H_2S and the filtrate was concd. *in vacuo*, and 300 ml of 3% barium hydroxide solution was added. White precipitate deposited, and it changed gradually to viscous. The precipitate, 30 g, was suspended in 150 ml of water, and barium ion was removed by CO_2. The filtrate was concd. *in vacuo* to obtain 14 g of white powder. This powder was used as the crude preparation of the garlic carbohydrates. It did not reduce the Fehling's solution, but after hydrolysis with mineral acid it reduced the solution. It was hydrolyzed with 0.2N HCl (100°, 3 hr), and the hydrolyzate was carried out in the usual manner to obtain reducing sugar as sirup. From the *Rf* values on paper chromatogram and thin-layer chromatogram, the reducing sugar was found to be fructose. *Anal.* Calcd. for $C_6H_{12}O_6$: C, 40.00; H, 6.72. Found: C, 40.12; H, 6.69. Phenylhydrazone: mp 204—207°. *Anal.* Calcd. for $C_{12}H_{16}O_9N_2$: N, 15.39. Found: N, 15.31.

Action of Amylase on Garlic Carbohydrates and Action of Enzyme in Garlic on Starch and Dextrin——1) Amylase Action on Garlic Carbohydrates: To 5 ml of 0.5% aqueous solution of the garlic carbohydrates,

5) J. Gadamer, *Arch. Pharm.*, **119**, 376 (1985).
6) W. Schneider, *Ber.*, **47**, 1248, 2218 (1914); *ibid.*, **49**, 1634 (1916); *ibid.*, **52**, 2131 (1918).

2 ml of 0.5% amylase solution was added and incubated at 37° for 6 hr. The incubated solution did not reduce the Fehling's solution.

2) Preparation of Crude Garlic Enzyme: One kg of raw garlic, after peeling, was crushed and filtered with suction and about 200 ml of the filtrate was obtained. To the filtrate, 200 ml of a mixture of ethanol–ether (1:1) was added and resulting precipitate was dried *in vacuo*. This powder was used as the crude garlic enzyme.

3) Garlic Enzyme Action on Garlic Carbohydrate: To 30 ml of 0.5% aqueous solution of the garlic carbohydrate, starch or dextrin, 20 ml of 0.5% aqueous solution of the crude garlic enzyme was added, and the mixture was incubated for 4 hr at 38°, and resulting reducing sugar was determined by Bertran's method (Table II). In the case of starch and dextrin, no reducing sugar was observed.

Table II. Garlic Enzyme Action on Garlic Carbohydrates

Exp. No.	Garlic polysaccharides soln. (ml)[a]	Crude garlic enz. soln. (ml)[a]	hr	Temp. (°C)	Hexose produced (%)
1	30	20	4	38	66.67
2	30	20[b]	4	38	0
3	—	20	4	38	0
4	30	—	4	38	0

a) Each 0.5% aqueous solution were used.
b) Crude garlic enzyme solution was heated in a boiling water bath for 15 minutes.

Isolation of Glycoside (Scordinin A_1)——Ten kg of raw garlic was treated with 10 liter of boiling water for 15 min, crushed, and shook with 20 liter of methanol and filtered. The filtrate was concd. *in vacuo* below 50°, and the residue obtained was dissolved in water to make 10% solution. To this solution, 10% lead acetate solution was added until no more precipitate occures and filtered. The filtrate was passed by H_2S, filtered, H_2S was removed by suction, and pH of the solution was adjusted to 4—5. To the solution, 500 g of active carbon was added, shook well for 30 min and filtered. The active carbon was extracted with 20 liter of hot methanol and filtered. The eluate was concd. *in vacuo* to 150 ml, and 5 liter of ethanol was added to the solution, stirred and filtered. The filtrate was concd. *in vacuo* to dryness to obtain 75 g of crude scordinin A as sirup. The crude scordinin A was dissolved in 1 liter of water, added with 30 g of $Cu(OH)_2$, and boiled for 1 hr and filtered. The filtrate containing the cuprous salt of scordinin A_1 was concd. *in vacuo* to about a half volume, and added with 2 liter of methanol, filtered, and the filtrate was concd. *in vacuo* to dryness. The residue was dissolved in 1 liter of water and cuprous ion was removed by passing of H_2S and filtered.

H_2S in the filtrate was removed by suction, and 20 g of active carbon was added to the solution, shook for 30 min and filtered. Active carbon was extracted with 1 liter of hot methanol and filtered. The extracts was concd. *in vacuo* to obtain 3 g of scordinin A_1. IR and UV spectra of scordinin A_1 were shown in Chart 2 and 3.

Reactions of Scordinin A_1——After heating of scordinin A_1 in an aqueous solution of pH 8.5—9.0 for 30 min, it changed to yellow by the addition of ammonium molybdate in sulfuric acid. Results of the tests were as follows: pH 2.0 (5% aqueous solution), sodium fusion test: sulfur, nitrogen, and phosphorus were positive, Fehling's solution: negative, but after acid hydrolysis became positive, Ninhydrin reaction: positive, Sakaguchi's reaction: positive, bromine, iodine and potassium permanganate solution: decolorized, tetranitromethane: yellow, Weyl's reaction: positive, Jaffe's reaction: positive, not purple but deep reddish brown.

