Conclusion

Relating the results of the ester biosynthesis from 6-C^{14}-D-glucose to those of tyrosine metabolism, the possibility that methyl *p*-methoxycinnamate is synthesized by *Lentinus lepideus* from glucose *via* shikimic acid must be considered. However, in the ester biogenesis, the specific activity of carbon 1 underwent greater dilution, when compared with carbon 6. This probably is accounted for by an alternative oxidative decarboxylation of carbon 1 of glucose.

It also was observed that carbon 6 of glucose was markedly incorporated into the methoxyl carbon and the ester methyl carbon of the product. This result indicates that the methyl donor may not be a compound which could be derived from the citric acid cycle by this fungus. The unsymmetrical incorporation of carbons 1 and 6 of glucose into these positions gives further support of the occurrence in our organism of a pathway other than E.M.P. glycolysis. However, these considerations may be limited to the cultural conditions under which methyl *p*-methoxycinnamate is produced by *Lentinus lepideus*.

Acknowledgments.—The authors thank Dr. Wm. J. Robbins of the New York Botanical Gardens for the culture of *Lentinus lepideus* used in this study, which was supported by grants of the National Seience Foundation, the U.S. Public Health Service and the U.S. Atomic Energy Commission.

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[CONTRIBUTION FROM THE CHEMISTRY DIVISION OF THE BRITISH COLUMBIA RESEARCH COUNCIL]

Phosphorylated Sugars. V.[1] Syntheses of Arabinofuranose and Arabinopyranose 1-Phosphates

BY R. S. WRIGHT AND H. G. KHORANA

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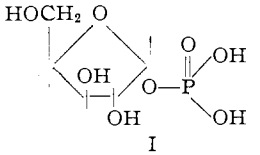
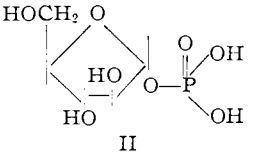
D- and L-arabinofuranose 1-phosphates have been synthesized by treatment of 2,3,5-tri-*O*-acylarabinofuranosyl bromides with triethylammonium dibenzyl phosphate, followed by hydrogenation and alkaline hydrolysis to remove, respectively, the benzyl and acyl groups. The products consisted largely of the α-anomers. The corresponding pyranose 1-phosphates were synthesized by analogous procedures using the appropriate tri-*O*-acylpyranosyl bromides. Methyl 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside was prepared as a crystalline substance in 50% yield by treatment of D-arabinose with methyl alcoholic hydrogen chloride followed by benzoylation and fractional crystallization of the products. Acetylation of D-arabinose in pyridine was shown to give mixtures of furanose and pyranose tetraacetates, with elevated temperatures favoring the formation of the furanose derivatives.

Since the first demonstration by Kalckar[2] of the enzymatic phosphorolysis of certain purine ribonucleosides, a number of investigations have dealt with nucleoside phosphorylases.[3] However, definitive information on important questions such as the mechanism of the action of such enzymes and their substrate specificities, especially with regard to sugar 1-phosphates, has largely been lacking. In recent papers from this Laboratory the syntheses of the anomeric D-ribofuranose 1-phosphates[4-6] were reported and from both chemical and enzymatic evidence it was established that the synthetic α-anomer[6] was identical with the ribose 1-phosphate obtained by the enzymatic phosphorolysis of ribonucleosides. It was thus clear that the ribonucleoside phosphorylases, at least those investigated,[4] brought about an inversion at the glycosidic center during the reaction that they catalyzed.[7] Further work directed to the question of substrate specificities of enzymes of this group required highly purified enzymes and some work along these lines will be reported elsewhere.[10] It also was necessary to make available synthetically some closely related sugar 1-phosphates for testing their suitability as substrates. The work reported in the present communication was therefore undertaken.

