to produce significant amounts of *cis*-vaccenic acid under our experimental conditions.

Formate incorporation into *L. arabinosus* fatty acids was demonstrated in a second series of experiments in which sodium [^{14}C]formate ($3.8 \cdot 10^6$ disintegrations/min) was incubated with a cell suspension (5 g wet wt.) in 0.03 *M* phosphate, pH 6.8 (50 ml) containing 1 % glucose. The total fatty acids isolated from these cells exhibited a radioactivity of 1550 disintegrations/min. On chromatography, the major proportion of this radioactivity accumulated in the lactobacillic acid fraction (Table I).

The radioactivity present in the dihydroxy fraction was located in a sharp band preceding the major dihydroxyoctadecanoic acid peak suggesting that an intermediate in the conversion of *cis*-vaccenic into lactobacillic acid may be involved.

The predominant incorporation of formate carbon into lactobacillic acid is in excellent agreement with our hypothesis[1] pertaining to lactobacillic acid biosynthesis. Experiments to locate the radioactivity of the biosynthetic lactobacillic acid in the methylene-bridge carbon atom are in progress. A procedure for this purpose has been described recently[5].

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The synthesis of D-arabinose-5-phosphate

A chemical source of D-arabinose-5-phosphate was needed for studies of the enzymic dissimilation of this compound by *Propionibacterium pentosaceum*. Early work concerned with the enzymic formation of A-5-P was hampered by the difficulty in obtaining or preparing the compound. The synthesis reported by LEVENE AND CHRISTMAN[1] involved the formation of 7 intermediary compounds between the starting material, D-arabinose, and the product. The synthesis reported here is a simple 2-step procedure for the synthesis of A-5-P from glucosamine.

Glucosamine-6-phosphate was prepared by phosphorylation of glucosamine by yeast hexokinase as described by BROWN[2]. The reaction mixture for the synthesis of A-5-P contained 400 μmoles glucosamine-6-phosphate, 1000 μmoles triketohydrindene hydrate, and 1500 μmoles citrate buffer, pH 4.7, in a final vol. of 60 ml. The yield of A-5-P remained constant between pH 4.2 and 5.2, but decreased markedly on either side of this pH range. The reaction mixture was placed in a boiling-water bath. Pentose formation was followed by means of the orcinol test[3]. As shown in Fig. 1 a maximum yield of pentose was obtained after a 40-min period at 100°.

Abbreviation: A-5-P, D-arabinose-5-phosphate.

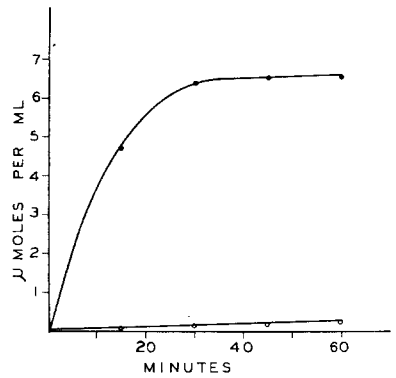

Fig. 1. The system contained 400 μmoles glucosamine-6-phosphate, 1500 μmoles citrate buffer, pH 4.7, and 1000 μmoles triketohydrindene hydrate in a total vol. of 60 ml. The mixture was placed in a boiling-water bath and the amount of pentose formed was determined at times indicated. Free pentose was determined after adsorption of phosphorylated pentose on Dowex-1 formate. ● total pentose; ○ free pentose.

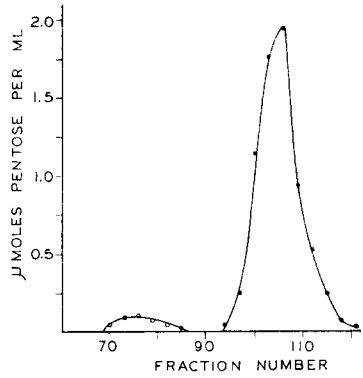

Fig. 2. The reaction mixture contained 400 μmoles glucosamine-6-phosphate, 1500 μmoles citrate buffer, and 1000 μmoles triketohydrindene hydrate in a total vol. of 60 ml. After heating at 100° for 40 min, the reaction mixture was cooled and adsorbed directly on a Dowex-1 formate column. Elution was achieved with 0.2 *N* formic acid containing 0.02 *M* ammonium formate. ○ Fraction 1; ● Fraction 2.

