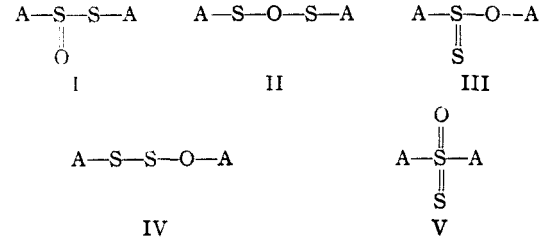
[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF WINTHROP CHEMICAL COMPANY, INC.]

Allicin, the Antibacterial Principle of *Allium sativum*. II. Determination of the Chemical Structure

BY CHESTER J. CAVALLITO, JOHANNES S. BUCK AND C. M. SUTER

The isolation and some of the physical properties of allicin have been described in the preceding paper.[1] By means of cryoscopic measurements, the molecular weight has been found to be approximately 167. Together with other analytical data, this would indicate an empirical formula of $C_6H_{10}OS_2$ of molecular weight 162.

As reported in part I, alkaline hydrolysis of allicin yielded sulfur dioxide and some allyl disulfide. This indicates that the C_6H_{10}-portion of the molecule consists of two allyl groups, which is not surprising considering the nature of the plant source. The structure of allicin could then be any of the five given below in which A represents the allyl group.



In order for sulfur dioxide and allyl disulfide to be formed, a dismutation must occur in alkaline solution. Approximately 0.4 mole of each is formed from each mole of allicin. No hydrogen sulfide or free sulfur is formed. The quantity of sulfur dioxide liberated during this reaction is not affected by the concentration of alkali or by the reaction time allowed.

Non-alkaline, aqueous or non-aqueous solutions or a dry preparation of allicin undergo a chemical change on standing at room temperature, yielding an inactive viscous liquid (VI) which is no longer water soluble and cannot be distilled. The change is usually complete in two days. Cryoscopic molecular weight determinations indicated a molecular weight of approximately 485. The antibacterial agent appears to have undergone an intermolecular reaction involving three molecules. Only minor quantities of sulfur dioxide are formed during this change, probably arising from a side reaction.

Some of the reactions of allicin are mentioned under the Experimental section. Of these, the reaction with cysteine was the most clear cut. An aqueous solution of allicin reacts rapidly with cysteine and at *p*H 6 yields a white crystalline precipitate, VII, which is soluble in acids and bases and slowly dissolves in hot water. The analytical data indicate VII to be C_3H_5—S—S—CH_2—$CH(NH_2)$—COOH. From each mole of allicin there was obtained two moles of VII, definitely showing that the two allyl groups are attached to different sulfur atoms. Structures III, IV and V for allicin are thereby eliminated. The water solubility favors structure I rather than II.

The molecular refractivity of allicin was calculated according to the Lorenz and Lorentz equation on the basis of the $n^{20}D$ and D_{20} values reported in part I and was found to be 47.17. Using the carbon (2.418), hydrogen (1.100), oxygen (1.982) and carbon–carbon double bond (1.733) values for refractivity given by Landolt–Börnstein[2] and the sulfur value (8.17) from organic disulfides as given by Bezzi,[3] the calculated molecular refractivity is 47.30. This calculation, however, ignores any effect of the sulfoxide type of bond on the refractivity inasmuch as no data are available on —S(=O)—S— structures.

Spectral absorption measurements showed that allicin gave no selective absorption between λ 224–440 mμ.

We are indebted to Mr. Jerry McCormick for the data represented by Fig. 1, a polarogram of a solution of allicin in 0.5 molar sodium phosphate buffer of *p*H 6.5. The curves show the effect of time on the current–voltage relationship.

Experimental

Molecular Weight and Analysis of Allicin.—A solution of 0.332 g. of allicin in 20.160 g. of benzene gave a freezing point depression of 0.280°; 1.369 g. in 14.553 g. of benzene gave a depression of 2.770°. The former reading shows a molecular weight of 165, the second, 169. *Anal.* Calcd. for $C_6H_{10}OS_2$: mol. wt., 162; C, 44.44; H, 6.17; S, 39.51. Range of values found: C, 44.12 to 44.59; H, 6.30 to 6.34; S, 39.69 to 40.90.

