

latum gauze announced a year ago is now implanted in production. Intermediates for sulfa drugs and antimalarials have been synthesized and put at the disposal of medicinal research organizations. Pentanedione is available to the pharmaceutical industry. Special amines have been made in a pilot plant for use in the synthesis of a new anesthetic. A novel process for preparing ethyl-phenyl malonic ester has been worked out. The synthesis of intermediates for theophylline production has been improved and the synthesis of papaverine is undergoing research along new lines.

The range-finding test is offered as a discerning solution to the problem of probing the toxic hazards of new chemical compounds before their uses justify large expenditures upon detailed studies. Coal-tar derivatives of promise as disinfectants have been appraised and ways of using them are under study. A new insecticide mentioned last year is in small-scale production. The synthesis of insect repellents has been supplemented and progress has been accomplished in the search for satisfactory means of utilizing existing repellents by combat forces. The repelling property of "Hubbellite" for specific insects is another project.

What Is Being Done in the Maintenance of Manpower. Industrial Hygiene Foundation, which has its headquarters at Mellon Institute, has added to the

forward health movement through its numerous services and activities for the maintenance of manpower among its member companies and the industrial fields in general. Most important have been plant hygiene investigations to appraise working conditions for their possible effects upon health, and to provide preventive engineering measures to control noxious and obnoxious exposures in workplaces. Sixty of these surveys were conducted during the year, mainly in the East and Middle West, and the projects ranged from several days to over a month in duration.

Numerous studies have been made of soldering operations. Several chemical dermatitis problems have been overcome through the coordination of field investigations with experimental studies by ten dermatologists. In other research electron photomicrographs of air-borne asbestos dust have disclosed that the particles are preponderantly fibrous and that the ultimate fiber is about 0.01 micron in diameter. In addition to its plant hygiene surveys and research, the foundation, with the collaboration of the U. S. Public Health Service, has carried on a program, started in 1940, to help reduce sick absences in the industries. Systematic course of action in industrial hygiene demands concrete knowledge not only of the amount of sick absenteeism, but also of when, where and why it occurs.

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SPECIAL ARTICLES

CRYSTALLIZATION OF SOUTHERN BEAN MOSAIC VIRUS

SINCE the first chemical isolation of tobacco mosaic virus in 1935,¹ a fairly large number of plant^{2,3} and animal^{4,5,6} viruses and certain bacteriophages⁷ have been obtained in more or less pure form. In spite, however, of widespread success of the purification procedures, only three viruses, tobacco mosaic,¹ tomato bushy stunt⁸ and tobacco necrosis⁹ viruses, have been

¹ W. M. Stanley, *SCIENCE*, 81: 644, 1935; *Phytopath.*, 26: 305, 1936.

² W. M. Stanley and R. W. G. Wyckoff, *SCIENCE*, 85: 181, 1937; W. M. Stanley, *Jour. Biol. Chem.*, 129: 405, 1939.

³ H. S. Loring and R. W. G. Wyckoff, *Jour. Biol. Chem.*, 121: 225, 1937; F. C. Bawden and N. W. Pirie, *Brit. Jour. Exp. Path.*, 19: 66, 1938; H. S. Loring, *Jour. Biol. Chem.*, 126: 455, 1938.

⁴ J. W. Beard and R. W. G. Wyckoff, *SCIENCE*, 85: 201, 1937.

⁵ J. W. Beard, H. Finkelstein and R. W. G. Wyckoff, *SCIENCE*, 86: 331, 1937.

⁶ R. W. G. Wyckoff, *Proc. Soc. Exp. Biol. and Med.*, 36: 771, 1937.

⁷ H. Bechold and M. Schlesinger, *Biochem. Zeitschr.*, 236: 388, 1931; J. H. Northrop, *Jour. Gen. Physiol.*, 21: 335, 1938.