It was considered, on the basis of the results already obtained, that a possible substrate for the nucleoside phosphorylases should possess the furanose ring form and that the configuration of the phosphate group at C_1 be α. The two compounds that appeared of immediate interest were D-xylofuranose 1- (I) and D-arabinofuranose 1-phosphates (II). The synthesis of the latter was undertaken first for a number of reasons. Firstly, it differs



I



II

(1) Paper IV in this series, J. G. Moffatt and H. G. Khorana, THIS JOURNAL, **79**, 1194 (1957).

(2) H. M. Kalckar, *J. Biol. Chem.*, **167**, 477 (1947).

(3) Some selected references are: (a) M. Friedkin and H. M. Kalckar, *ibid.*, **184**, 437 (1950); (b) M. Friedkin and D. Roberts, *ibid.*, **207**, 245 (1954); (c) J. O. Lampen, "Phosphorus Metabolism," Vol. II, John Hopkins Press, Baltimore, Md., 1951, p. 363; (d) L. M. Paege and F. Schlenk, *Arch. Biochem. Biophys.*, **40**, 42 (1952).

(4) R. S. Wright and H. G. Khorana, THIS JOURNAL, **77**, 3423 (1955); **78**, 811 (1956).

(5) G. M. Tener and H. G. Khorana, *ibid.*, **79**, 437 (1957).

(6) G. M. Tener, R. S. Wright and H. G. Khorana, *ibid.*, **78**, 506 (1956); **79**, 441 (1957).

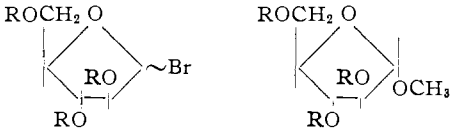
(7) It is worth noting that the recently discovered ribonucleotide pyrophosphorylases, which catalyze the reaction, purine or pyrimidine + 5 phosphoryl ribofuranose α-1-pyrophospbate[8,9] $\rightleftarrows$ ribonucleoside 5'-phosphate + pyrophosphate, also bring about an inversion at the glycosyl bond.

(8) A. Kornberg, I. Lieberman and E. S. Simms, *J. Biol. Chem.*, **215**, 389 (1955).

(9) C. N. Remy, W. T. Remy and J. M. Buchanan, *ibid.*, **217**, 885 (1955).

(10) W. E. Razzell and H. G. Khorana, *Biochim. Biophys. Acta*, in press.

from 2-deoxyribose 1-phosphate,[11] the natural substrate for the deoxyribonucleoside phosphorylases, in the single respect of having a hydroxyl group at C_2 in place of hydrogen. Secondly, the recent discovery by Bergmann and Burke[12] of naturally occurring pyrimidine β-D-arabinofuranosides (spongothymidine and spongouridine) made it of some interest to test the possible enzymatic syntheses of these nucleosides by using the appropriate pyrimidines and II. Finally, from the chemical standpoint, the synthesis of II was anticipated to be relatively straightforward since phosphorylation of a polyacylarabinofuranosyl halide (III) would be expected, by virtue of the participation effect[4,13] of the acyl group at C_2 to give the α-anomer (II). While this work was in progress, Hassid and co-workers[14] reported their interesting discovery in mung bean seedlings of uridine diphosphate arabinose and demonstrated the enzymatic synthesis of this substance from L-arabinopyranose 1-phosphate and uridine 5′-triphosphate. The present work was, therefore, extended to the syntheses of the D- and L-arabinopyranose 1-phosphates.



