

circles of equal diameters represent their original positions. The area between the circles will reach a maximum when the circles are rearranged, as in d; on further compression the area will start to decrease, shown in e, returning finally to its original value, as in f, equal to that of b.

To verify the above explanation an experimental set-up was made with a 100 cc rubber bulb between the parallel jaws of a milling machine vise. The height of the water level was measured by means of a meter stick arranged parallel to the vertical tubing. The tube was calibrated by measuring the volume of water that filled a certain length of the tube. The distance *d*, between the jaws, was determined from the pitch of the screw. The data are presented in form of three curves in Fig. 2. Curve 1 was obtained for a bulb filled with water only. Curve 2 shows how the water level changes with the distance *d*, when the bulb is tightly packed with the sand. In Curve 3, the bulb was filled with small glass spheres, ranging from .0215" to .0224" in diameter. These small spheres are produced by atomizing molten glass and are

solidified in the air. Surface tension is responsible for their perfect sphericity. They are used for decorative purposes under the name of "Crystalline" or "Glascherben." These curves definitely show the minimum volume for water and sand or glass spheres and an increase in volume on further decrease in *d*. As was expected, for the same change in *d*, the minimum is more pronounced with the glass spheres. Because of larger frictional forces between the irregular grains of sand the interspacial volume for sand increases more rapidly and reaches larger value than in the case of glass spheres. The limit of compression was set by the strength of the rubber bulb.

The phenomenon described here is a familiar experience to those at the seashore who have noticed how rapidly the water "dries up" around foot prints when walking on the wet sand.

Any of the readers who can give the reference to any publications concerning the described effect are kindly asked to communicate with the author.

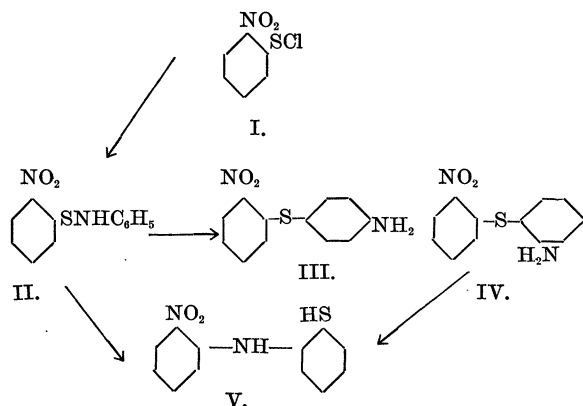
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SPECIAL ARTICLES

MOLECULAR REARRANGEMENTS OF SULFANILIDES

DURING recent work in this laboratory on the development of improved methods for the synthesis of new sulphide phenol compounds possessing high antiseptic and germicidal power, it was discovered that certain sulphur compounds of the sulfanilide type are capable of undergoing profound molecular changes. Two types of transformations have thus far been revealed in the course of our researches, which may be illustrated by the following example: The starting point serving for our illustration is *o*-nitrophenylsul-



furchloride I, which is easily prepared by the action of chlorine on *o*-nitrophenyldisulphide. This sulfurchloride I interacts smoothly with aniline to form in

good yield *o*-nitrophenylsulfanilide II. We find that this latter compound II undergoes two types of molecular change, depending upon the experimental conditions employed. (1) If the sulfanilide II is heated at a definite temperature, or if it is digested with its respective amine (aniline) at its boiling point, it is transformed into its isomeric 2-nitro-4'-aminodiphenylsulfide III. In some cases we have also observed a corresponding *ortho* rearrangement IV. (2) On the other hand, when the sulfanilide II is warmed in alcohol in the presence of sodium hydroxide it undergoes an entirely different type of change and is transformed smoothly into a mercapto-diphenylamine derivative corresponding to formula V. Regarding the mechanism of this last change we are not prepared to offer a decisive explanation. The simplest postulation, that we are dealing here with the intermediate formation of an *ortho*-aminosulphide (formula IV), which then rearranges to the isomeric diphenylamine V, does not satisfy as the correct explanation. The English chemist, Dr. Smiles, and his co-workers report¹ that *o*-aminosulphides of this type, studied by them, are unattacked by alcoholic sodium hydroxide, while the corresponding acetyl- and benzoyl-derivatives, for example, rearrange easily to diphenylamine derivatives under the same experimental conditions. Two types of reaction products, therefore, are possible of

¹ See Evans and Smiles, *Jour. Chem. Soc. (London)*, p. 183, 1935.

formation by molecular rearrangement of a given sulfanilide. We are interested in determining the structural configurations limiting the practical application of this double molecular rearrangement.

