[Chem. Pharm. Bull.
17(11)2198—2200(1969)]

UDC 581. 19 : 615. 322 : 582. 572. 2 : 547. 917. 02

Studies on Biological Active Component in Garlic (*Allium Scorodoprasm* L. or *Allium Sativum*). II.[1] Chemical Structure of Scordinin A_1

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(Received September 25, 1968)

In the previous paper, scordinin A_1 was isolated from boiled garlic, and it was seemed as the biological active component in garlic. In this paper the constituents of scordinin A_1 were described from the hydrolysis products of scordinin A_1. By acid hydrolysis of scordinin A_1, fructuronic acid was detected, and by garlic enzyme allyl mercaptan was proved. And by hydrolysis with barium hydroxide, one kind of peptide, scormin, was isolated. From the above results, the author presumed that allyl thiofructuronic acid combined with scormin to form scordinin A_1, and on the chemical structure of scormin will be discussed in Part III in this series.

In the previous paper in this series,[1] scordinin A_1 was presumed to be a biological active component in garlic. Further studies were continued and it was proved that scordinin A_1 was consisted from three components as follows: allyl thioglycoside, a peptide which combined with thioglycoside, and creatinyl thiazole combined with above mentioned peptide.

By the hydrolysis of scordinin A_1 using 2% mineral acid solution, ketocarboxylic acid was obtained. The melting point of potassium salt of this ketoacid agreed with potassium salt of fructuronic acid reported by Ohle.[3] Allyl mercaptan has formed by hydrolysis with garlic enzyme which was described in Part I. So the presence of allyl thiofructuronic acid was postulated. Scordinin A_1 was also positive for halochromy and carboxyl reagents, and it was proved that it has allyl and carbonyl residues which has the double bond in α- and β- position. From the above results and infrared (IR) spectrum, this compound can be thought as one kind of thioglycoside in which calcium allyl thiofructosiduronate combined with glutamic acid residue in the peptide.

The peptide including glutamic acid moiety was named as scormin by the present author. Calcium content in the molecule agreed with theoretical amount. The occurence of $R-CH:CH-CH_2-SH$ was admitted by the reaction with heavy metals as described in Part I in this series. The combination of calcium allyl thiofructosiduronate and peptide might be seemed as interesting linkage in view of biochemistry.

Experimental

Hydrolysis of Scordinin A_1 with Mineral Acid——Scordinin A_1 was dissolved in 2% sulfuric acid and hydrolyzed on a boiling water bath for 8 hr in an atmosphere of CO_2. Garlic odor came out and the solution was colorized, and after hydrolysis sulfuric acid was neutralized with barium hydroxide solution. The filtrate reduced the Fehling's solution. The filtrate was concd. *in vacuo*, and a sirupy substance which failed to crystalize was obtained. Its barium salt was also sirup. Calcium salt which was produced by the addition of calcium hydroxide solution was obtained as needles, recrystallized from 85% ethanol, mp 182—183°. *Anal.* Calcd. for $C_6H_9O_7 \cdot \frac{1}{2}Ca$: Ca, 9.02. Found: Ca, 9.00. The crystals were not positive for naphthoresorcinol reaction, which was a specific reaction for glucuronic acid, but positive for Seliwanoff's reaction. The crystals, 40—50 mg, was heated with a piece of resorcinol and 10 ml of 1 N HCl, it changed to blood red, and Pinoff reaction was also positive. Potassium salt of the compound was obtained from Ca salt, mp 152°, and

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[1] Part I: K. Kominato, *Chem. Pharm. Bull.* (Tokyo), 17, 2193 (1969); A part of this manuscript was reported at the 88th Annual Meeting of Pharmaceutical Society of Japan, April 1968.

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[3] Ohle, *Ber.*, 58, 2577 (1925); *ibid.*, 63, 843 (1927).

agreed with Ohle's report.[3] On the other hand, when a solution of calcium salt of scordinin A_1 was kept in the air for a long time, sometimes crystals were separated at the bottom of the flask. This was proved as calcium fructuronate. *Anal.* Calcd. for $C_6H_{10}O_7$ (free acid): C, 37.11; H, 5.15. Found: C, 37.08; H, 5.24. From the above data, this ketocarboxylic acid was proved as D-fructuronic acid.

Hydrolysis with the Crude Garlic Enzyme——Ten grams of scordinin A_1 was dissolved in dil. acetic acid of pH 4.0, and made up to 50 ml. The crude garlic enzyme, 0.2 g, previously reported was suspended in 5 ml of water and used this solution as an enzyme solution. The enzyme solution was mixed with scordinin A_1 solution with 1—2 drops of toluene, and kept in an incubator at 36° for 3 hr. Special odor came out. The reaction mixture was transferred into a steam distillation flask, and distilled with the aid of steam. The distillate was transparent and stimulative, and was extracted with ether, and 0.28 g of oil was obtained. *Anal.* Calcd. for C_3H_6S: C, 48.65; H, 8.11; S, 43.24. Found: C, 48.43; H, 8.23; S, 43.11.

Calcium Allyl Thiofructosiduronate——Scordinin A_1 was dissolved in 5% barium hydroxide solution and kept for 4 hr at 70—80°. After neutralization with dil. sulfuric acid, $BaSO_4$ formed was filtered off. The solution was concd. to a small volume, and treated with active carbon for several times. The solution of non-adsorbed substances was negative for ninhydrin reaction and Sakaguchi's reaction respectively. This solution was concd. to sirup and kept it in a cold place for several days, but crystals did not separated. *Anal.* Calcd. for $C_9H_{13}O_6S \cdot \frac{1}{2}Ca$: S, 11.94; Ca, 7.43. Found: S, 11.87; Ca, 7.00. This sirup was neutral, and after hydrolysis, reduced remarkably the Fehling's solution. By the addition of ammonium oxalate solution, white precipitate of calcium oxalate occured. With lead acetate solution, the solution changed to black and black precipitate was formed. From the results of analyses, it was proved that this sirup was calcium salt of allyl thiofructosiduronic acid as shown in Chart 1, and its content in scordinin A_1 was 7.36%.

