108°), previously prepared by Haworth and coworkers through the dicarbonate [Haworth and Porter, *J. Chem. Soc.*, 649 (1930)] and direct from mannose and methyl alcoholic hydrogen chloride [Haworth, Hirst and Webb, *ibid.*, 651 (1930)]. When the mother liquor of our preparation was treated with a saturated solution of calcium chloride, a non-reducing, crystalline substance with $[\alpha]^{20}D$ −58° in water solution separated. The analytical results and hydrolysis experiments indicated that this substance was a calcium chloride addition compound of β-methylmannofuranoside with the composition of $C_7H_8O_6 \cdot CaCl_2 \cdot 3H_2O$. Further investigations revealed also that the sirupy residue of the preparation of α-methylmannofuranoside from mannose and methyl alcoholic hydrogen chloride would combine with calcium chloride to produce the same addition compound with $[\alpha]^{20}D$ −58°. Removal of the calcium chloride from the double compound by silver oxalate yielded pure β-methylmannofuranoside, which was secured in crystalline condition from ethyl acetate; m. p. 47°; $[\alpha]^{20}D$ −107° in water solution. On the basis of the formula $C_7H_8O_5 \cdot CaCl_2 \cdot 3H_2O$, the rotation of −106° is calculated for the glycoside portion. The two methylmannofuranosides represent the first α,β-pair of methylglycofuranosides that has been obtained in crystalline condition. The half of the rotational difference (107°) of the two isomers is much closer to the value (97°) for the methylglucopyranosides, than to the abnormally low value (74°) for the methylmannopyranosides. It is suggested that the nonvalidity of Hudson's isorotation rules, in the case of the mannopyranosides, is largely due to unequal contents of differently puckered rings in the α- and β-isomers. Since a five-membered ring as it occurs in the furanosides is practically flat, both α- and β-mannofuranosides are considered as being derived from nearly identical rings. Then the small but real deviation (10°) from the "normal" value (97°) for the glucopyranosides can be attributed mainly to an effect produced by the *cis*-hydroxyl groups at carbon atoms 2 and 3 in mannose.

Incidental to this investigation, the crystalline dimethyl acetal of *d*-mannose was prepared from the pentaacetate of mannosediethylmercaptal by the above-mentioned method. After recrystallization from ethyl acetate, the acetal had m. p. 101° and $[\alpha]^{20}D$ 0.6° in water solution.

A detailed account of this work will be published shortly.

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RECEIVED JANUARY 3, 1939

THE SULFUR AND PHOSPHORUS CONTENTS OF TOBACCO MOSAIC VIRUS

Sir:

Tobacco mosaic virus protein isolated by chemical means has been reported to contain from 0.0 to 0.59% sulfur and from 0.0 to 0.55% phosphorus.[1] The amounts found vary with the chemical treatment and the manner in which the sample is prepared for analysis. It has now been found that virus protein isolated by the physical method of differential centrifugation and prepared for analysis by drying from the frozen state contains uniformly approximately 0.24% sulfur and 0.60% phosphorus. Neither element is removed by dialysis against water at *p*H 9.3, a result in accordance with that reported from this Laboratory by Loring.[2] It seems likely that in a previous experiment,[3] in which removal of these two elements was secured, the preparation was more alkaline than *p*H 9.3. Although the native protein does not give a color reaction with nitroprusside, the denatured protein gives positive tests for sulfhydryl groups. Since sulfur and phosphorus appear to be in organic combination, the nature of their distribution in the protein has been studied.

Sulfur occurs in the form of cysteine or cystine, methionine and sulfate sulfur, and phosphorus, in accordance with previous work, in the form of nucleic acid. Recent unpublished work of Loring indicates that the phosphorus in the virus protein can be accounted for by that isolated in the form of nucleic acid. Baernstein's procedure as modified by Kassell and Brand[4] gives values of 0.04% methionine sulfur, 0.0–0.04% sulfate sulfur, and 0.14% cystine plus cysteine sulfur, thus accounting for practically all of the sulfur. Application of Sullivan's method to hydrochloric–formic acid hydrolysates of the protein indicates that 0.11% sulfur is present as cystine or cysteine, while Lugg's modification[5] of the Folin–Marenzi