⁸ F. C. Bawden and N. W. Pirie, *Nature*, 141: 513, 1938; *Brit. Jour. Exp. Path.*, 19: 251, 1938.

crystallized. Considerable interest is therefore attached to the isolation of a fourth virus, southern bean mosaic virus, in crystalline form. This virus, which was first described and studied by Zaumeyer and Harter,¹⁰ is sufficiently stable that it may be purified and concentrated by the methods commonly employed for the purification of proteins. Perhaps it is of significance that some of the crystalline forms, the shape of the particles and the thermostability of southern bean mosaic, tomato bushy stunt and tobacco necrosis viruses are similar. On the other hand, their host ranges and cross protection tests indicate that they are not closely related.

Purified preparations of southern bean mosaic virus were obtained by fractionating the juice from infected Bountiful bean plants. The fractionation was carried out either by alternating cycles of high- and low-speed centrifugation or by chemical treatment. The chemi-

⁹ N. W. Pirie, K. M. Smith, E. T. C. Spooner and W. D. McClement, *Parasitology*, 30: 543, 1938; F. C. Bawden and N. W. Pirie, *Brit. Jour. Exp. Path.*, 23: 314, 1942.

¹⁰ W. J. Zaumeyer and L. L. Harter, *Phytopath.*, 32: 438, 1942; *Jour. Agr. Res.*, 67: 305, 1943.

cal fractionation consisted of concentration by precipitation with $(\text{NH}_4)_2\text{SO}_4$ and then resolution in water, removal of certain impurities by treatment with 30 per cent. alcohol in which the virus remained soluble, and then removal of the virus by a second precipitation with $(\text{NH}_4)_2\text{SO}_4$. Further purification was sometimes accomplished by precipitation with MgSO_4 .

Crystallization was carried out in the following manner. Highly purified preparations containing about 15 or 20 mg/cc of the virus were brought to about 20 per cent. saturation with respect to $(\text{NH}_4)_2\text{SO}_4$ or MgSO_4 by addition of the solid salt and centrifuged for three hours in a Bauer and Pickels type¹¹ high-speed centrifuge at 24,000 r.p.m. The supernatant, which contained practically no virus activity, was poured off and discarded. To the clear, glassy pellets in the bottom of the centrifuge tubes was added one or two drops of distilled water and the tubes were then held overnight at about 3° C. As the pellets absorbed water they slowly became opaque and on examination were found to contain masses of crystals. The crystals were of one of two types, the type obtained depending upon conditions not yet fully known. Careful examination under the microscope suggests that both types belong in the orthorhombic system, yet this conclusion is only tentative since accurate measurements of the interfacial angles have not been made. They may be referred to as a rhombic prism (Fig. 1) and as a rhombic bipyramid combined with two pinacoids (Fig. 2).

The suspension containing the crystals was removed and more water was added, two drops at a time, until all the virus in the tubes was removed. In this way there was obtained a mass of crystalline material, which settled out on standing. The mother liquor was pipetted off. The crystalline fraction, which still contained a small amount of mother liquor, was suspended in 0.5 saturated MgSO_4 or $(\text{NH}_4)_2\text{SO}_4$. On addition of the salt solution, the small amount of virus that had remained in solution precipitated as an amorphous fraction. On dilution with an equal volume of water the amorphous material rapidly dissolved and the crystals were then removed by slow-speed centrifugation. Several washings in this manner served to remove all traces of the amorphous material. The washings were done rapidly in order to prevent the crystals from dissolving, since they are readily soluble in water or dilute salt solutions.