Molecular Weight of VI.—A solution of 0.345 g. of VI in 16.571 g. of benzene gave a freezing point depression of 0.215°. This corresponds to a molecular weight of 485.

The analytical values for this compound may not be very reliable as the compound did not lend itself to purification other than washing and drying.

Anal. Found: C, 49.13; H, 5.99; S, 42.63.

Alkaline Hydrolysis of Allicin.—The experiment described in Part I was repeated using 0.1 *N* and 0.5 *N* sodium hydroxide solutions and taking from five minutes to three hours as reaction time. The quantity of sulfur dioxide formed is the same in each case, and approximately an equimolar quantity of allyl disulfide was formed (about 0.4 mole from each mole of allicin).

Reaction of Allicin with Cysteine to Give S-(Thioallyl)-cysteine (VII).—To a solution of 452 mg. of allicin (2.79 mmoles) in 40 cc. of water, was added 2 g. of *l*-cysteine hydrochloride (12.7 mmoles) and enough sodium bicarbonate to raise the *p*H to 6. In a matter of seconds, a white

(1) Cavallito and Bailey, THIS JOURNAL, **66**, 1950 (1944).

(2) Landolt-Börnstein, "Physikalisch-chemische Tabellen," 5th ed., Vol. II, p. 985.

(3) Bezzi, *Gass. chim. ital.*, **65**, 701 (1935).

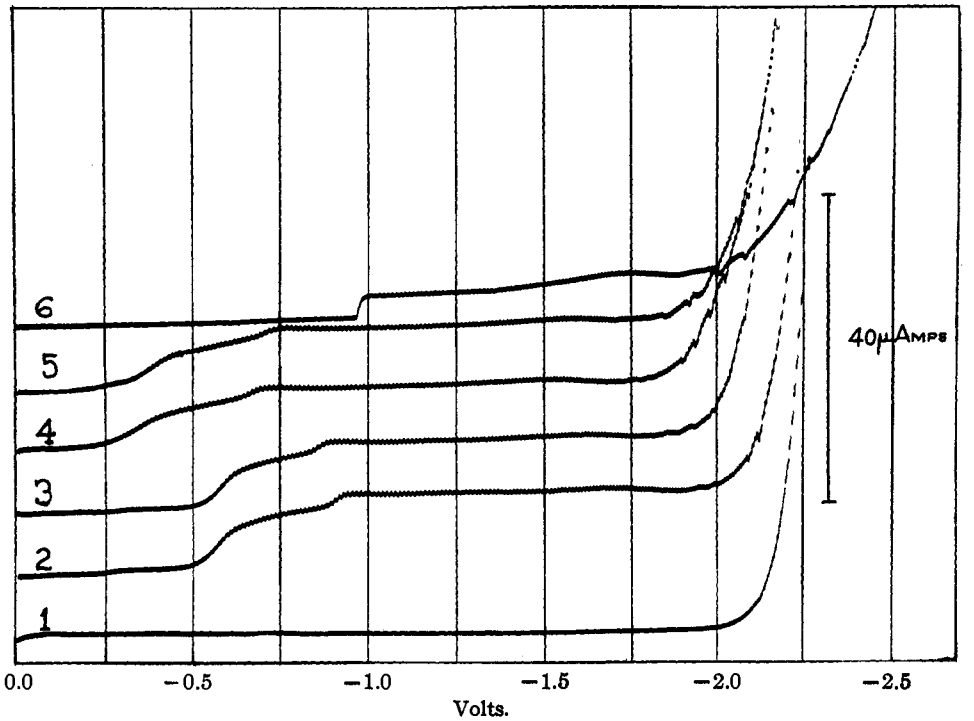


Fig. 1.—Polarograms showing the effect of aging on the reduction curve for allicin. Solutions for aging consisted of 0.03% allicin in air-free 0.5 M. phosphate buffer of *p*H 6.5: 1, phosphate buffer; 2, allicin solution immediately after making up; 3, after one-half hour at 30°; 4, after one and one-half hours; 5, after three hours; 6, after eighteen hours.

crystalline precipitate appeared and the odor of allyl mercaptan could be detected. After standing for twenty minutes, the precipitate was filtered off, washed with water, then with ether, dried and weighed. The aqueous filtrate showed a faint turbidity and extraction with ether yielded 70 mg. of an oil with the odor of allyl mercaptan. The yield of VII was 1.010 g., the theoretical yield is 1.077 g. The crystals were purified by dissolving in dilute hydrochloric acid solution, extraction with ether, separation and re-precipitation of VII from the aqueous solution by slowly raising the *p*H to 6.

