and recrystallized from 50% acetic acid. The product, 3.2 g. (20%) melted at 165.5–167°. Prepared by another procedure[16] this product has been reported as melting at 167°.

*Anal.* Calcd. for $C_{20}H_{22}O_3$: C, 77.21; H, 7.14. Found: C, 76.92; H, 7.14.

**Diethylstilbene.**—A mixture of 150 g. of 3,4-diphenylhexanol-3[8] and 50 g. of fused potassium acid sulfate was heated at 180–200° for one-half hour. The product was extracted with ether, and the ethereal extracts concentrated. Distillation of the residue gave 120 g. of clear colorless fairly viscous oil, b. p. 128–131° at 0.8 mm. The oil was diluted with two volumes of methanol and placed in the ice-box overnight. The crystalline precipitate after two recrystallizations from methanol melted at 57–58°.

(16) Jaeger and Robinson, *J. Chem. Soc.*, 744 (1941).

*Anal.* Calcd. for $C_{18}H_{20}$: C, 91.49; H, 8.51. Found: C, 90.96; H, 8.32.

We wish to thank Mr. A. Kozlowski for technical assistance and Mr. S. Gottlieb for the microanalyses reported here.

## Summary

1. The preparation of several variants of diethylstilbestrol in which one or more of the phenolic hydroxyl groups have been replaced by $-NH_2$, —Br, —COOH and $-OCH_3$ groups has been described.

2. The estrogenic and bacteriostatic activities of these compounds has been determined.

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

# Allicin, the Antibacterial Principle of *Allium sativum*. I. Isolation, Physical Properties and Antibacterial Action

BY CHESTER J. CAVALLITO AND JOHN HAYS BAILEY

While investigating plant extracts for antibacterial activity, it was observed that a freshly prepared infusion of ground garlic cloves possessed high antibacterial activity when tested by the cylinder-plate method[1] used for the assay of penicillin.

A literature investigation showed that *Allium sativum*, the common garlic, has been endowed with therapeutic virtues both in legend and in the scientific literature. Most of the claims have been poorly substantiated. Several investigators have observed antibacterial activity of garlic extracts and have attributed this activity to diallyl sulfide,[2,3] unstable sulfur in alkyl polysulfides,[4] a bacteriophage,[5] acrolein or some similar unsaturated aldehyde,[6,7] and recently to a chemically undefined group of substances designated as phytoncides.[8]

A preparation of oil of garlic obtained by steam distillation of the crushed cloves was subjected to fractional distillation and the fractions separated as described by Semmler.[9] When tested by the cylinder-plate method, the natural diallyl disulfide and diallyl polysulfides showed practically no antibacterial action. Synthetic diallyl sulfide was equally ineffective. Aqueous solutions of acrolein and crotonaldehyde showed a diffuse reaction and no sharply defined zones of inhibition. The garlic antibacterial, hereinafter called allicin, showed a sharp zone of inhibition with the periphery accentuated by a line of heavy growth. The phytoncides as described[8] appear to be more volatile and less stable than allicin, which has been obtained thus far only from species of garlic.[10]

It was found convenient during this investigation to use the penicillin[1] assay method and penicillin as a reference standard for the assay of crude garlic preparations.

Having shown allicin to differ from previously claimed garlic antibacterials, tests were conducted on the stability of infusions of garlic. These solutions showed rapid loss of activity when heated, considerable stability when refrigerated and immediate inactivation upon addition of alkalies. Dilute acids had no effect.

The antibacterial agent, allicin, has been isolated in the pure state as a colorless liquid.[11]

The compound contains approximately 40% sulfur, and no nitrogen or halogens. The oil cannot be dry distilled without decomposition. It is soluble in water to the extent of approximately 2.5% at 10°, is miscible with alcohol, benzene and ether, and fairly insoluble in the Skellysolves. It has a $d^{20}$ of 1.112; $n^{20}D$ 1.561, and is optically inactive. The pure product is irritating to the skin and the odor is much more characteristically that of garlic than is that of the various allyl sulfides.

