

The effect of tertiary amines on this type of phosphorylation is illustrated as follows. When weak bases such as pyridine or 2,6-lutidine were used in the above experiment, the reaction proceeded slowly and the expected phosphates were obtained in low yields; on the other hand, strong bases such as hexamethylenetetramine and triethylenediamine were also found to be ineffective, because the preferential formation of stable mercury-amine complexes prevented the reaction.

In conclusion, all the common alcohols were easily phosphorylated in good yields by treating phosphorous acid with mercuric chloride and triethylamine in acetonitrile. Further, this procedure was proved to be applicable for the phosphorylation of alcohols available in only small amounts.

### Experimental Section

**Materials.**—Phosphorous acid and mercury compounds were obtained from a commercial source. They were ground and dried over  $P_2O_5$  *in vacuo* before use. Alcohols and solvents used were purified by the conventional procedures.

**General Procedures for the Preparation of Monoalkyl Dihydrogen Phosphates. A. The Reaction of Mercuric Chloride with Phosphorous Acid and Alcohols.**—Into a solution of mercuric chloride (2.72 g, 0.01 mole) and phosphorous acid (0.82 g, 0.01 mole) in dry alcohols (15 ml) was added excess triethylamine (5–6 ml) in one portion. The mixture was heated on a water bath at 80° for 15 min with vigorous stirring. After removal of metallic mercury (1.65–1.94 g, 82–96%) and triethylamine hydrochloride, the filtrate was concentrated and then acetone (10 ml) was added to remove residual ammonium salt (total yields amounted to more than 80%). The acetone solution was passed through an IR-200 ion-exchange column (25 × 1.8 cm) already prepared in water, and eluted with ethanol (50%). The eluates were concentrated, then ethanol (5 ml) and aniline (1.5 ml) were added. Recrystallization of the resulting anilinium salts from ethanol once or twice gave analytical samples. Crude yields amounted to 69–92%. Some of the mixture melting points were not depressed with authentic samples.<sup>6</sup> Both physical and analytical data are summarized in Table I.

(6) O. Mitsunobu, T. Obata, and T. Mukayama, *J. Org. Chem.*, **30**, 1071 (1965).

**B. The Reaction of Mercurous Chloride with Phosphorous Acid and Alcohols.**—To anhydrous solutions of mercurous chloride (4.78 g, 0.01 mole) and phosphorous acid (0.82 g, 0.01 mole) in alcohols (15 ml) was added triethylamine (6 ml) in one portion. The mixtures were heated at 80° and stirred constantly for an additional 30 min. After separation of mercury and ammonium salt, monoanilinium salts of the corresponding monoalkyl dihydrogen phosphates were obtained from those filtrates through the same procedure described in the above reaction.

**Phosphorylation of Benzyl Alcohol and Borneol in Acetonitrile by the Reaction of Mercuric Chloride and Phosphorous Acid.**—Into a clear solution of benzyl alcohol (1.4 g, 0.013 mole), phosphorous acid (0.82 g, 0.01 mole), and mercuric chloride (3.0 g, 0.011 mole) in dry acetonitrile (20 ml) was poured triethylamine (6 ml) in one portion, and the mixture was quickly warmed to 80°. After additional stirring for 30 min, the mixture was cooled. Precipitated metallic mercury (1.63 g, 82%) and triethylamine hydrochloride (1.18 g, 40%) were filtered out. The filtrate was evaporated under reduced pressure, and the oil was desalted through an IR-200 acidic ion-exchange resin column (25 × 1.8 cm) already prepared in 50% ethanol, followed by elution with the same solvent. The collected eluate (between 40 and 120-ml fractions) was concentrated, then the oil was chromatographed on a Whatman Column Chromedia CF 11 column (17 × 3 cm) which was prepared in water and then saturated with *n*-butyl alcohol. The first 40–90-ml fraction, eluted with *n*-butyl alcohol, contained free monobenzyl dihydrogen phosphate, which was characterized as its anilinium salt: mp 150–152°, 2.95 g (80%). *Anal.* Calcd for  $C_{19}H_{23}N_2O_4P$ : C, 61.79; H, 4.94; N, 7.48. Found: C, 60.36; H, 5.01; N, 7.50. The mixture melting point was not depressed with an authentic sample.

Similarly, bornyl dihydrogen phosphate was synthesized from 1.3 mole equiv of borneol, and it was isolated as the monoanilinium salt: mp 194–196°, 1.54 g (47%). *Anal.* Calcd for  $C_{16}H_{22}NO_4P$ : C, 58.72; H, 8.02; N, 4.28. Found: C, 57.94; H, 8.09; N, 4.31.

