most toxic part of this plant thus far tested was the kernel of the mature seed. The Durham and red-color tests for rotenone were negative. The marked paralytic action of the ground dry seeds suggested the presence of pyrethrins or similar compounds and led to the isolation of the toxic fraction and studies of its character with the following results.

Similarity to pyrethrins was noted in the solubility of the toxic material in various organic liquids. Petroleum ether extractives could be handled like pyrethrins for the removal of fats, waxes and fatty acids in the manner described by LaForge and Haller,[2] with the final residue, corresponding to a concentrate of pyrethrins I and II, containing the toxic principle intact. Considerable insoluble matter was deposited when the resins were left in petroleum ether at room temperature. Hydrolysis of the purified resin by refluxing one and one-half hours with 0.5 *N* sodium hydroxide followed by acidification and extraction with petroleum ether, resulted in a non-toxic resin. Inasmuch as the original toxic fraction did not contain nitrogen and therefore its toxicity was not due to an alkaloid or an amide, the evidence indicated that the toxicity may be due to an ester whose component alcohol and acid were non-toxic. The powder also was found to give an analysis for pyrethrins I and II of 0.05 and 0.14%, respectively, by the AOAC method [3] which, however, is not a positive test for pyrethrins in material other than that from *Chrysanthemum cinerariaefolium* Vis.

A principal point of difference from pyrethrins was in the change of color that took place during analysis for pyrethrins; upon the addition of Denigè's reagent the mamey material remained yellow, while pyrethrins change color from white to pink, to red, to violet and finally to blue. The absence of methoxyl in the resins was shown by analysis. The preparation of the semicarbazone from the resins by the method of Staudinger and Harder as given by Gnadinger[4] yielded no crystallizable derivative. However, after purification of the resins by nitromethane extraction of the toxic principle from a petroleum ether solution, the solvent was displaced by methanol and treatment with semicarbazone hydrochloride and sodium acetate gave a non-crystalline, nitrogen-containing derivative that could not be washed out with water. Upon subjection of this fraction to methanolic alkaline hydrolysis, a precipitate formed which was soluble in water. Addition of acid to the solution caused turbidity which could be taken up in ether. This was, therefore, an acid and, titrated electrometrically, appeared to be monobasic. This further suggests that the toxic material was an ester.

Although some of the chemical behavior of the toxic fraction suggested pyrethrins, preliminary biological tests indicated that the toxicity could not be due to actual pyrethrin content. These tests, using methods previously outlined,[1] consisted of a comparison of an extract of mamey seed powder with that of pyrethrum flowers at several levels of concentration using two different test insects. Aliquots of both materials were mixed with an insecticidally inert marc to give powders after evaporation of the solvent. When the toxicities obtained were plotted against concentration, the use of probits and log concentration did not make the curves straight. Nevertheless, it could be seen that the curves diverged sharply as the concentration (and toxicity) increased when tested on larvae of *Diaphania hyalinata* (L.), but were practically parallel when adults of *Diabrotica bivittata* F. were used. Thus, with the former test insect the concentration, in terms of percentage of original resins restored, necessary to give 40% toxicity was 13.8 for mamey seed and 2.6 for pyrethrum, signifying that the latter was 5.3 times as toxic as the former; to obtain 60% toxicity required 33% restoration for mamey seed as against 4.4% restoration for pyrethrum, thus giving a toxicity ratio of pyrethrum to mamey seed of 7.5. Against *Diabrotica* the pyrethrum was about five times as toxic as mamey seed at all levels of toxicity. If the toxicity of mamey seed were due to pyrethrins, it would be half that of the pyrethrum preparation according to analysis, and the curves representing change of toxicity with concentration for both materials would be parallel regardless of the test insect.

At the present stage of our investigations, it may be said that the toxicity of mamey seed is not due to pyrethrins but may be due to a somewhat similar type of substance.

FEDERAL EXPERIMENT STATION
MAYAGUEZ, PUERTO RICO RECEIVED AUGUST 14, 1945

(2) F. B. LaForge and H. L. Haller, THIS JOURNAL, **57**, 1893–1896 (1935).

(3) Association of Official Agricultural Chemists, "Official and Tentative Methods of Analysis," 5th ed., Washington, D. C., 1940, pp. 66–67.

(4) H. Staudinger and H. Harder, *Ann. Acad. Sci. Fennicae*, **A29**, (18), 1–14 (1927). In C. B. Gnadinger, "Pyrethrum Flowers," 2nd ed., Minneapolis, 1936.

Note on a New Color Reaction of β-Chlorovinyldichloroarsine

BY HOWARD S. MASON

A project recently carried out in this Laboratory required a simple, rapid, and highly sensitive test for β-chlorovinyldichloroarsine. A test of this character has not been described in the current literature, but the findings of Baranger and Mercier[1] (who showed that in dry chloroform solution ergosterol and methyldichloroarsine produced a golden yellow color) provided the basis for an indicating reaction which met the above requirements.

It was observed that granular silica gel upon

(1) Baranger and Mercier, *Biochem. J.*, **36**, 703 (1942).

which ergosterol had been adsorbed gave an immediate violet coloration on contact with traces of β-chlorovinyldichloroarsine vapor, changing to a deep green in the presence of larger amounts. A similar but much fainter color change was produced by β,β'-dichlorodiethylsulfide only upon prolonged exposure of the adsorbate to air saturated with its vapor. Hydrochloric acid vapor caused the reagent to change to a deep rust brown color.

