served since they solidified immediately at this low temperature.

Extraction of Quinones. These quinones are decomposed by light, heat, moisture, and some organic solvents. They may change into undefinable polymers or oily brown masses. Also, they are extremely reactive toward compounds occurring in living matter; they form, for instance, additional compounds with proteins including nucleoproteins, amino acids, enzymes, and vitamins (*5*).

Previous procedures of isolating the beetle secretion (*4*) are time consuming and yield very little of the unchanged quinones. We employ the mild conditions and efficient procedure of high-vacuum extraction (*6*). The apparatus consists of a 1-l flask, a series of 3 traps, and a high-vacuum pump. The flask is kept at 40° C by a water bath while the traps are thermostated to 0, −20, and −78° C, progressively. About 50,000 frozen insects (200 cc by volume) are placed in the flask. Upon evacuating, yellow quinones begin to deposit in the first trap. The main portion is collected in a few hours. After 24 hr there are practically no quinones left in the beetles, but a slight additional amount may be recovered by increasing the temperature of the flask to 95° C. Finally, the yellow compounds including some yellow oil are found in the first 2 traps, while the remaining volatile matter, mainly water, is found in the coldest trap. The crude quinones melt between 29 and 53° C. They may be purified by extracting with pentane, evaporating the solvent, and subliming under vacuum.

One insect weighs about 1.8 mg (*7*). Almost 60% of its weight consists of matter volatile under high vacuum and it produces about 25 gamma of crude quinones. We have observed also traces of reddish, greenish, and dark blue substances along with some sweet smelling colorless oil in the extractable portion of these beetles.

For studying the effects of these insects upon and in contact with flour, the volatile matter is adsorbed directly on flour. The traps are replaced by a 1-l flask with finely ground flour spread in a thin layer over its bottom and cooled by dry ice-alcohol. During extraction, the volatile matter deposits on the flour and the inner walls of the flask. After extraction, the flask is removed and lightly stoppered to allow for expansion of the gases while the flask is warming to room temperature. After several hours the extracted quinones complete their chemical reaction with the flour as indicated by the reddish color developing on the flour, if sufficient insects have been used in relation to the amount of flour. No visible color change is ordinarily detectable on 1 lb of bleached white flour at concentrations up to 10 mg of beetle secretion as obtained from about 400 insects.

References

1. GOOD, N. E. USDA Techn. Bull., 498, Washington, D. C. (1936).
2. ALEXANDER, P., and BARTON, D. M. R. *Biochem. J.*, **37**, 463 (1943); HACKMAN, R. H., *et al., Ibid.*, **43**, 474 (1948); LOCONTI, J. D., and ROTH, L. M. *Ann. Entomol. Soc. Amer.*, **46**, 281 (1953).
3. GULLAND, J. H. *Biochem. J.*, **26**, 32 (1932); TAKIZAWA, N. *Proc. Imp. Acad. (Tokyo)*, **16**, 309 (1940); GEIGER, W. B. *Arch. Biochem.*, **3**, 176 (1947); LEHMAN, F. E. *Exptl. Cell Research* Suppl. **1**, 156 (1949); MAYER, R. L. *Experientia*, **6**, 241 (1950).
4. PAYNE, N. H. *J. Econ. Entomol.*, **18**, 737 (1925); HOLDAWAY, F. G. *Ecol. Monographs*, **2**, 261 (1932); PARK, T. *J. Exptl. Zool.*, **65**, 17 (1933); ROTH, L. M., and HOWLAND, R. B. *Ann. Entomol. Soc. Amer.*, **34**, 151 (1941); PEARL, R., *et al. Am. Naturalist*, **75**, 5 (1941); BOYCE, J. M. *Ecology*, **27**, 290 (1946).
5. KENSLER, C. J., *et al. Cancer Research*, **2**, 1 (1942); WIGGLESWORTH, V. B. *Experientia*, **2**, 210 (1946); BEILER, J. H., *et al. Arch. Biochem.*, **26**, 72 (1950); MAYER, R. L. *Experientia*, **6**, 241 (1950); LORA TAMAYO, M., *et al.* Paper presented at XII Intern. Congr. Pure and Appl. Chem., New York City (Sept. 1951).
6. LADISCH, R. K., and BERNHARDT, P. L. "Studies on the secretion of odoriferous glands of *Tribolium castaneum*," Quartermaster General Laboratories, Philadelphia, Pa. Unpublished report (May 1949).
7. ROTH, L. M., and WILLIS, E. R. *J. Exptl. Zool.*, **118**, 337 (1951).

