ular weight of the bulk of these two fractions was approximately 100,000 and 250,000. At *p*H 6 a mixture of these almost non-infective components (10:1) was shown to reconstitute active virus. Typical TMV lesions were produced in susceptible plants, indicative of regeneration of up to 1%, and in more recent experiments of 1–5%, of the original infectivity. Electron micrographs showed rod-shaped particles 15 mμ in diameter. Many of these were 300 mμ long and appeared indistinguishable from the original TMV particles. These rods contained about 5% nucleic acid, as a central core.

It is the purpose of this communication to report on mixed reconstitution experiments performed with protein and nucleic acid fractions isolated from different strains of TMV. Native protein has been obtained only from common TMV and the masked (M) strain, active nucleic acid from these as well as yellow aucuba (YA) and Holmes ribgrass (HR). Five of the six possible combinations of these have given active virus which in each case, has produced on Turkish tobacco plants only those symptoms which are characteristic of the strain supplying the nucleic acid.

Of particular interest was the "hybrid" virus reconstituted from TMV-protein with HR-nucleic acid, because HR protein differs markedly from TMV protein in amino acid composition and in antigenic specificity.[3] When this hybrid was treated with anti-TMV serum its infectivity was largely neutralized while anti-HR serum did not appear to affect it. Immunologically, therefore, the hybrid resembled TMV, the virus which has supplied the protein coat, while its symptoms in the plant were only those of the HR-strain, which supplied the nucleic acid core. This finding of a viral activity showing properties characteristic of each of the parent strains appears to rule out any interpretation of the mode of virus reconstitution other than the *in vitro* production of active particles from the two components. The progeny produced from this hybrid (TMV-protein + HR-nucleic acid) in the plant, and in particular its protein were very similar to, if not identical with, HR virus and protein. The progeny protein, like HR and unlike TMV,[3] contained methionine and histidine, and much tyrosine, but quantitative analyses remain to be done. Thus the ribonucleic acid seems to represent the main genetic determinant even for the progeny protein in the TMV strains.

In attempts to free the original protein and nucleic acid fractions from any traces of undegraded virus, assays at high concentrations (up to 0.05%) in a very sensitive variety of *nicotiana* test plants indicated the presence of no more than about 0.0003% of active virus in either. Yet, when the nucleic acid was tested in a plant variety less sensitive to TMV (Holmes necrotic), sufficient lesions were sometimes produced to indicate the presence of about 0.1% of TMV. This residual infectivity differed from the virus in its relative activity on different hosts and at different levels, in its marked instability, and in its suceptibility to ribonuclease. Virus rods could not be found to account for this residual infectivity and it is therefore now regarded as a characteristic property of the nucleic acid itself. However, it is found only in those fresh preparations which are also able to combine with virus protein to give, with at least a 200-fold increase in activity, a much more stable and virulent infectious agent, the reconstituted virus.

(3) C. A. Knight, *J. Biol. Chem.*, **171**, 297 (1947); **145**, 11 (1942).

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D-XYLOSE-3-PHOSPHATE

Sir:

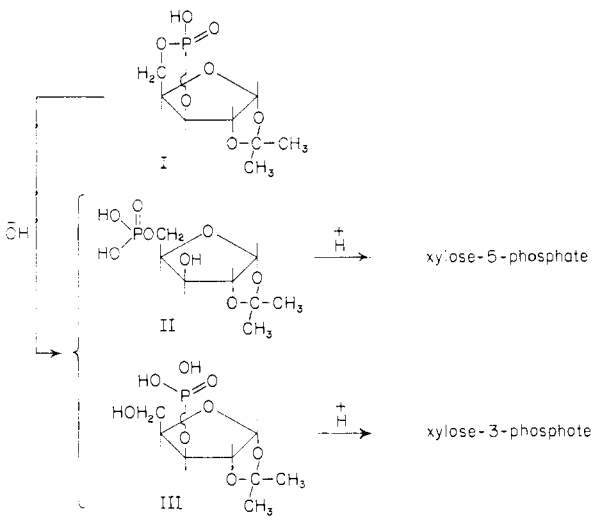
Levene and Raymond[1] have recorded unsuccessful attempts to synthesize D-xylose-3-phosphate. Recently, Watson and Barnell[2] have suggested that this compound might be formed from X-xylose-5-phosphate as a result of a phosphoryl group migration from the 5- to the 3-hydroxyl group. In this communication we wish to report the synthesis and characterization of D-xylose-3-phosphate and to show that the change in the optical rotation of the solutions of xylose-5-phosphate observed by Watson and Barnell is due to the formation of D-xylulose-5-phosphate rather than to a phosphoryl group migration.

The crystalline 1,2-isopropylidene-D-xylofuranose-5-diphenylphosphate[3] (2.11 g., 5 mM.) was treated with a mixture of 2 *N* sodium hydroxide (15 ml.) and dioxane (5 ml.) at room temperature. The conversion to 1,2-isopropylidenexylofuranose-3,5-cyclic phosphate (I) was complete in 72 hours and the excess of alkali was then removed by the addition of Dowex 50 (H^+) resin. The solution of the sodium salt of the six-membered cyclic phosphate (I) was passed through a cyclohexylammonium-Dowex 50 column and the effluent evaporated to dryness. The cyclohexylamine salt crystallized from a mixture of acetone and ether in a yield of 85%; m.p. 217–219° with dec. $[\alpha]^{21}D$ +14.1 (*C* 1.9 in water) *Anal.* Calcd. for: $C_{14}H_{26}O_7NP$: C, 47.86; H, 7.46; N, 3.99. Found: C, 47.79; H, 7.50; N, 3.61. Potentiometric titration showed the absence of a secondary phosphoryl dissociation. The cyclic phosphate was hydrolyzed quantitatively by 1 *N* sodium hydroxide at 100° in 20 hours to, presumably, a mixture of II and III. An aqueous solution of the free acids (II and III), prepared by passing the alkaline solution through a Dowex 50-H^+ column, was heated at

(1) P. A. Levene and A. L. Raymond, *J. Biol. Chem.*, **102**, 317, 331, 347 (1933); **107**, 75 (1934).