III, R = acyl IV, R = benzoyl

In the first approach to the synthesis of D-arabinofuranose 1-phosphate an investigation of the methylation of D-arabinose with methanolic hydrogen chloride and subsequent benzoylation of the mixture of products was undertaken along the lines of the researches of Fletcher and co-workers into the syntheses of 2,3,5-tri-*O*-benzoyl-D-ribose[15] and 1,2,3,5-tetra-*O*-benzoyl-D-xylofuranose.[16] Thus D-arabinose was methylated with methanolic hydrogen chloride at room temperature for 7 hours and the sirupy product treated with excess of benzoyl chloride in pyridine. A solution of the resulting product in 95% ethyl alcohol deposited crystalline material which was shown by elemental analysis and by alkaline hydrolysis to methyl α-D-arabinofuranoside,[17,18] to be methyl 2,3,5-tri-*O*-benzoyl α-D-arabinofuranoside. Since this product can be isolated readily in 50% yield in a pure state, the procedure described represents a simple and convenient synthesis of this useful derivative of D-arabinose.[19] IV was converted to the corresponding oily bromide (III, R = benzoyl) by treatment with a mixture of hydrogen bromide in acetic acid.

Recently, Dr. H. G. Fletcher kindly informed us of the work carried out in his laboratory on the benzoylated derivatives of D-arabinofuranose. Drs. Ness and Fletcher have, in fact, been successful in obtaining both anomers of 2,3,5-tri-*O*-benzoyl-D-arabinofuranosyl bromide[19] in a crystalline state.

The alternative starting material, 2,3,5-tri-*O*-acetyl-D-arabinofuranosyl bromide (III, R = acetyl) used in the present work, already has been prepared by Bristow and Lythgoe.[13] The procedure described by these authors was followed except that the intermediate 1,2,3-tri-*O*-acetyl-5-*O*-trityl-D-arabinofuranose was converted to 1,2,3,-5-tetra-*O*-acetyl-D-arabinofuranose in one step by treatment with acetyl bromide in acetic anhydride.[20]

Treatment of 2,3,5-tri-*O*-benzoyl- or acetyl-D-arabinofuranosyl bromide with one equivalent of triethylammonium dibenzyl phosphate[4] in benzene, followed by hydrogenolysis and mild alkaline hydrolysis, to remove the benzyl and the acyl groups, respectively, gave D-arabinofuranose 1-phosphate which was isolated and purified as the barium salt. The yield (50%) obtained using the benzoyl derivative (II, R = benzoyl) was higher than that (35%) obtained with the tri-*O*-acetyl bromide (III, R = acetyl). The analytical data for the products were as expected for an arabinose monophosphate. The products also were characterized by their lability in acidic solution, being hydrolyzed in 0.01 *N* hydrochloric acid at room temperature to the extent of 47% in 4 hours. In this respect D-arabinofuranose 1-phosphate closely resembled the α- and β-D-ribofuranose 1-phosphates[4,6] and, as expected, was more labile than D-arabinopyranose 1-phosphate (see below) which was hydrolyzed to the extent of only 7% under these conditions.

The configuration of the synthetic D-arabinofuranose 1-phosphate was anticipated, as mentioned above, to be α, by analogy with the exclusive formation of β-D-ribofuranose 1-phosphate from 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide.[4] Actually, the evidence obtained from the study of the reactions of the various synthetic samples with dicyclohexyl carbodiimide (DCC) indicated that they, while consisting largely of the expected α-anomer, were contaminated by varying amounts of the β-1-phosphate (V). On the basis of the previous experience of reactions of sugar phosphates,[4,21] *e.g.*, α-D-ribofuranose 1-phosphate, with DCC in aqueous pyridine, V would be expected to react rapidly to form, first, a five-membered cyclic phosphate VI, which would react further to form a phosphorylurea VII. Treatment of samples of pyridinium arabinofuranose 1-phosphates with DCC under standard conditions[21] for 20 hours revealed only a minor spot of a fast travelling material corresponding to a phosphorylurea (presumably VII). Since the reactions proceeding accord-

(11) Although no definite evidence is available so far for the anomeric configuration of this substance, it might be expected, by analogy with the enzymatically prepared ribofuranose 1-phosphate, to have the α-configuration.

(12) W. Bergmann and D. C. Burke, *J. Org. Chem.*, **20**, 1501 (1955).