(1) Stanley, *Phytopathology*, **26**, 305 (1936); Bawden and Pirie, *Proc. Roy. Soc.* (London), **B123**, 274 (1937); Loring and Stanley, *J. Biol. Chem.*, **117**, 733 (1937).
(2) Loring, *ibid.*, **123**, 126 (1938).
(3) Stanley, *ibid.*, **117**, 325 (1937).
(4) Kassell and Brand, *ibid.*, **125**, 145 (1938).
(5) Lugg, *Biochem. J.*, **26**, 2160 (1932).

method gives still lower results, unless nucleic acid is removed from the protein. The lower results are probably due to the formation of much humin, for when cystine is added before hydrolysis it is incompletely recovered by the latter methods. Removal of nucleic acid from the protein by treatment with 5% sodium hydroxide for one minute at 0° does not eliminate humin formation and does not alter the cystine plus cysteine values obtained by the Baernstein and Sullivan procedures. Removal of nucleic acid results in a partial loss of methionine and complete elimination of sulfate sulfur. Virus nucleic acid gives only negligible or no volatile iodide, homocysteine, cysteine or sulfate titrations. In the methionine determinations on protein the volatile iodide and homocysteine titrations agree, yet the results following treatment with alkali make further work necessary to establish definitely the presence or absence of methionine as a part of the intact virus protein molecule.

Virus activity is unaffected following treatment of the protein with reducing agents,[6] hence labile disulfide groups either are not present or their reduction does not affect virus activity. Although free sulfhydryl groups have not been demonstrated in active protein, they appear following denaturation by even mild means. Furthermore, activity is lost following mild oxidation.[6] The possibility that sulfhydryl groups may be correlated with activity is being investigated.

(6) Stanley, *Phytopathology*, **25**, 899 (1935).

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PRINCETON, N. J. W. M. STANLEY

RECEIVED JANUARY 20, 1939

A VOLATILE COMPOUND OF ALUMINUM, BORON AND HYDROGEN

Sir:

As a result of successive treatments of aluminum methyl with excess of diborane at temperatures up to 80°, we have obtained a new compound AlB_3H_{12}. Vapor density measurements using 33.5, 94.6, and 55.5 mg. gave molecular weights of 71.5, 71.6, and 71.1, respectively; calculated, 71.5. Hydrolysis of two samples (4.50 and 4.35 cc. at S. C.) gave quantities of hydrogen corresponding, respectively, to 11.9 and 12.2 times the volumes of the samples; theory, 12.0 times. After hydrolysis, aluminum was precipitated as 8-hydroxyquinolinate, giving, respectively, 91.5 and 91.0 mg. of the salt (theory, 92.3 and 89.2 mg.). Another sample (3.17 cc.) was hydrolyzed; boron was removed from the residue as methyl borate and then titrated as boric acid: found, 27.9mg., calcd., 26.3.

For further confirmation of the composition, 5.30 cc. of the compound was treated with hydrogen chloride at −80°, yielding 17.2 cc. of hydrogen, aluminum chloride and a volatile mixture of diborane and chlorodiborane. This mixture was hydrolyzed, giving 43.6 mg. of boric acid (theory, 43.9 mg.) and 46.7 cc. of hydrogen. Total hydrogen thus is 63.9 cc.; calculated, 63.6 (12 volumes).

Physical properties determined are: m. p. −64.5 ± 0.5°; vapor tension at 0°, 119 mm.; b. p. (extrapolated) 44°. The gaseous substance undergoes little change within a relatively long time and is not rapidly affected even at 100°; in the liquid phase, slow polymerization seems to occur at room temperature.

With an equimolecular quantity of methyl ether the substance gives a liquid of low vapor tension, having the composition $AlB_3H_{12}\cdot(CH_3)_2O$. From this, methane is evolved without formation of diborane. With ammonia, a series of products containing up to four moles of ammonia is obtained, but the individuals have not yet been isolated.

The reaction of AlB_3H_{12} with trimethylamine has been studied in more detail. At room temperature, four moles of trimethylamine are taken up, but the resulting product seems to be a mixture of solids which, though moderately volatile, are difficult to separate. That one of the products is borine trimethylamine, $BH_3\cdot N(CH_3)_3$, is indicated by the fact that this substance could be isolated when the reaction was carried out under somewhat different conditions. Furthermore, it seems probable that at lower temperatures trimethylamine removes borine groups stepwise from AlB_3H_{12}. We are continuing the study of the reaction of trimethylamine to determine whether an addition compound of the amine and aluminum hydride is present in the final product.

We have found that diborane reacts with alkyls of metals other than aluminum, giving in some cases alkyldiboranes and other as yet unidentified products. The investigation is being carried on along the lines herein indicated.

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