(2) R. W. Watson and J. L. Barnell, *Chem. and Industry*, 1089 (1955).

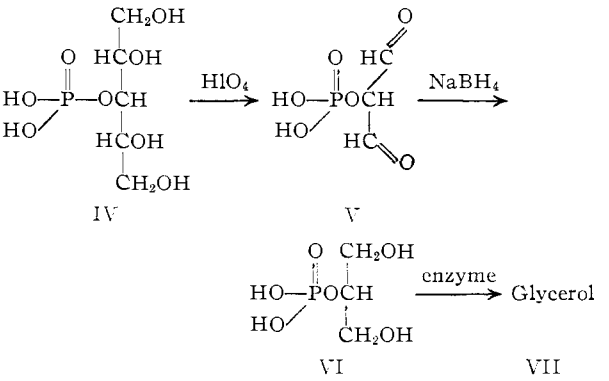
(3) J. L. Barnell, W. A. Saunders and R. W. Watson, *Can. J. Chem.*, **33**, 711 (1955); P. A. J. Gorin, L. Hough and J. K. N. Jones, *J. Chem. Soc.*, 585 (1955).



100° for 10 min. under its own *p*H (*ca.* 1.5) to remove the isopropylidene group, and the resulting mixture of xylose-3- and -5-phosphates applied to a Dowex 2-(formate) column. Elution with 0.1 *M* sodium formate–formic acid buffer, *p*H 3, gave, first, xylose-5-phosphate and then xylose-3-phosphate as completely separated peaks. The latter compound was isolated as the barium salt in 15% yield: $[\alpha]^{22}D$ +1.27 (*C* 5.13 in water) *Anal.* Calcd. for $C_5H_9O_8P\cdot Ba$: xylose, 41.1; P, 8.47. Found: xylose, 41.5; P, 8.40. The following evidence rigidly establishes the structure of this compound: (1) Dephosphorylation with acid phosphatase[4] liberated only xylose, identified by paper chromatography in several suitable solvent systems. (2) Xylose-3-phosphate can be distinguished from the isomeric 5-phosphate on paper chromatograms developed in isopropyl alcohol–ammonia–water (70–10–20, v./v.) through its slightly, but consistently, higher mobility and by the appearance of its characteristic pink spot with the aniline–phthalate spray. (Xylose-5-phosphate appears as a brown spot.) (3) Reduction of xylose-3-phosphate with sodium borohydride in aqueous solution gave, as expected, the optically inactive xylitol-3-phosphate. (4) The above D-xylitol-3-phosphate (IV) was treated for 30′ at neutral *p*H with sodium periodate (3 mols., theoretical requirement 2 mols.) and the reaction mixture treated with sodium borohydride for 24 hours. The phosphorus-containing product was isolated by chromatography on Whatman No. 4 paper in the isopropyl alcohol–ammonia system (see above) and dephosphorylated enzymatically. Glycerol, the expected product (IV–VII), was identified by paper chromatography.[5] After the same series of reactions, D-xylitol-5-phosphate gave, as expected, only ethylene glycol.

(4) Worthington Biochemicals Corp., Freehold, N. J.

(5) M. Visconti, D. Hoch and P. Karrer, *Helv. Chim. Acta*, **38**, 642 (1955).



Studies of the rates of oxidation of xylose-5- and -3-phosphates by periodic acid and the reaction of these compounds with dicyclohexylcarbodiimide have led us to conclude that xylose-3-phosphate exists in solution in the pyranose form (CI conformation[6]). This conclusion warranted a careful study of the problem of phosphoryl group migration on acidic treatment of this substance and a reinterpretion of some of Levene and Raymond's[1] results. These aspects of the chemistry of xylose-3-phosphate will be discussed in a forthcoming publication.

The nature of the reaction responsible for the change[2] in the optical rotation of a solution of D-xylose-5-phosphate was investigated because (a) the suggested[2] phosphoryl group migration under neutral conditions appeared highly improbable, and (b) the properties of our sample of xylose-3-phosphate were completely different from those of solutions of D-xylose-5-phosphate treated according to Watson and Barnell.[2] It was thought that the data recorded by these authors[2] could be explained by assuming the formation of D-xylulose-5-phosphate from xylose-5-phosphate.[7] This was found to be so by the following tests on a solution (*p*H 6.2) of sodium D-xylose-5-phosphate heated at 50° for 2 hours. (1) Dephosphorylation with acid phosphatase liberated xylulose in addition to xylose as shown by paper chromatography in three solvent systems[8] and by the $FeCl_3$–orcinol spectrum.[8] (2) Ethylene glycol was the only product obtained after the degradative reactions described above for xylitol phosphates.

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(6) R. E. Reeves, THIS JOURNAL, **72**, 1499 (1950).

(7) The partial transformation at room temperature of barium ribose-5-phosphate to a ribulose-containing compound has already been noted: B. Axelrod and R. Jang, *J. Biol. Chem.*, **209**, 847 (1954).

(8) S. Mitsuhashi and J. O. Lampen, *ibid.*, **204**, 1011 (1953); R. M. Hochster and R. W. Watson, *Arch. Biochem. Biophys.*, **48**, 120 (1954); M. W. Slein, THIS JOURNAL, **77**, 1663 (1955).