Crystals that were washed free of non-crystallized virus and dissolved in H_2O to the extent of about 1 mg/cc gave a positive biuret and xanthoproteic test and a negative test with Molisch's and Fehling's solu-

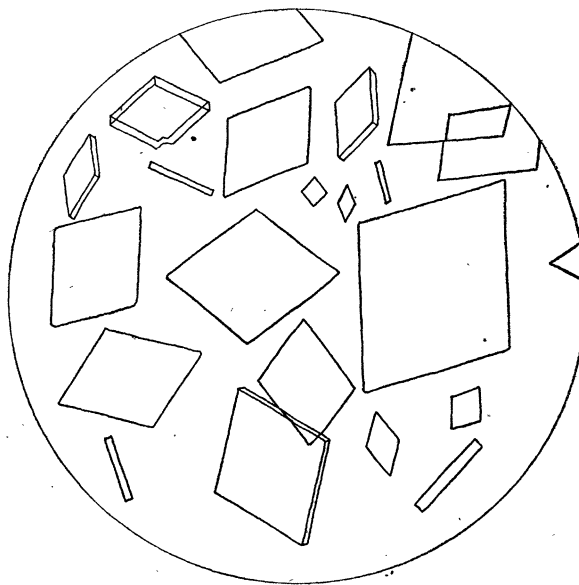


Fig. 1.

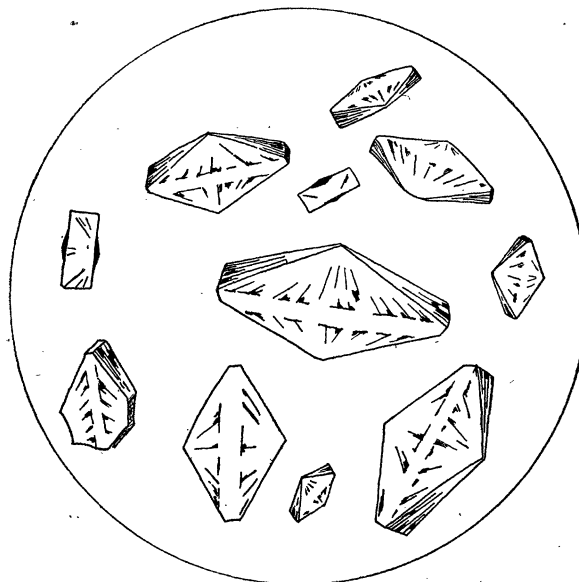


Fig. 2.

FIGS. 1 and 2. Diagrammatic representation of crystals of southern bean mosaic virus. Fig. 1 shows the rhombic prisms magnified about 500 times. Fig. 2 represents the rhombic bipyramids at a magnification of approximately 750. Solutions of both types of crystals were infectious at a concentration of about 10^{-8} gm/cc.

tions. With Millon's reagent such solutions formed a precipitate that failed to turn red on heating. The glyoxylic reaction was negative in dilute solutions, but concentrated solutions of the virus gave a positive reaction very much weaker than that of egg albumin.

Solutions of the crystalline material produced characteristic symptoms of southern bean mosaic when used to inoculate Bountiful beans. On Early Golden

¹¹ J. H. Bauer and E. G. Pickels, *Jour. Exp. Med.*, 64: 503, 1936.

Cluster beans they produced lesions at concentrations as low as 10^{-8} gm/cc. Table 1 shows the results of

TABLE 1

ACTIVITY OF THE BIPYRAMIDAL CRYSTALS (A), THEIR
MOTHER LIQUOR (B), THE RHOMBIC PRISMS (C) AND
THEIR MOTHER LIQUOR (D) AT CONCENTRATIONS
BETWEEN 10^{-4} AND 10^{-8} GMS/CC AS INDICATED
BY THE NUMBER OF LESIONS PRODUCED
ON 24 LEAVES OF EARLY GOLDEN
CLUSTER BEANS

Log. Concentration	Lesions			
	A	B	C	D
-4	4060	2584	3350	2565
-5	1362	810	1098	694
-6	148	231	238	67
-7	33	31	14	6
-8	27	10	11	2

one test of the comparative infectivities of the rhombic prisms and bipyramidal crystals and the mother liquors from each of these. In the preparations used for the test much of the virus had remained in the mother liquors, only from 10 to 30 per cent. of the total having been obtained as crystals. The data show no significant differences between the activities of the crystals and their mother liquors. All four preparations were highly active.