When a solution of allicin at *p*H 6.5 is added to an unbuffered solution of cysteine at *p*H 6.5, no *p*H change occurs during the reaction. This indicates that a sulfenic acid is not liberated; however, it does not bar the possibility of the formation of an isomeric compound $C_3H_5—\underset{\underset{O}{\|}}{S}—H$ which could react further with cysteine. For a discussion of a similar reaction one may refer to the work of Toennies and Lavine.[4]

The α_D for VII in 1.0 *N* hydrochloric acid solution is approximately −150° (cystine is −214°); m. p. dec. > 185°.

Anal. Calcd. for $C_6H_{11}O_2NS_2$: C, 37.31; H, 5.70; N, 7.25; S, 33.16. Found: C, 37.25; H, 5.51; N, 7.66; S, 33.30.

Reactions of Allicin.—Allicin loses its antibacterial activity when treated with sodium cyanide or cysteine at *p*H 6. N-Acetylcysteine reacts with allicin nearly as readily as does cysteine, whereas S-methylcysteine shows no reaction. Potassium permanganate solution and bromine water are rapidly decolorized, the latter yielding an oily precipitate. Mercuric chloride solution gives a white precipitate with liberation of some sulfur dioxide. Sodium hydrosulfite produces rapid inactivation.

Grote's reagent[5] yields no immediate color change but, in a few minutes, an evanescent green color appears. Hydrogen peroxide inhibits formation of VI from allicin in aqueous solutions and does not cause rapid inactivation. Allicin is not rapidly decomposed in pyridine (aqueous or anhydrous) solution as in the stronger alkaline media. The antibacterial agent oxidizes hydriodic acid; however, the iodine liberated reacts with the other products in solution.

Discussion

The mechanism whereby $(C_3H_5S)_2O$ can undergo hydrolysis to yield 0.4 molar equivalent each of allyl disulfide and sulfur dioxide cannot readily be explained on the basis of any one particular reaction. At least one other reaction product must be formed, and a small quantity of unidentified resinous water-soluble residue has been obtained upon evaporation of the mother liquors after removal of the sulfur dioxide and allyl disulfide. The sulfur dioxide might arise from the decomposition of a sulfinic acid[6,7] which might be formed along with allyl disulfide from a dismutation of a sulfenic acid liberated by the hydrolysis. One might postulate part of the reaction to be

$$(C_3H_5S)_2O + H_2O \longrightarrow 2[C_3H_5SOH]$$
$$3[C_3H_5SOH] \longrightarrow [C_3H_5SO_2H] + (C_3H_5S)_2 + H_2O$$

The principal reaction of allicin with cysteine is $C_3H_5—SO—S—C_3H_5 + 2HSCH_2—CH(NH_2)—$

(4) Toennies and Lavine, *J. Biol. Chem.*, **113**, 571 (1936).

(5) Grote, *ibid.*, **93**, 25 (1931).

(6) Reuterskiold, *J. prakt. Chem.*, **127**, 269 (1930).

(7) Fromm and Palma, *Ber.*, **39**, 3308 (1906).

COOH → $2C_3H_5S—S—CH_2—CH(NH_2)—COOH$ + H_2O and a secondary reaction may be $C_3H_5SO—S—C_3H_5$ + $HSCH_2—CH(NH_2)—COOH$ → C_3H_5SH + $C_3H_5SO—S—CH_2—CH(NH_2)—COOH$.