Manuscript received May 15, 1953.

Products of the Reaction between Thiamine and Ingredients of the Plants of *Allium* Genus: Detection of Allithiamine and Its Homologs

Taizo Matsukawa, Shojiro Yurugi, and Toshiro Matsuoka[1]

Research Laboratory,
Takeda Pharmaceutical Industries, Ltd., Osaka, Japan

Fujiwara *et al.* (*1*) reported that when thiamine is heated at pH 8 with the alcoholic extract of the garlic (*Allium sativum* L. var. *japonicum* Kitamura), its thiochrome test becomes negative but it is restored by treating with cysteine, and that a crude product of this reaction, in spite of its negative thiochrome test, still possesses a remarkable activity similar to that of thiamine and is absorbed from the intestinal canal more rapidly than thiamine. They named the product allithiamine.

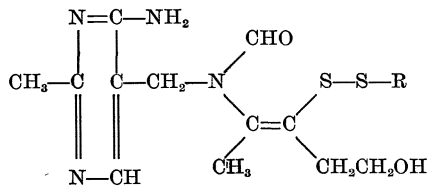
The present authors (*2*) have succeeded in isolating pure allithiamine and have established its structure as 2-(2′methyl-4′-aminopyrimidyl (5′))-methyl-formamino-5-hydroxy-Δ^2-pentenyl (*3*) allyldisulfide(I).

However, in this reaction, besides allithiamine, the formation of its homologs having other alkyl groups than allyl may be assumed, especially in the reaction of thiamine and other plants of *Allium* genus whose sulfur-containing ingredients have not been clarified. For confirmation of the formation of such homologs it is necessary to find a method which can detect them distinctly.

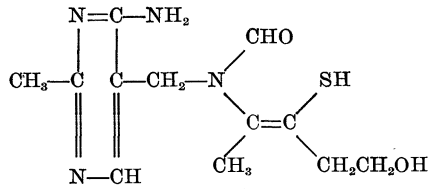
On the other hand, the study of the mechanism of the reduction of allithiamine revealed that allithiamine(I) was reduced by cysteine(IV) into thiamine(V) and at the same time, the liberated allylmercapto group combined with cysteine to form S-allylmercapto-cysteine(VI), which had already been obtained

[1] The authors wish to thank Y. Asahina and S. Kuwada, for encouragement during this investigation, and M. Togasi for his great contribution to collection of the plants.

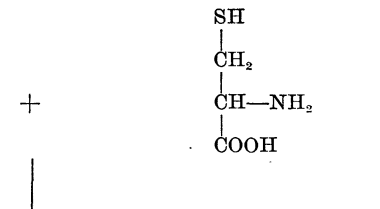
by Cavallito *et al.* (*3*) from allicin(XII) and cysteine(IV). It was also found that the same mechanism was true of the homologs of allithiamine.



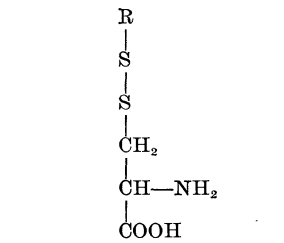
I R = —CH_2—CH=CH_2
II R = —CH_3
III R = —CH_2—CH_2—CH_3



V



IV



VI R = —CH_2—CH=CH_2
VII R = —CH_3
VIII R = —CH_2—CH_2—CH_3

The present authors have pursued this peculiar reaction with great interest and have found that the resulting S-alkylmercapto-cysteines produce their own spots when subjected to paper partition chromatography.

When acetic acid-butanol-water (1 : 4 : 5) is used as the developing solvent, and Ninhydrin and the thiol agent (*4*) as the detecting agent, the Rf values of cystine, cysteine, (VII), (VI), and (VIII) are 0.03, 0.23, 0.43, 0.61, and 0.7, and those of S-ethyl- and S-butylmercapto-cysteine are 0.56 and 0.78, respectively. By employing this method all allithiamine homologs can be detected, in whatever proportion they may be mixed.