**Registry No.**— $C_{19}H_{23}N_2O_4P$ , 7721-82-6;  $C_{16}H_{22}NO_4P$ , 7704-43-0;  $C_7H_{12}NO_4P$ , 7704-44-1;  $C_8H_{14}NO_4P$ , 2180-42-9;  $C_9H_{13}N_2O_4P$ , 7704-46-3;  $C_9H_{16}NO_4P$  (mp 137–139), 2180-41-8;  $C_9H_{16}NO_4P$  (mp 159–160), 1992-41-2;  $C_{10}H_{18}NO_4P$  (mp 138–140), 1992-40-1;  $C_{10}H_{18}NO_4P$  (mp 155–156), 7704-50-9;  $C_{11}H_{20}NO_4P$  (mp 135–137), 7704-51-0;  $C_{11}H_{20}NO_4P$  (mp 149–151), 7704-52-1;  $C_{12}H_{20}NO_4P$ , 7704-53-2;  $C_{14}H_{26}NO_4P$ , 7704-54-3.

## Synthesis of D-erythro-Pentulose 1-Phosphate (D-Ribulose 1-Phosphate)

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The chemical synthesis of D-erythro-pentulose 1-phosphate (D-ribulose 1-phosphate) has been accomplished by the phosphorylation of 3,4,5-tri-O-benzoyl-D-erythro-pentulose dimethyl acetal, followed by the appropriate removal of the blocking groups. The pentulose acetal was prepared from the known 3,4,5-tri-O-benzoyl-1-deoxy-1-diazo-D-erythro-pentulose by hydrolysis to 3,4,5-tri-O-benzoyl-D-erythro-pentulose which was acetalated with trimethyl orthoformate.

The synthesis of phosphate esters of sugars has, since the early experiments of Fischer and Baer,<sup>1</sup> been a subject to challenge the imagination and ingenuity of chemists and biochemists. Many successful syntheses are recorded, but few general methods are known, particularly for the preparation of esters of reducing sugars with three, four, and five carbon atoms.

One method of rather wide applicability, developed mainly in this laboratory, makes use of the acyclic acetal derivatives of reducing sugars.<sup>2</sup> This approach eliminates interference from the oxane ring of the sugar,

freeing all hydroxyl groups for reaction. In addition, the acetal structure stabilizes the final product against the decomposition characteristic of carbonyl phosphate esters, and, being sensitive to acid hydrolysis, permits the ready conversion of the derivative to the free sugar phosphate, as desired.

The success of this approach is attested by published syntheses of glycolaldehyde phosphate,<sup>3</sup> D-glyceralde-

(2) C. E. Ballou and D. L. MacDonald, "Methods in Carbohydrate Chemistry," Vol. 2, R. L. Whistler and M. L. Wolfson, Eds., Academic Press Inc., New York, N. Y., 1963, p 270.

(3) C. E. Ballou, *Arch. Biochem. Biophys.*, **78**, 328 (1958).

(1) H. O. L. Fischer and E. Baer, *Ber.*, **65**, 337, 1040 (1932).

hyde 3-phosphate,<sup>4</sup> dihydroxyacetone phosphate,<sup>5</sup> hydroxypyruvic acid phosphate,<sup>6</sup> D-erythrose 4-phosphate,<sup>7</sup> D- and L-glycero-tetralose 1- and 4-phosphate,<sup>8,9</sup> D-glycero-tetralose 1,4-diphosphate,<sup>10</sup> and 2-deoxy-D-erythro-pentose 5-phosphate.<sup>11</sup>

The key to these syntheses is the preparation of the acyclic acetal. In every case this requires a circuitous route to overcome the tendency of reducing sugars to form either monomeric or dimeric cyclic hemiacetals. The synthesis of ketose phosphates presents the additional problem that the ketose sugars themselves are not readily available as starting materials and must be prepared from some available intermediate.

An excellent procedure for the preparation of ketoses, and one which at the same time yields intermediates which can be converted to the acyclic acetal, is based on a long series of investigations by Wolfrom and co-workers.<sup>12</sup> This procedure utilizes the reaction of acetylated glyconic acid chlorides with diazomethane. The usefulness and flexibility of this approach have been demonstrated by our previous syntheses of the mono- and diphosphates of D-glycero- and L-glycero-tetralose.<sup>8-10</sup> In this paper we describe attempts, in part successful, to extend this methodology to the synthesis of D-erythro-pentulose phosphates (ribulose phosphates).