Reaction of Scordinin A_1 with Silver Nitrate and Mercuric Chloride——An aqueous solution of $AgNO_3$ was added into an aqueous solution of scordinin A_1, colloidal precipitate gradually occured. The precipitate was collected and washed with 0.1% $AgNO_3$ solution and suspended in a small volume of water. The suspension was treated with H_2S, filtered and the filtrate was concd. *in vacuo* at a lower temperature to obtain

Table III. Sterilization Activity of Aglycon of Scordinin A_1 to *E. coli*.

Dilution	After 24 hr	After 48 hr	Dilution	After 24 hr	After 48 hr
20	−	−	5000	−	−
200	−	−	10000	±	−
2000	−	−	control	−	++

−; bacterial growth could not be observed ++; bacterial growth could be observed clearly

crystals, mp 165—170°. The crystals was strong acidic and had stimulant mercaptan odor, and also showed the reducing powder. By the addition of $FeCl_3$ solution, it turned to green.

A methanol solution of mercuric chloride was added into an aqueous solution of scordinin A_1, colloidal precipitate occured, and the precipitate was washed with 0.01% $HgCl_2$ aqueous solution and then was decomposed with H_2S and filtered. The filtrate was concd. *in vacuo* to obtain sirup.

Sterilization Effect of the Aglycon of Scordinin A_1——*E. coli* (2 mg, about 2.5×10^9 cells) was incubated in various concentrations of the aglycon of scordinin A_1 which was obtained by silver nitrate method. The results were shown in Table III.