Aqueous solutions of allicin have a *p*H of approximately 6.5 and, upon standing, an oily precipitate forms; the acidity slowly increases from formation of small quantities of sulfur dioxide and

(1) Abraham, Chain, *et al.*, *Lancet*, [2] **241**, 177 (1941).
(2) Uemori, *Chem. Abst.*, **24**, 2191 (1930).
(3) Dittmar, *Z. Krebsforsch.*, **49**, 515 (1939).
(4) Kitagawa and Amano, *Chem. Abst.*, **30**, 3019 (1936).
(5) L. M. Jacobson, *ibid.*, **31**, 6689 (1937).
(6) Vollrath, Walton and Lindegren, *Proc. Soc. Exptl. Biol. Med.*, **36**, 55 (1937).
(7) Carl, McKnight, Scott and Lindegren, *Am. J. Hyg.*, **29**, 32 (1939).
(8) B. Tokin, *Amer. Review Soviet Med.*, **1**, 237 (1944).
(9) Semmler, *Archiv. der Pharmazie*, **230**, 434 (1893).

(10) *Allium vineale* also showed antibacterial activity. Osborn, *Brit. J. Exp. Path.*, **24**, 227 (1943), also reports antibacterial activity from *Allium ursinum* and *Allium triquetrum*. We had tested approximately two hundred species of plants up to the time of appearance of the article by Osborn.

(11) The chemistry of allicin is to be discussed in subsequent papers.

the activity of the solution decreases. Addition of alkalies leads to immediate inactivation with precipitation of allyl disulfide and formation of an alkali sulfite.

The action of allicin is considerably more bacteriostatic than bactericidal. It is about equally effective against Gram positive and Gram negative organisms. By the cylinder-plate method against *Staphylococcus aureus*, allicin shows an activity equivalent to about 15 Oxford penicillin units per milligram, which is about 1% of the activity of penicillin; however, allicin is equally effective against the Gram negative organisms which are practically unaffected by penicillin. The antibacterial activity is unaffected by the presence of *p*-aminobenzoic acid. The action of allicin on a number of organisms is shown in Table I.

TABLE I

Presence of growth, —: absence, 0.

| Organism | Dilution in broth[a] 1:85,000 | 1:125,000 | 1:250,000 |
|---|---|---|---|
| *Staphylococcus aureus* | 0 | 0 | — |
| *Streptococcus hemolyticus* | 0 | — | — |
| *Streptococcus viridans* | 0 | 0 | — |
| *B. subtilis* | 0 | 0 | — |
| *B. typhosus* | 0 | 0 | — |
| *B. paratyphosus A* | 0 | 0 | — |
| *B. paratyphosus B* | 0 | 0 | — |
| *B. paratyphosus Kunzendorf* | 0 | 0 | — |
| *B. morgani* | 0 | 0 | — |
| *B. enteriditis* | 0 | 0 | — |
| *B. typhi-murium* | 0 | 0 | — |
| *B. dysenteriae Shiga* | 0 | 0 | — |
| *B. dysenteriae Flexner* | 0 | 0 | — |
| *B. dysenteriae Sonne* | 0 | 0 | — |
| *V. cholerae* | 0 | 0 | — |

[a] Meat extract both, *p*H 6.8, except for the Streptococci in which a 0.5% dextrose–veal infusion broth was employed.

Under the conditions of the tests in Table I, sulfaguanidine at 1:5000 did not inhibit growth of *B. typhosus* or of *S. aureus*. Penicillin did not inhibit *B. typhosus* at 1:5000.

Toxicity tests in mice indicate the $LD_{50}$ for allicin in aqueous solutions to be of the order of 60 mg. per kg. intravenously and 120 mg. per kg. by subcutaneous administration.