The preparation of D-erythro-pentulose by the "diazomethane synthesis" was reported by MacDonald, *et al.*, in 1958.<sup>13</sup> By coupling 2,3,4-tri-O-benzoyl-D-erythronyl chloride (I) with diazomethane, the expected product, 3,4,5-tri-O-benzoyl-1-deoxy-1-diazo-D-erythro-pentulose (II), was obtained. Although II can be converted to 1,3,4,5-tri-O-benzoyl-D-erythro-pentulose,<sup>13</sup> such derivatives have not proved useful in the past for phosphate ester synthesis since the ketone cannot be acetalated. The unreactivity of such ketones was first recorded by Fischer and Baer,<sup>14</sup> who attempted to acetalate 1,3-diacetoxypropanone. On the other hand, the reaction occurs readily with 1-hydroxy-3-acetoxypropanone,<sup>14</sup> and it appeared that such acetalations required an unsubstituted hydroxyl group on one side of the carbonyl group. This we have confirmed<sup>8</sup> with the finding that 3,4-di-O-benzoyl-D-glycero-tetralose is converted smoothly to the dimethyl acetal on treatment with trimethyl orthoformate, methanol, and hydrogen chloride.

This synthetic approach requires the direct hydrolysis of the benzoyldiazo ketone (*i.e.*, II) to a benzoyl-hydroxy ketone [*i.e.*, 3,4,5-tri-O-benzoyl-D-erythro-pentulose (III)]. We have reported previously<sup>8</sup> that this reaction occurs readily in the tetralose series, and we have now found that under appropriate conditions the reaction II  $\rightarrow$  III will go also in good yield.

## Results and Discussion

The synthesis of 3,4,5-tri-O-benzoyl-1-deoxy-1-diazo-D-erythro-pentulose (II) according to MacDonald, *et al.*, gives variable results and the product requires purification by chromatography on Magnesol-Celite.<sup>13</sup> Although numerous modifications of the procedure were tried, no significant improvement resulted. Hydrolysis of II to III gave crystalline hydroxy ketone in moderate yield.

The synthesis of III was mentioned in a footnote to the paper by Chü and Ballou in 1961,<sup>8</sup> with the comment that "the ketalation step had given anomalous results that precluded synthesis of pentulose phosphates by this procedure." In fact, the attempted ketalation of III failed, using trimethyl orthoformate, methanol, and 1% hydrogen chloride. When carried out at room temperature, no reaction occurred. When the reaction was refluxed, the solution became levorotatory, methyl benzoate was produced, and no other identifiable product was isolated. Subsequently, it was discovered that if sulfuric acid was used in place of hydrogen chloride as the catalyst, the solution became strongly dextrorotatory on refluxing, and a good yield of the dimethyl acetal (IV) was obtained.

Direct phosphorylation of IV by diphenylphosphorochloridate, followed by removal of the blocking groups, yielded D-erythro-pentulose 1-phosphate dimethyl acetal as a crystalline salt (V). The acid hydrolysis of this compound yields D-erythro-pentulose 1-phosphate. This hydrolysis reaction, like that of 2-deoxy-D-erythro-pentose 5-phosphate dimethyl acetal,<sup>11</sup> is complicated by alternative pathways involving other intermediates.

This synthesis makes D-erythro-pentulose 1-phosphate available for the first time. It has not been found in nature, although the analogous tetralose 1-phosphate and fructose 1-phosphate are known.

## Experimental Section

Except where specified to the contrary, reagents were of analytical grade, or were purified by standard procedures. Analyses were done by the Microchemical Laboratory, University of California at Berkeley. Melting points are uncorrected.

**3,4,5-Tri-O-benzoyl-1-deoxy-1-diazo-D-erythro-pentulose (II).**—This substance was prepared by a modification of the procedure of MacDonald, Crum, and Barker.<sup>13</sup> To a solution of diazomethane (35 mmoles) in 100 ml of anhydrous ether at  $-25^{\circ}$  was added 6.9 g of 2,3,4-tri-O-benzoyl-D-erythronyl chloride (I, mp  $88-91^{\circ}$ ,<sup>13</sup> 15 mmoles) in 120 ml of anhydrous ether at the same temperature. The reaction mixture was stirred, and the addition took 20 min, during which time the temperature was allowed to rise to  $-15^{\circ}$ . The solution was allowed to stand for 45 min at  $0^{\circ}$  and then for 45 min at room temperature. It was evaporated to dryness, the solid was dissolved in 20 ml of anhydrous benzene, and the solution was applied to a Magnesol-Celite column (4:1, w/w, Magnesol-Celite,  $6.5 \times 32$  cm). Elution was carried out with 1900 ml (two column volumes) of benzene containing 0.33% ethanol. The column material was extruded using compressed air, and was streaked with a 1% solution of potassium permanganate in 2.5 N sodium hydroxide. The main oxidizable band was cut out and the product was extracted from the adsorbent with acetone. After evaporation of the acetone, the resulting syrup was crystallized from ether. The yield of II was 3.6 g (52%) and a sample recrystallized once more from ether had mp  $94-96^{\circ}$ . The reported value<sup>13</sup> is  $95-96^{\circ}$ ,  $[\alpha]_D^{25} -28^{\circ}$  (*c* 2.0, chloroform).