In order to utilize this method, bulbs of a plant of *Allium* genus are ground, extracted with alcohol (*5*), and the extract is reacted with thiamine at 50–60° C, pH 8. The solvent is distilled under reduced pressure and the residue is extracted with ether. The ethereal extract, after drying, is evaporated to leave a syrupy substance. The substance is dissolved in 3% acetic acid, and insolubles are filtered off to obtain a yellow-greenish solution as a standard sample, part of which is subjected to paper partition chromatography to determine the Rf value of the common amino acid. The standard sample is then adjusted to pH 6, cysteine hydrochloride is added and, this mixture is allowed to stand at room temperature for a while. The reaction mixture is adjusted again to pH 1 and subjected to paper partition chromatography. The resulting spots, which are positive to the thiol agent and are located differently from that of the common amino acid, represent S-alkylmercapto-cysteines and indicate the formation of the corresponding allithiamine homologs (Table 1).

The same reaction was carried out with many other plants such as *A. cepa* L. (onion), *A. porrum* L., *A. schoenoprasum* L., *A. fistulosum* L., *A. fistulosum* L. var. *caespitosum* Makino, *A. fistulosum* L. var. *giganteum* Makino, *A. albopilosum, Nothoscórdum fràgrans* Kunth (*A. fràgrans* Vent), and *Brodia uniflora* Baker. In all cases the formation of (VII) was detected weakly and in some cases (VI) and (VIII) were too.

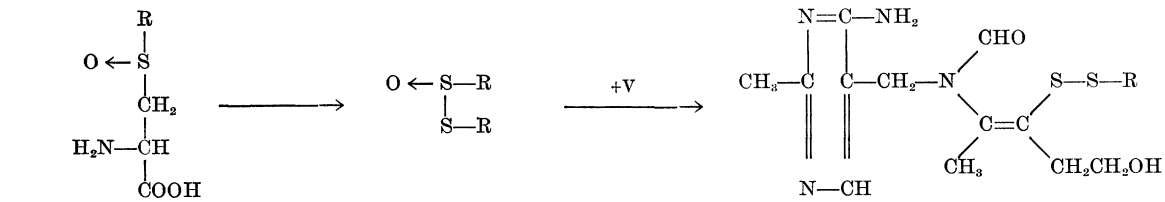
It is interesting to find from the above results that the garlic and *A. victorialis* in reacting with thiamine produce, besides allithiamine(I), considerable amounts of its methyl homolog (II) and a small quantity of propyl homolog (III), whereas *A. tuberosum, A. bakeri, A. grayi, A. thunbergii, A. togasii,* and *A. rosenbachianum* produce more (II) than (I).

TABLE 1

Plants	Detection of R-S-S-cysteine in ether extracts			Corresponding allithiamine homologs
A. sativum L. var. *japonicum* Kitamura (garlic)	VI,***	VII,	(VIII)	I,[a] II, III
A. sativum L. (garlic)	VI,**	VII,*		I, II,
A. tuberosum Rottl. (*A. odorum* L.)	VI,*	VII,**		I,[a] II,[a]
A. victorialis L. var. *platyphyllum* Hultén	VI,**	VII,**	(VIII)	I,[a] II,[a] III
A. bakeri Regel	(VI),	VII,**	(VIII)	I, II,[a] III
A. grayi Regel	(VI),	VII,**	(VIII)	I, II, III
A. thunbergii Don	VI,	VII,*	(VIII)	I, II, III
A. togasii Hara (*6*)	(VI),	VII,**	(VIII)	I, II, III
A. rosenbachianum		VII**		

Note. Parentheses ( ) imply weak detection and no mark; *, **, and *** indicate that the degree of the detection increases in this order.

[a] The formation of the allithiamine and its methyl homolog has been proved by obtaining them in crystals.



IX R = —CH_2—CH=CH_2
X R = —CH_3
XI R = —CH_2—CH_2—CH_3

XII R = —CH_2—CH=CH_2

I R = —CH_2—CH=CH_2
II R = —CH_3
III R = —CH_2—CH_2—CH_3

The present authors have already established that allithiamine (I) is formed by the reaction between the thiol form thiamine(V) and allicin(XII) (*7*), a secondary ingredient of the garlic produced by the enzymatic decomposition of alliin (*8*).

On the other hand, it was found that, when the plants other than the garlic given in Table 1 were treated beforehand with boiling water to inactivate the enzyme, they lost the ability to form allithiamine or its homologs. This fact shows that in the reactions of thiamine and these plants, the secondary ingredients produced by the enzymatic decomposition of the original ingredients take part in the reactions.