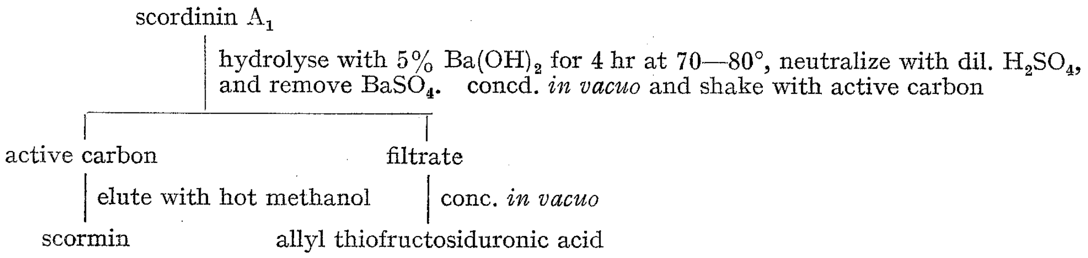

Chart 1. Separation of Scormin and Allyl Thiofrutosiduronic Acid from Scordinin A_1

Isolation of Scormin——Scormin, a peptide moiety of scordinin A_1, was isolated from the hydrolyzate of scordinin A_1 using barium hydroxide. Active carbon described above was extracted with hot methanol successively, and the methanol solution was concd. *in vacuo* to obtain acidic sirup. It was soluble in water, in hot methanol, but almost insoluble in acetone and glacial acetic acid. Crystallized from a mixture of ethanol–methanol (1:1), needles or prisms. By the qualitative analyses, nitrogen, sulfur and phosphorus were detected, and also it was positive for ninhydrin reaction, Sakaguchi's reaction and Jaffe's reaction (Chart 1). Analysis and the chemical structure of scormin will be reported in next report.

Acid Hydrolysis of Scormin——Ten grams of the crude scormin obtained above, was dissolved in 200 ml of 6N sulfuric acid, and boiled for 10 hr in an oil bath. After neutralizing with barium hydroxide solution, adjusted to pH 3.2, and concd. *in vacuo* to about a half volume. Each 2 g of active carbon was added and shook well for several times, and the mixture was filtered. The filtrate was combined and concd. *in vacuo* to obtain glutamic acid as crystals (0.2 g). The sirup, after removing glutamic acid, was adjusted to pH 11,

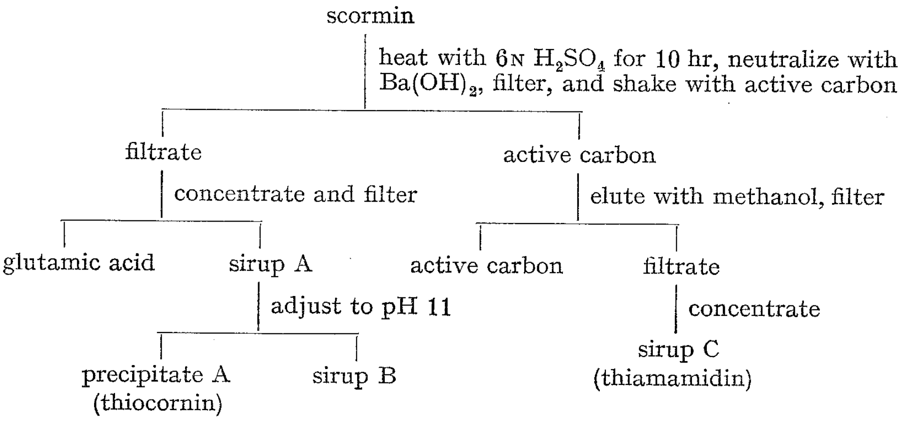

Chart 2. Separation of Acid Hydrolyzates of Scormin

and resulting precipitate was collected (Precipitate A, named as thiocornin) (0.78 g). The filtrate was concd. again *in vacuo* to obtain Sirup B. Active carbon, described above, was extracted with total 200 ml of hot methanol successively, and the extracts were combined and concd. *in vacuo* to obtain Sirup C (containing thiamamidin, named by author and details were reported later) (0.42 g). Separation of the scormin acid hydrolyzate was shown in Chart 2.

Discussion

Fructuronic acid by acid hydrolysis, allyl mercaptan by garlic enzyme hydrolysis, and allyl thiofructosiduronic acid by alkali hydrolysis were obtained from scordinin A_1. And from the alkali hydrolyzate of scordinin A_1, scormin, one kind of peptide, was isolated using active carbon adsorption procedure. As will be reported in Part III in this series, the chemical structure of scormin may be one kind of tripeptide and it consisted of glutamic moiety, thiamamidin which has thiazol and guanidyl group, and thiocornin which is one kind of a new amino acid. Therefore, allyl thiofructosiduronic acid combines with scormin to form scordinin A_1 as shown in Fig. 1.

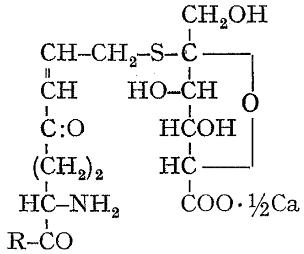

Fig. 1. Chemical Structure of Scordinin A_1