*Anal.* Calcd for  $C_{26}H_{26}N_2O_7$  (472.3): C, 66.1; H, 4.25; N, 5.94. Found: C, 65.8; H, 4.42; N, 6.28.

**3,4,5-Tri-O-benzoyl-D-erythro-pentulose (III).**—To a solution of II, (8.6 g) in 100 ml of dioxane, 32 ml of 3 N sulfuric acid was added. The mixture was heated at  $80-82^{\circ}$  for 90 min and

(4) C. E. Ballou and H. O. L. Fischer, *J. Am. Chem. Soc.*, **77**, 3329 (1955).

(5) C. E. Ballou and H. O. L. Fischer, *ibid.*, **78**, 1659 (1956).

(6) C. E. Ballou and R. Hesse, *ibid.*, **78**, 3718 (1956).

(7) C. E. Ballou, H. O. L. Fischer, and D. L. MacDonald, *ibid.*, **77**, 5967 (1955).

(8) N. J. Chü and C. E. Ballou, *ibid.*, **83**, 1711 (1961).

(9) J. W. Gillett and C. E. Ballou, *Biochemistry*, **2**, 547 (1963).

(10) G. A. Taylor and C. E. Ballou, *ibid.*, **2**, 553 (1963).

(11) D. L. MacDonald and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, **81**, 3719 (1959).

(12) M. L. Wolfrom and R. B. Bennett, *J. Org. Chem.*, **30**, 458 (1965).

(13) D. L. MacDonald, J. D. Crum, and R. Barker, *J. Am. Chem. Soc.*, **80**, 3379 (1958).

(14) H. O. L. Fischer and E. Baer, *Ber.*, **65**, 345 (1932).

then poured into 300 ml of ice water. The product was extracted with benzene (three portions of about 150 ml each), and the combined benzene layer was washed with 200 ml of water, dried over sodium sulfate, and evaporated to a syrup. This was dissolved in warm ether, the solution was decolorized with charcoal, and the product was allowed to crystallize from ether solution. The yield of III was 5.05 g (60%), and a sample recrystallized from ether had mp 123–125°. It showed  $[\alpha]_{589}^{20} -2.2^\circ$  and  $[\alpha]_{400}^{20} -25.9^\circ$  (*c* 2.25, chloroform).

*Anal.* Calcd for  $C_{26}H_{22}O_8$  (462.3): C, 67.7; H, 4.76. Found: C, 67.4; H, 4.91.

Compound III was also prepared from I without the isolation of II. The over-all yield by this procedure was 39%, compared to 32%, but the quality of the product was not quite so good (mp 119.5–121.5° after one crystallization).

**3,4,5-Tri-*O*-benzoyl-*D*-erythro-pentulose Dimethyl Acetal (IV).**—To a solution of III (1.75 g) in 25 ml of anhydrous methanol, were added 25 ml of redistilled trimethyl orthoformate and 4 drops of concentrated sulfuric acid. The reaction mixture was left at room temperature until the optical rotation reached a constant value,  $[\alpha]_{589}^{20} +88^\circ$  after 48 hr, calculated as the acetal. The solution was poured into a separatory funnel containing 80 ml of 0.1 *N* aqueous ammonia, and the mixture was extracted with ether. The ether layer was washed with water, dried over sodium sulfate, and evaporated to a syrup which was dried in a vacuum at room temperature for 48 hr. The yield was 1.75 g (92%), and the substance showed  $[\alpha]_{589}^{20} +81^\circ$  (*c* 0.7, chloroform). Solution in anhydrous ether and reprecipitation with petroleum ether (bp 30–60°) gave 1.33 g.

*Anal.* Calcd for  $C_{28}H_{28}O_9$  (508):  $OCH_3$ , 12.2. Found:  $OCH_3$ , 11.1.