From these facts it is presumed that the plants which produce (II) and (III) would then contain new alliin-like ingredients having methyl(X) and propyl(XI) groups respectively, instead of the allyl group of alliin(IX) and that the methyl homolog (X) exists in the plants of *Allium* genus more extensively than alliin(IX).

References

1. Fujiwara, M., and Watanabe, H. *Proc. Jap. Acad.*, **28**, 156 (1952).
2. Matsukawa, T., and Yurugi, S. *Ibid.*, **28**, 146 (1952); *J. Pharm. Soc. Japan*, **72**, 1602, 1616 (1952).
3. Cavallito, C. J., Buck, J. S., and Suter, C. M. *J. Am. Chem. Soc.*, **66**, 1952 (1944).
4. Chargaff, E., Levine, C., and Green, C. *J. Biol. Chem.*, **175**, 67 (1948).
5. Raghunandana Rao, R. *Chem. Abstr.*, **41**, 2461 (1947).
6. Hara, K. *J. Japan. Bota.*, **28**, 62 (1953).
7. Cavallito, C. J., and Bailey, J. H. *J. Am. Chem. Soc.*, **66**, 1950 (1944).
8. Stoll, A., and Seebeck, E. *Helv. Chim. Acta*, **31**, 189 (1948); **32**, 197, 866 (1949).

Manuscript received April 1, 1953.

The Iron(III)-Phenol Complex in Aqueous Solution

Therald Moeller and Richard W. Shellman

Noyes Chemical Laboratory, University of Illinois, Urbana

The nature of purple complex produced by the reaction of phenol with iron(III) chloride in aqueous solution still remains in some question in spite of the many studies made upon the system. The composition $[Fe(OC_6H_5)_3]$ was perhaps first suggested by Raschig's early observations (*1*), using a substituted phenol, and was apparently confirmed by the colorimetric studies of Claasz (*2*). On the other hand, reaction in a 4:1 mole ratio to give an acidic species, $H[Fe(OC_6H_5)_4]$, was indicated by the work of Weinland and Binder (*3*). In a comprehensive study of a large number of systems derived from various phenols and iron(III) chloride, Wesp and Brode (*4*) obtained a single broad band in the absorption spectrum of each system, the position of the band depending upon the phenol used. With phenol itself, this absorption band centered at 558 mμ, the intensity of the absorption at this wavelength being increased by either added iron(III) or phenol, and obeying Beer's law in moderate concentrations. Wesp and Brode reported further that the colored species is destroyed by either acid or alkali and concluded on the basis of ion migration behavior that the composition of the species is $[Fe(OC_6H_5)_6]^{-3}$. Babko (*5*), on the other hand, suggested the presence of the cation $[Fe(OC_6H_5)]^{+2}$ and found its complete formation to be impossible.

On the basis of thermometric and conductimetric titration data, Banerjee and Haldar (*6*) reported reaction of phenol with iron(III) chloride in both 3:1 and 6:1 mole ratios. Inasmuch as Job's method of continuous variations (*7, 8*) showed reaction in only the 3:1 ratio, no partition between aqueous and organic phases could be detected, and ion migration indicated the presence of anionic iron, it was proposed that the colored species has the composition $Fe[Fe(OC_6H_5)_6]$. More recently, however, it has been shown that *m*-cresol gives a neutral 3:1 species with iron (III) and no anionic $[Fe(OAr)_6]^{-3}$ species (*9*). On the other hand, spectrophotometric data have also indicated formation of a number of species of the type $[Fe(OAr)]^{+2}$ with phenols and naphthols (*10*), although the phenol complex itself was not studied because of its instability.

Lack of consistency among these reports prompted a further study of the phenol-iron(III) chloride system. In aqueous solutions, the color is destroyed by heat and by alkalies, precipitation of hydrous iron(III) oxide occurring in both instances. The color is stable in the pH range 1.7–3.0 but is destroyed by additional acid. Best results in producing color systems of reasonable stability were obtained at pH 2.2. Even at optimum pH, the color intensity first increases for some 2 hr and then decreases with time until after some 12 hr or more a purple solid is precipitated. Dialysis also yields a purple flocculate. Microanalyses