(13) R. S. Tipson, *J. Biol. Chem.*, **130**, 55 (1939); H. S. Isbell, *Ann. Revs. Biochem.*, **9**, 65 (1940). Thus, Bristow and Lythgoe (*J. Chem. Soc.*, 2306 (1949)) obtained purine α-arabinofuranosides by the condensation of silver salts of purines with tri-*O*-acetylarabinofuranosyl bromide.

(14) V. Ginsburg, P. K. Stumpf and W. Z. Hassid, *J. Biol. Chem.*, **223**, 977 (1956); E. F. Neufeld, V. Ginsburg, E. W. Putman, D. Fanshier and W. Z. Hassid, *Arch. Biochem. Biophys.*, **69**, 602 (1957).

(15) R. K. Ness, D. W. Diehl and H. G. Fletcher, Jr., THIS JOURNAL, **76**, 763 (1954).

(16) H. G. Fletcher, Jr., *ibid.*, **75**, 2624 (1953).

(17) E. M. Montgomery and C. S. Hudson, *ibid.*, **59**, 992 (1937).

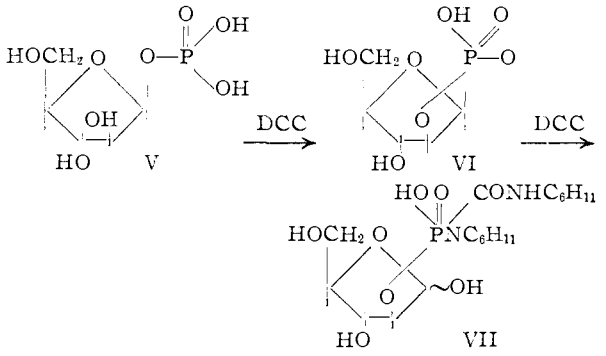
(18) I. Augestad and E. Berner, *Acta. Chem. Scand.*, **8**, 251 (1954).

(19) R. K. Ness and H. G. Fletcher, Jr., THIS JOURNAL, **80**, 2007 (1958).

(20) P. Chang and B. Lythgoe, *J. Chem. Soc.*, 1992 (1950).

(21) H. G. Khorana, G. M. Tener, R. S. Wright and J. G. Moffatt, *ibid.*, **79**, 430 (1957).

ing to V → VI → VII were expected to be complete in the time given, the above result is taken to indicate the presence of only a small amount (5–10%) of V in the synthetic products. The bulk of the starting material remained unchanged, except for some simple hydrolysis to arabinose and inorganic phosphate.[22]

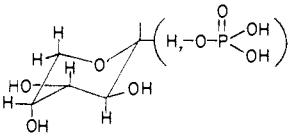


The specific rotations observed for the different synthetic samples of D-arabinofuranose 1-phosphate varied ([α]D −9.7 to +6.4°) and, so, further indicated the lack of configurational purity of the products. In one experiment, a batch of material with $[\alpha]^{22}D$ −5.7° was treated with DCC in aqueous pyridine and the unchanged material, presumably the α-anomer, was separated by chromatography on paper sheets. The rotation of the product, purified as the barium salt, was [α]D +2.2°. The small increase in the rotation thus obtained supports the above conclusion that the synthetic product consisted largely of the α-anomer. The present results would also indicate a small positive rotation for the α-anomer and a relatively high negative rotation for the β-anomer.

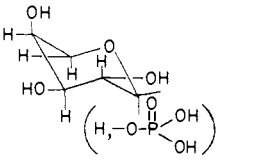
For the preparation of D-arabinopyranose 1-phosphate, D-arabinose was acetylated in pyridine at 0° with acetic anhydride and the sirupy product treated with an excess of hydrogen bromide in acetic acid. The product set to a crystalline mass from which 2,3,4-tri-*O*-acetyl-β-D-arabinopyranosyl bromide[23] was isolated and purified by crystallization in 46% yield. Phosphorylation of this product and then removal of the protecting groups gave D-arabinopyranose 1-phosphate which was first isolated as the barium salt (38%) and then converted to the crystalline dicyclohexylammonium salt.