***D*-erythro-Pentulose 1-Phosphate Dimethyl Acetal (V).**—To a solution of 1.8 g of IV in anhydrous pyridine, 4.8 ml of diphenylphosphorochloridate was added dropwise with mixing over a period of 10 min while the solution was cooled in ice-water. The mixture was kept at 4° for 2 days, then a few drops of water were added to destroy excess of reagent, and after 10 min the solution was poured into 50 ml of ice-water. The mixture was extracted with benzene (three 100-ml portions) and the benzene layer was washed with dilute sodium bisulfate, aqueous sodium bicarbonate, and water, and then dried over sodium sulfate. The solution was decolorized with charcoal and evaporated to give 2.62 g of pale yellow syrup (93%).

The syrup was hydrogenated in 250 ml of absolute ethanol, using 2.4 g of platinum dioxide catalyst. The calculated uptake was 1695 ml, while the actual uptake was 1670 ml. The catalyst was removed by centrifugation, and to the supernatant solution was added 90 ml of 0.15 *M* barium hydroxide solution. The mixture was left overnight at room temperature, and was then evaporated to remove ethanol. A solution of 4.06 g of cyclohexylammonium sulfate in water was added, the mixture was filtered through Celite to remove the precipitate of barium sulfate, and the filtrate was evaporated to dryness. The residue was extracted with 250 ml of acetone, and the insoluble fraction was extracted with 200 ml of absolute ethanol. The ethanol-insoluble material was discarded. The ethanol-soluble fraction (820 mg) was crystallized from water by addition of acetone. The total yield of V was 655 mg (39%), and the substance showed  $[\alpha]_{589}^{20} +19.7^\circ$  (*c* 1, water).

*Anal.* Calcd for  $C_{19}H_{43}N_2O_9P \cdot 2H_2O$  (510): C, 44.7; H, 9.2; N, 5.5; P, 6.1. Found: C, 44.8; H, 9.2; N, 5.7; P, 5.8.

In agreement with the assigned structure, the compound consumed 1.9 moles (calcd, 2.0) of periodate per mole. The product of periodate oxidation was reduced with sodium borohydride. On paper chromatography, the reduced material had the same  $R_f$  value as authentic dihydroxyacetone phosphate dimethyl acetal. Treatment with acid to hydrolyze the acetal structure, followed by enzymic assay for free dihydroxyacetone phosphate, gave a value representing a quantitative yield of the three-carbon unit from the original pentulose 1-phosphate.

The phosphate group was hydrolyzed in 1 *N* hydrochloric acid at 100° with a half-time of 7 min.

***D*-erythro-Pentulose 1-Phosphate (*D*-Ribulose 1-Phosphate) (VI).**—Five milligrams of V was dissolved in 0.5 ml of water. A small amount of Dowex-50(H) was added and the solution was heated at 100° in a water bath to hydrolyze the acetal structure. Aliquots were removed at 1-min intervals and spotted on Whatman No. 1 paper for chromatography with the solvent isopropyl alcohol–ammonia–water (7:1:2). The starting acetal (V) had  $R_f$  0.58, and all of V was converted within 1 min to a second substance with  $R_f$  0.36, a value reasonable for the expected free ketose (VI). The new substance was the major phosphate-containing component produced, and it reacted strongly on the paper with a silver nitrate–sodium hydroxide dip reagent, whereas V reacted very weakly, as would a methyl glycofuranoside.

The solution of *D*-erythro-pentulose 1-phosphate was reduced with sodium borohydride for 2 hr. Then it was treated with Dowex-50(H) to remove cations, concentrated to dryness, and evaporated twice with addition of methanol to remove boric acid. The residue was dissolved in 1 ml of concentrated ammonia and the solution was sealed in a glass tube and heated at 180° for 18 hr for dephosphorylation. The hydrolysate was evaporated to dryness, and the residue was chromatographed on Whatman No. 1 paper with the solvent ethyl acetate–pyridine–aqueous boric acid.<sup>15</sup> Two spots were observed, revealed by the periodate–benzidine reagents, which corresponded to authentic arabinol and ribitol.

The pentulose 1-phosphate was decomposed by 1 *N* sodium hydroxide at 40° with the release of 75–80% of the phosphate as inorganic phosphate.

The ribulose 1-phosphate was inactive as a substrate for ribulose diphosphate carboxylase from spinach leaves.<sup>16</sup>

**Registry No.**—II, 10043-38-6; III, 7721-51-9; IV, 7721-52-0; V, 7721-53-1; VI, 7721-50-8.

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(15) C. Grado and C. E. Ballou, *J. Biol. Chem.*, **236**, 54 (1961).

(16) E. Racker, "Methods in Enzymology," Vol. V, S. P. Colowick and N. P. Kaplan, Ed., Academic Press Inc., New York, N. Y., 1962, p 266.