The presence of a chemical substance as unstable as allicin in garlic which has been stored for several months to a year raises the question as to the nature of its state in garlic. If this oxide exists in a bound form, it has been impossible to prevent its liberation by grinding the garlic under alcohol or acetone. If it is formed by oxidation of allyl disulfide, the reaction is not inhibited by grinding under the organic solvents which should prevent enzymatic catalysis of the oxidation. There is also posed the question as to whether the degradation of allicin in garlic leads to formation of the other sulfides present, or whether the antibacterial agent arises from oxidation of the sulfides. The other sulfides could well arise from allicin inasmuch as garlic contains from 0.3 to 0.5% of this compound as determined by antibacterial activity. We also believe that the characteristic odor of garlic should be ascribed to allicin rather than to the allyl sulfides.

The mechanism by which allicin acts as an antibacterial agent may be suggested by its reaction with cysteine. The sulfhydryl group is postulated to be a specific stimulator of cell multiplication.[8] Since allicin is considerably more bacteriostatic than bactericidal in action, it may operate by destroying —SH groups essential to bacterial proliferation, thus inhibiting growth. The heavy line of growth surrounding the zone of inhibition in cup-plate tests may be the result of the stimulating action of —SH groups in products formed in the degradation of the antibacterial agent. Hammett[8] points out that whereas SH is stimulating and sulfonates are inert, the intermediate stages of sulfide oxidation, such as the sulfoxides, are inhibitory to cellular proliferation in marine animals.

Summary

The antibacterial principle from *Allium sativum* has been assigned the structure allyl—S(=O)—S—allyl with the structure allyl—S—O—S—allyl not entirely eliminated. A discussion of its reactions is included.

(8) Hammett and co-workers, *Protoplasma*, **10**, 382 (1930); **13**, 261 (1931); **15**, 59 (1932); **16**, 253 (1932).

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Studies in the Santonin Series. II. The Bromination Products of Desmotropo-santonins and Desmotropo-santonous Acids[1]

BY HUANG MINLON,[2] C. P. LO AND LUCY J. Y. CHU

In a previous paper[3] it has been shown that santonin can be transformed into *l*-α-desmotropo-santonin acetate[4] through enol acetylation and that the four known optically active isomers of desmotropo-santonins can be converted into each other by treating with acid or alkali or by alternating the treatments. It is therefore desirable to study whether the halo-santonin and the halo-desmotropo-santonins can be similarly transformed and converted or not. It was found that when monobromosantonin (III), the constitution of which was well established by Wedekind,[5] was treated with acetic anhydride and sulfuric acid, it changed into the bromo-*l*-α-desmotropo-santonin acetate (IV). This gave the bromo-*l*-α-desmotropo-santonin (V) upon saponification. The same compound could also be obtained by the direct bromination of *l*-α-desmotropo-santonin (VI). It may be concluded from the first series of reactions that the bromine atom of the bromo-*l*-α-desmotropo-santonin must be in the aromatic ring. From the second series of reactions it is obvious that the bromine atom must occupy the position ortho to the phenolic hydroxyl group, since there is one and only one free position in the aromatic ring of *l*-α-desmotropo-santonin. The designation of this product as 2-bromo-*l*-α-desmotropo-santonin is therefore beyond any doubt.

The bromo-desmotropo-santonins are still unknown in the literature. These compounds can now be easily prepared by the direct bromination of the corresponding desmotropo-santonins. The yields are generally satisfactory.

The conversion of the bromo-α-desmotropo-santonins into the bromo-β-desmotropo-santonins by treating with acid was not possible. On the other hand the high melting bromo-desmotropo-santonins could be converted into the low melting ones by fusing with alkali. Thus we are able to

(1) Publication of this manuscript was at first postponed pending the submission of analytical data for the new compounds described. We have now learned from the authors that it has been impossible in China for a year or more to make the stipulated analyses and that there is little likelihood of the situation improving until after the conclusion of hostilities. In view of this situation and since the new substances had been tentatively identified by conversion into known derivatives, the manuscript was accepted for publication.—*The Editor.*

(2) Research Fellow, Associate Research Fellow, and Assistant Fellow, respectively.

(3) Huang Minlon, Lo and Chu, THIS JOURNAL, **65**, 1780 (1943).

(4) For nomenclature of desmotropo-santonins and desmotropo-santonous acids, see previous paper, ref. 3.

(5) Wedekind, *Ber.*, **41**, 364 (1908).