Purified virus preparations appeared to be essentially homogeneous when studied in a high-speed centrifuge, an electrophoresis apparatus and a diffusion apparatus.¹² Concentrated solutions did not show stream double refraction when allowed to flow under crossed polaroid plates. This indicates that the virus particles are not elongated rods or plates, a conclusion which is borne out by calculation of the shape of the particles from sedimentation and diffusion data. In the presence of MgSO_4 or $(\text{NH}_4)_2\text{SO}_4$, the virus is considerably more soluble at low than at high temperatures. Virus completely precipitated as an amorphous fraction with a minimum amount of salt at room temperature goes into solution in about an hour at 3° C. In this respect it is similar to tomato bushy stunt⁸ and tobacco necrosis⁹ viruses.

The chemical tests that have been made indicate that the crystalline material is mostly protein. The homogeneity of the material indicates it to be relatively free from impurities. That the material is not a normal constituent of bean plants is shown by the failure on several occasions to isolate any such material by subjecting the juice of healthy bean plants to the treatments described above. The fact that thoroughly washed crystals are infectious when diluted to 10^{-8} gm/cc indicates that they represent the virus itself and not some by-product of virus activity.

The results therefore show that southern bean mosaic virus consists of essentially spherical particles small enough and uniform enough to crystallize under appropriate conditions. The fact that the virus has been crystallized is not considered sufficient evidence

¹² Data to be published.

on which to decide whether it is animate or inanimate in nature.

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INACTIVATION OF THE IRRITANT TOXICANTS OF POISON IVY AND RELATED COMPOUNDS BY TYROSINASE

WORK of the past years, chiefly by Majima,¹ Hill *et al.*,² and Mason and Schwartz,³ has resulted in the isolation, identification and synthesis of certain of the skin irritants of poison ivy, poison oak and related plants of the *Anacardiaceae*. These compounds have been shown to be phenols or catechols characterized by a long unsaturated side chain attached to the ring (see Fig. 1). Since the toxic properties of the molecule are due in part to the presence of OH groups

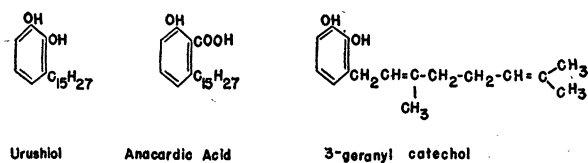


FIG. 1

in the ring,⁴ one of the better methods of treatment of skin poison ivy involves oxidation of such groups with strong oxidants such as ferric chloride and potassium permanganate. It seemed possible, however, that the same results might be obtained using innocuous agents such as enzymes. Since the phenol oxidases are not highly specific⁵ (attacking such different compounds as mono- and polyphenols, tyrosine and even certain of the sex hormones⁶), it seemed possible that this type of enzyme might also oxidize phenolic groups of the toxic poison ivy irritant as well as related compounds.

In this study were used a large number of partially purified and concentrated commercial poison ivy extracts as well as pure compounds of structure and toxic properties known to be related to the active principle (urushiol) of poison ivy. The enzyme solutions employed were very active and highly purified mushroom tyrosinase.⁷ The reaction was studied by measuring oxygen consumption in the Barcroft res-

¹ R. Majima, *Ber.*, 55 B: 172, 1922.

² G. A. Hill, V. Mattacotti and W. D. Graham, *Jour. Am. Chem. Soc.*, 56: 2736, 1934.

³ H. S. Mason and L. Schwartz, *Jour. Am. Chem. Soc.*, 64: 3058, 1942.

⁴ (a) I. Toyama, *Jour. Cut. Dis.*, 36: 157, 1918. (b) H. Keil, D. Wasserman and C. R. Dawson, *Jour. Exp. Med.*, 80: 275, 1944.

⁵ J. M. Nelson and C. R. Dawson, *Adv. in Enz.*, 4: 99, 1944.

⁶ S. Ansbacher, "Vitamins and Hormones," 2: 215, 1944.

⁷ We are greatly indebted to Dr. J. F. Nelson of Columbia University for the tyrosinase preparations.