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ISOLATION OF A CRYSTALLINE PROTEIN POSSESSING THE PROPERTIES OF TOBACCO-MOSAIC VIRUS

A CRYSTALLINE material, which has the properties of tobacco-mosaic virus, has been isolated from the juice of Turkish tobacco plants infected with this virus. The crystalline material contains 20 per cent. nitrogen and 1 per cent. ash, and a solution containing 1 milligram per cubic centimeter gives a positive test with Millon's biuret, xanthoproteic, glyoxylic acid and Folin's tyrosine reagents. The Molisch and Fehlings tests are negative, even with concentrated solutions. The material is precipitated by 0.4 saturated ammonium sulfate, by saturated magnesium sulfate, or by safranin, ethyl alcohol, acetone, trichloroacetic acid, tannic acid, phosphotungstic acid and lead acetate. The crystalline protein is practically insoluble in water and is soluble in dilute acid, alkali or salt solutions. Solutions containing from 0.1 per cent. to 2 per cent. of the protein are opalescent. They are fairly clear between pH 6 and 11 and between pH 1 and 4, and take on a dense whitish appearance between pH 4 and 6.

The infectivity, chemical composition and optical rotation of the crystalline protein were unchanged after 10 successive crystallizations. In a fractional crystallization experiment the activity of the first small portion of crystals to come out of solution was the same as the activity of the mother liquor. When solutions are made more alkaline than about pH 11.8 the opalescence disappears and they become clear. Such solutions are devoid of activity and it was shown by solubility tests that the protein had been denatured. The material is also denatured and its activity lost when solutions are made more acid than about pH 1. It is completely coagulated and the activity lost on heating to 94° C. Preliminary experiments, in which the amorphous form of the protein was partially digested with pepsin, or partially coagulated by heat, indicate that the loss in activity is about proportional to the loss of native protein. The molecular weight of the protein, as determined by two preliminary experiments on osmotic pressure and diffusion, is of the order of a few millions. That the molecule is quite large is also indicated by the fact that the protein is held back by collodion filters through which proteins such as egg

albumin readily pass. Collodion filters which fail to allow the protein to pass also fail to allow the active agent to pass. The material readily passes a Berkefeld "W" filter.

The crystals are over 100 times more active than the suspension made by grinding up diseased Turkish tobacco leaves, and about 1,000 times more active than the twice-frozen juice from diseased plants. One cubic centimeter of a 1 to 1,000,000,000 dilution of the crystals has usually proved infectious. The disease produced by this, as well as more concentrated solutions, has proved to be typical tobacco mosaic. Activity measurements were made by comparing the number of lesions produced on one half of the leaves of plants of Early Golden Cluster bean, *Nicotiana glutinosa* L., or *N. langsdorffii* Schrank after inoculation with dilutions of a solution of the crystals, with the number of lesions produced on the other halves of the same leaves after inoculation with dilutions of a virus preparation used for comparison.

The sera of animals injected with tobacco-mosaic virus give a precipitate when mixed with a solution of the crystals diluted as high as 1 part in 100,000. The sera of animals injected with juice from healthy tobacco plants give no precipitate when mixed with a solution of the crystals. Injection of solutions of the crystals into animals causes the production of a precipitin that is active for solutions of the crystals and juice of plants containing tobacco-mosaic virus but that is inactive for juice of normal plants.

The material herein described is quite different from the active crystalline material mentioned by Vinson and Petre¹ and by Barton-Wright and McBain,² which consisted, as Caldwell³ has demonstrated, largely of inorganic matter having no connection with the activity. These preparations were less active than ordinary juice from diseased plants, and the activity they possessed diminished on further crystallizations.

The crystalline protein described in this paper was prepared from the juice of Turkish tobacco plants infected with tobacco-mosaic virus. The juice was brought to 0.4 saturation with ammonium sulfate and the precipitated globulin fraction thus obtained was removed by filtration. The dark brown globulin portion was repeatedly fractionated with ammonium sulfate and then most of the remaining color was removed by precipitation with a small amount of lead subacetate at pH 8.7. An inactive protein fraction was removed from the light yellow colored filtrate by adjusting to pH 4.5 and adding 2 per cent. by weight of standard celite. The celite was removed, suspended in

¹ C. G. Vinson and A. W. Petre, *Contrib. Boyce Thompson Inst.*, 3: 131, 1931.

² E. Barton-Wright and A. McBain, *Nature*, 132: 1003, 1933.

³ J. Caldwell, *Nature*, 133: 177, 1934.