Crystalline dicyclohexylammonium L-arabinopyranose 1-phosphate was prepared in an analogous fashion, starting either with 2,3,4-tri-*O*-acetyl-β-L-arabinopyranosyl bromide[23] or with 2,3,4-tri-*O*-benzoyl-L-arabinopyranosyl bromide,[24] which, in turn, was prepared from 1,2,3,4-tetra-*O*-benzoyl-α-L-arabinopyranose.[25]

With regard to the configurations of the above pyranose 1-phosphates, no definitive information could be drawn from the study of their reactions with DCC. As was shown earlier,[21] in compounds of this type cyclization to form a five-membered cyclic phosphate and subsequent reaction to form N-phosphorylureas occur both when the relationship of a phosphate group to the adjacent hydroxyl function is axial to equatorial (*cis*) and when this relationship is equatorial to equatorial (*trans*). If as is likely the synthetic D- and L-arabinopyranose 1-phosphates have the IC (VIII) and C1 (IX) conformation, respectively, then the hydroxyl groups at C_2 will be equatorial and cyclization will occur, both when the phosphate groups have the α- or the β-configurations. Both the synthetic samples were, in fact, found to give first the cyclic phosphates and then the phosphorylureas.



VIII, D-Arabinopyranose 1-phosphate



IX, L-Arabinopyranose 1-phosphate

Preparations of L-arabinopyranose 1-phosphates have been carried out independently by Dr. Hassid and his co-workers,[26] starting with 2,3,4-tri-*O*-acetyl-β-L-arabinopyranosyl bromide and using two different methods of phosphorylation. Using Reithel's[27] method which would be expected to give the α-anomer they obtained a sample with [α]D +30.8°, while using the Cori, *et al.*,[28] procedure which should give the β-anomer, a sample with [α]D +91° was obtained. On the basis of these results our crystalline L-arabinopyranose 1-phosphate would appear to be, predominantly, of the α-configuration ([α]D +40.4°). Similarly, our synthetic D-arabinopyranose 1-phosphate ([α]D −39.1°) would be of the same configuration.

During the course of this work, synthetic arabinopyranose and arabinofuranose 1-phosphates were found to separate well on paper chromatograms developed in the solvent system isopropyl alcohol–ammonia–water (7–1–2, v./v.). This finding was made the basis of an alternative and more direct preparation of both these substances. Thus, D-arabinose was acetylated to give a mixture of the furanose and pyranose tetraacetates, which was converted to the corresponding triacetylarabinosyl bromides. This mixture was directly phosphorylated and, after removal of the protecting groups, the pyranose and furanose 1-phosphates were separated on sheets of Whatman 3 MM paper. Table I (Experimental) shows the amounts of the isomeric phosphates obtained under different conditions of acetylation of the pentose. It will be seen that the higher the temperature at which the acetylation is carried out, the greater is the proportion of the furanose 1-phosphate in the final product. The high temperature acetylation thus favored the for-

(22) It seems best to confine the reaction period for the DCC reaction to 24 hours. With longer reaction periods hydrolysis of the pentose phosphate is extensive and also the paper chromatographic picture becomes more complex.

(23) M. Gehrke and F. X. Aichner, *Ber.*, **60**, 918 (1927).

(24) 2,3,4-Tri-*O*-benzoyl-D-arabinopyranosyl bromide was first described by H. G. Fletcher, Jr., and C. S. Hudson, THIS JOURNAL, **72**, 4173 (1950).

(25) M. L. Wolfrom and C. C. Christman, *ibid.*, **58**, 39 (1936).

(26) We are grateful to Dr. W. Z. Hassid for communicating these results, which have now been published: E. W. Putman and W. Z. Hassid, *ibid.*, **79**, 5057 (1957).