At the end of the heating period, the dark-blue mixture was cooled and adsorbed on a Dowex-1 formate column (2 cm × 20 cm). (It should be noted that considerably larger amounts can be handled if one uses a larger Dowex-1 formate column or if the A-5-P is precipitated at this point as its alcohol insoluble barium salt and then redissolved and adsorbed on a Dowex-1 formate column.) The column was eluted with 0.2 *N* formic acid containing 0.02 *M* ammonium formate. Fig. 2 illustrates the elution pattern of the pentoses. It can be seen that the phosphorylated material giving a positive orcinol test was eluted in 2 fractions. Fraction 1 comprised less than 1 % of the total pentose and is believed to contain both ribose and fructose phosphates. The material in Fraction 2 (334 μmoles pentose phosphate) was pooled and neutralized to pH 7.5 with KOH. $BaCl_2$ (1000 μmoles) and ethanol (to a final concn. of 80 %) were added. After overnight at 2°, the barium salt was collected by centrifugation and dissolved in water. The total amount of pentose phosphate at this point was 197 μmoles.

In order to identify the pentose phosphate in Fraction 2, 2000 μmoles glucosamine-6-phosphate were treated as outlined above. After chromatography on a Dowex-1 formate column, Fraction 2 (1670 μmoles) was pooled and precipitated as the alcohol-insoluble barium salt. After overnight at 2°, the precipitated barium salt was collected by centrifugation, dissolved in water, freed of barium by the addition of K_2SO_4, and treated with acid phosphatase in acetate buffer, pH 5.0, for 4 h. At this time the reaction mixture was made 0.005 *M* with $Na_2B_4O_7$, adjusted to pH 8.0, and adsorbed directly on a Dowex-1 borate column (1 cm × 15 cm). The column was eluted with 0.02 *M* $Na_2B_4O_7$, and 20-ml fractions were collected. The single pentose fraction was pooled, passed through a Dowex-50 (H^+) column to convert the borate to boric acid, and lyophilized to dryness. The boric acid was removed as the methyl borate[4]. The diphenylhydrazone of the pentose was prepared from the residual syrup according to the procedure of BROWNE[5]. The m.p. of the diphenylhydrazone was

203°. Authentic D-arabinose diphenylhydrazone melted at 202°, and the mixed m.p. was 202°.

Since the arabinose phosphate was synthesized from glucosamine-6-phosphate the phosphate on the arabinose would be expected to be limited to the 5 position. This conclusion is supported by the observation that only 16 % of the phosphate of the synthesized arabinose phosphate was hydrolyzed in 1 h in 1 *N* H_2SO_4 at 100°.

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A colorimetric method of estimation of shikimic acid*

Procedures for the estimation and detection of shikimic acid, involving oxidation by periodate, have been reported[1–3]. During periodate oxidation of shikimic acid, formation of a conjugated aldehyde occurs[4]. Since thiobarbituric acid selectively couples with conjugated aldehydes to produce colored products[5], it appeared feasible to devise a method of estimation of shikimic acid based on periodate oxidation and subsequent reaction with thiobarbituric acid. The reagents employed have been previously described[6], and the behavior of various aldehydes upon reaction with thiobarbituric acid has been reviewed[5].

Mix 0.5 ml of a solution of 0.025 *M* HIO_4 in 0.125 *N* H_2SO_4 with 3.5 ml of an aqueous solution containing 2.5–60.0 μg of shikimic acid** in a test tube. Heat the tube in a water bath at 56° for 60 min. Remove the tube, add 1.0 ml of 2 % reagent-grade $NaAsO_2$ in 0.5 *N* HCl, and mix well. After 2 min, transfer an aliquot of 1.0 ml, or less, of the resulting solution to a test tube containing 2.0 ml 0.6 % thiobarbituric acid (pH 2.0). Bring the volume to 3.0 ml with distilled water and mix the contents of the tube. Prepare a blank by adding 1.0 ml of distilled water to 2.0 ml of the thiobarbituric acid reagent. Heat the tubes for 60–65 sec in a boiling-water bath and immediately immerse them in cold water. After 1–5 min, add 0.3 ml 1.0 *N* NaOH, mix well, and within 3 min, immerse the tubes for 60–65 sec in a boiling-water bath. Cool the tubes in cold water for 1 min and measure the intensity of the blue solution at the absorption maximum of 660 mμ (Fig. 1) with a Beckman DU spectrophotometer.

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