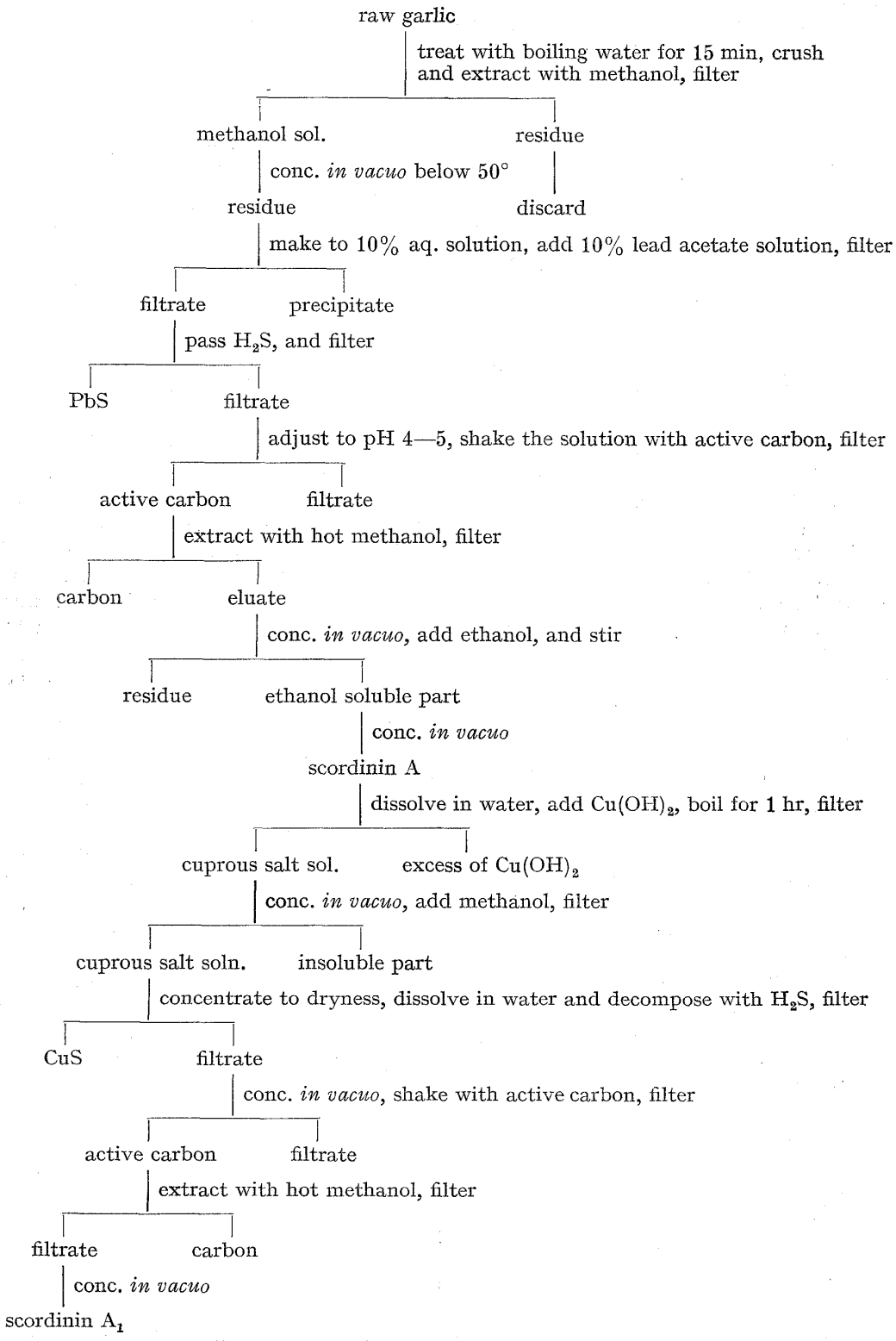

Chart 1. Isolating Procedure of Scordinin A_1

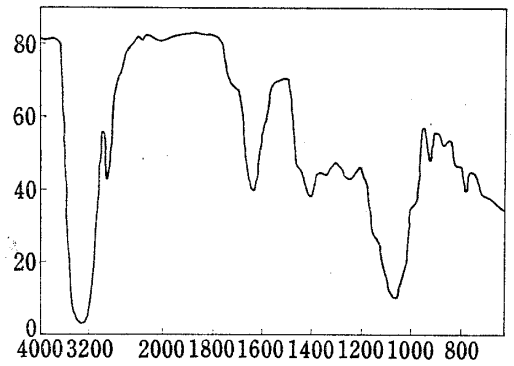

Chart 2. Infrared Spectrum of Scordinin A_1 (KBr)

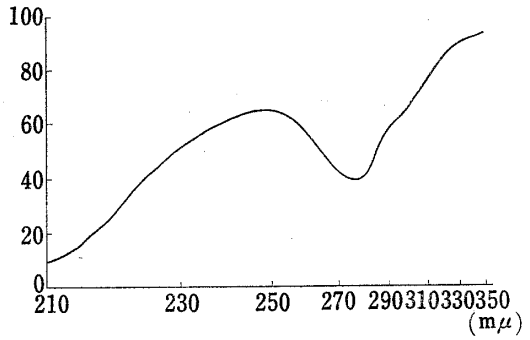

Chart 3. Ultraviolet Spectrum of Scordinin A_1 (Water)

Discussion

A great parts of the carbohydrate contained in garlic was proved as fructosan, and it was hydrolyzed to fructose with the crude garlic enzyme, but the enzyme did not hydrolyze starch and dextrin. As the enzyme also hydrolyzed the thioglycoside to produce allyl mercaptan, the thioglycoside was obtained from garlic which was heated in boiling water.

Chemical structure of the thioglycoside, named as scordinin A_1, and its physiological actions will be reported in Part II and V in this series respectively, and this compound was seemed as one of the biological active component in garlic. One group of Albino rat was fed with boiled garlic and the other group with raw garlic. The raw garlic group lost their appetite and became thinner, but the boiled garlic group increased their body weight and was rather in good nourishment.

By the treatment with silver nitrate, an aglycon of scordinin A_1 was obtained as crystals. Schneider, in his research on mustard, found one kind of glycoside, sinigrin and its aglycon was isolated by the addition of silver nitrate. The reaction of silver complex formation was reported as follows:

$$C_3H_5N{:}C\begin{matrix}SC_6H_{11}O_5\\OSO_2K\end{matrix} + 2AgNO_3 \longrightarrow C_3H_5N{:}C\begin{matrix}SAg\\OSO_2Ag\end{matrix} + HNO_3 + C_6H_{12}O_6$$

The same procedure may be applied to scordinin A_1 and the following equations could be described.

$$R\text{–}CH{:}CH\text{–}CH_2\text{–}S\text{–}C_6H_8O_6\cdot\tfrac{1}{2}Ca + AgNO_3 + H_2O \longrightarrow$$

$$R\text{–}CH{:}CH\text{–}CH_2\text{–}S\text{–}Ag + HNO_3 + C_6H_9O_7\cdot\tfrac{1}{2}Ca \xrightarrow{H_2S} R\text{–}CH{:}CH\text{–}CH_2SH$$

The aglycon, R–CH:CH–CH_2SH, was easily oxidized in the presence of air, or condensed by heating, probably in the following equation.

$$2R\text{–}CH{:}CH\text{–}CH_2SH \xrightarrow{O_2} R\text{–}CH{:}CH_2\text{–}S\text{–}S\text{–}CH_2CH{:}CH\text{–}R$$

It was an acidic compound which had mercaptan odor and sterilization effect to *E. coli*, but this disulfide–type compound has no sterilization effect. Amylase did not hydrolyze the garlic carbohydrates and the crude garlic enzyme also did not hydrolyze starch or dextrin. From the above results, it was proved that the enzyme which has amylase action was not contained in raw garlic, and polysaccharides which was hydrolyzed by amylase was not present in garlic.