## Experimental

**Isolation of Allicin.**—To four kg. of ground garlic cloves was added five liters of 95% ethanol and the mixture stirred for thirty minutes. The mixture was then suction filtered, yielding about 5200 cc. of solution containing from 2.5 to 4 mg. per cc. of allicin on the basis of antibacterial activity. This extract was concentrated under reduced pressure (15–20 mm.) until most of the alcohol had been removed. The alcoholic distillate was discarded. The distillation was continued at 10–15 mm. pressure, collecting the aqueous distillate while maintaining the volume in the distillation flask at about 500 cc. by addition of water from a dropping funnel. The distillation was continued until the residual solution contained less than 10 units per cc. of active principle. About nine liters of aqueous distillate was collected.

Three-liter portions of the aqueous distillate containing the active principle were extracted once with 500 cc. of ethyl ether, then four times with 300-cc. portions of ether. The ether extracts were combined and the ether removed under reduced pressure. The residue consisted of some water and an oil. This residue was shaken thoroughly with about 250 cc. of water and 10 cc. of Skellysolve B. After separation and filtration, the aqueous solution was frozen and stored in Dry Ice until desired for further work.

The pure product was isolated by extracting the aqueous concentrate four times, each with one-fifth the volume of ether. The combined ether extract was cooled in a Dry Ice-bath and the ice crystals which separated were filtered off. The ether was removed under reduced pressure. The residual oil was dried by exposing to a vacuum of 0.5 mm. or less for thirty minutes at room temperature. The product thus obtained had a reproducible index of refraction, $n^{20}D$ 1.561. Approximately six grams of the oil was obtained from 4 kg. of garlic.

**Chemical Properties.**—Chemical analysis of the active principle showed C, 44.12–44.59; H, 6.30–6.34; S, 39.69–40.90.

Alkaline hydrolysis yielded one mole of sulfur dioxide from 400 to 450 mg. of allicin. This was determined by addition of an excess of standard alkali to a known concentration of the antibacterial agent in water and back titration with standard acid to *p*H 7.0. The sulfur dioxide was confirmed by acidification of the hydrolysis mixture with phosphoric acid, distillation in a stream of nitrogen and collection of the gas in standard alkali, then titration with standard acid to *p*H 7. All of the sulfur dioxide was thus recovered. An acidified aliquot of this solution gave the expected titration value with standard permanganate. Another aliquot was oxidized and then precipitated by the addition of barium chloride solution. The expected quantity of barium sulfate was obtained.

The insoluble oil formed from alkaline treatment of the allicin was extracted with ether, dried over calcium chloride, the ether removed, and the product distilled: b. p. at 0.5 mm., 53°; 16 mm., 78–80°. The latter is in agreement with the boiling point of allyl disulfide.[9] The refractive index $n^{20}D$ is 1.541.

*Anal.* Calcd. for $C_6H_{10}S_2$: C, 49.31; H, 6.85; S, 43.84. Found: C, 48.96; H, 6.87; S, 43.92.

**Antibacterial Tests.**—Sterile broth was pipetted aseptically into sterile tubes in amounts to give a final volume of 5 cc. when mixed with 1 cc. of a 1:1000 dilution of an eighteen-hour broth culture of the test organism and the required amount of allicin solution to give the dilutions described in Table I. All dilutions of the antibacterial agent were made in broth. Growth was observed after eighteen hours of incubation. The end-point for inhibition was very sharp, the effective concentrations showing no growth. The next tube in the series showed good growth. A concentration of 1:50,000 of allicin, which allowed no growth, was tested for stasis by removing 0.1 cc. from the test and adding to 5 cc. of fresh broth. All organisms grew.

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## Summary

A new type of antibacterial has been isolated from the cloves of *Allium sativum*. The product, which has been named allicin, is a colorless oil, approximately 2.5% soluble in water, and relatively unstable. The antibacterial action has been demonstrated against both Gram positive and Gram negative bacteria.

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