Experimental

To 15 cc. of purified chloroform was added 50 mg. of ergosterol (Montrose Chemical Company) and the temperature of the mixture raised to the boiling point for one minute. Dry, white silica gel, 80-mesh (Davison Chemical Corporation), 5 g., was added and the mixture allowed to stand for a minute; the solid was then separated from the supernatant liquid and spread out upon a filter paper to dry. As a result of this procedure the silica gel mixture became a pale steel gray; when heat was applied to facilitate the evaporation of the solvent a product varying in color from orange to green was obtained and in such cases the sensitivity of the test was greatly impaired. The dry material was loaded into glass tubes 4 mm. in diameter in columns about 1 cm. long, held in place with glass fiber plugs. Tubes so prepared were evacuated by means of an oil pump and sealed under vacuum. In use, the ends of the tubes were broken open and gas drawn through the impregnated gel with a rubber atomizer bulb, the valves of which had been reversed in such a manner as to provide suction instead of pressure.

A tube prepared and used in this manner indicated the presence of 10 micrograms of β-chlorovinyldichloroarsine in 150 cc. of gas by means of a stripe of readily visible color (violet to green) at the zone of first contact. Ammonia vapor converted this stripe to an orange band, while the unaffected part of the reagent turned straw yellow.

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Some Crystalline Forms of Amylose

BY RALPH W. KERR

Amylose has been reported to crystallize in various forms depending upon conditions, particularly the type of starch from which the amylose fraction is prepared and the solvent medium from which the fraction crystallizes. Schoch[1] noted that when an autoclaved starch solution is treated with an excess of butyl alcohol, a slow precipitation of amylose in the form of minute, semi-crystalline spheroids occurs at the alcohol–water interface. Those from corn starch[2] are described as six segmented spherulites about 15–50 microns in diameter and those from potato as well-formed, six-petalled rosets, 50-80 microns in diameter or occasionally clumps of hair-like needles. Kerr and Severson[3] crystallized that portion of amylose which is leached from starch by warm water, using butyl alcohol–water as the crystallizing medium and obtained rectangular platelets from corn and needles which formed star-like clusters from potato starch. Subsequently, needles in star-like clusters were obtained from tapioca starch by a similar procedure.[4] Wiegel[5] refluxed potato starch with 30% ethanol and obtained needle-like particles which on closer observation appeared to be very thin platelets. When the ethanol was evaporated from the extract and butyl alcohol added to the hot solution, the crystals which separated assumed an hour-glass shape. Aqueous isobutyl alcohol gave typical sphero-crystals.

Kerr and Severson believe that all amyloses are chemically heterogeneous and that the material obtained in a warm water extract of whole starch represents a subfraction of the particular amylose, which is possibly representative of the less branched and lowest degree of polymerization material in the total fraction. Thus, a warm water extract of corn starch contains a predominant proportion of relatively short unbranched amylose chains and these crystallize eventually, after careful purification, as well-formed rectangular platelets. Corresponding subfractions from potato and tapioca crystallize as needles but it is suggested by Kerr[4] that even these may be slightly branched and of greater molecular weight.

It becomes desirable to know whether amylose crystallizes from butyl alcohol–water mixtures in shapes which are largely determined by minor variables of the crystallization technique or whether an amylose, such as corn amylose, can be subfractionated so as to obtain crystal shapes which are inherently related to the predominating structure in these subfractions and its orientation with butyl alcohol. This report proposes a method for subfractionating the total butyl alcohol precipitate of starch and describes the crystal forms which are characteristic of the subfractions obtained from corn amylose.

Experimental

Nine grams of sphero-crystals of corn amylose (8.4 g. dry basis) which had been obtained from defatted corn starch by the method of Schoch[2] and by recrystallization from an aqueous butyl alcohol solution, was added with stirring to 183 ml. of ethylenediamine in a closed vessel at room temperature. After several days, the amylose had completely dissolved. Absolute ethyl ether was added dropwise with shaking at 25° until a distinct cloudiness developed. This required adding 53 ml. of ether. The flask was shaken intermittently for two days, whereupon the upper phase, richer in ethyl ether, was removed and poured into 500 ml. of absolute ether. The precipitate was washed by long standing in two additional 500-ml. portions of ether and decanting and then air dried to evaporate the ether. Then 300 ml. of warm water was added, the *p*H carefully adjusted to 6.2 with hydrochloric acid, the solution heated to 80°, filtered, saturated with butanol and slowly cooled over a period of several days. The crystals obtained were rectangular platelets.[6] These were centrifuged at 2000 r. p. m. and washed by standing

(1) T. J. Schoch, *Cereal Chem.*, 18, 121 (1941).
(2) T. J. Schoch, THIS JOURNAL, 64, 2957 (1942).
(3) R. W. Kerr and G. M. Severson, *ibid.*, 65, 193 (1943).
(4) R. W. Kerr, "Chemistry and Industry of Starch," Academic Press, Inc., New York, N. Y., 1944, p. 147.
(5) B. Wiegel, *Kolloid-Z.*, 102, 145 (1943).
(6) See ref. 4 for figure illustrating platelet-shaped crystals of corn amylose.