(27) F. J. Reitbel, *ibid.*, **67**, 1056 (1945).

(28) C. F. Cori, S. P. Colowick and G. T. Cori, *J. Biol. Chem.*, **121**, 465 (1937).

mation of the furanose tetraacetate. These results are on a parallel with those recorded by Schlubach and Prochownick[29] and by Zinner[30] on the high temperature acetylations, respectively, of D-galactose and D-ribose. Furthermore, it would appear from the present work that the "β-tetra-acetyl D-arabinose"[31] of Deriaz, *et al.*,[32] remaining after the separation of the crystalline α-tetra-*O*-acetyl-D-arabinopyranose, consists largely of 1,2,3,-5-tetra-*O*-acetyl-D-arabinofuranose.

Deriaz, *et al.*,[32] also reported on the acetylation of D-arabinose with acetic anhydride using perchloric acid as the catalyst. A crystalline product, different from the material obtained by the base-catalyzed acetylation, was obtained which was not identified. It appeared to us that under the strongly acidic conditions of this reaction, the equilibrium between the furanose and pyranose forms would be established rapidly and on the assumption that the primary hydroxyl function in the furanose form would be more rapidly acetylated, the furanose tetraacetate would predominate in the reaction product. The perchloric acid-catalyzed acetylation of D-arabinose was repeated and the resulting product, which, in our hands, failed to crystallize, was converted *via* the bromide to the 1-phosphates. The final product was shown by chromatography to consist of *ca.* 90% of the furanose 1-phosphate. It would appear therefore that the crystalline acetate obtained by Deriaz, *et al.*, was, in fact, 1,2,3,5-tetra-*O*-acetyl-D-arabinofuranose.

The synthetic D-arabinofuranose 1-phosphate was found to be inactive as a substrate for the pyrimidine deoxyriboside phosphorylase[10] of *E. coli* and for the purine nucleoside phosphorylase of fish muscle.[33] The synthetic L-arabinopyranose 1-phosphate was kindly tested enzymatically by Drs. Neufeld and Hassid and found to be active in the synthesis of uridine diphosphate arabinose. The corresponding D-arabinose ester as well as the furanose 1-phosphates were inactive.

Experimental

1,2,3,5-Tetra-*O*-acetyl-D-arabinofuranose.—1,2,3-Tri-*O*-acetyl-5-*O*-trityl-D-arabinofuranose was prepared according to Bristow and Lythgoe.[13] To a solution of this compound (14.2 g.) in acetic anhydride[20] (40 ml.) was added 10 ml. of freshly distilled acetyl bromide and the solution allowed to stand at room temperature for 30 minutes. The crystalline trityl bromide which had separated (5.4 g., m.p. 148–150°) was filtered off and the filtrate poured into ice-water (1 liter) with stirring, until the acetic anhyride had hydrolyzed. Trityl alcohol (1.3 g.) which had separated was removed and the solution extracted several times with chloroform. The chloroform solution was washed first with sodium bicarbonate solution then with water and dried over sodium sulfate when removal of the solvent *in vacuo* gave 6.9 g. (79%) of the gummy 1,2,3,5-tetra-*O*-acetyl-D-arabinofuranose.

Methyl 2,3,5-Tri-*O*-benzoyl-α-D-arabinofuranoside.—D-Arabinose (5 g.) was suspended in 200 ml. of freshly prepared 1% methanolic hydrogen chloride and the mixture shaken at room temperature. The solution was completely clear after *ca.* 1 hour and the reducing power had disappeared after *ca.* 2.5 hours. After shaking for a total period of 7 hours, pyridine (25 ml.) was added and the volatile material removed *in vacuo*, the last traces of methyl alcohol being removed by two evaporations with 20-ml. portions of pyridine *in vacuo*. The gummy residue was dissolved in 40 ml. of pyridine and 30 ml. of benzoyl chloride added slowly with cooling. After the reaction had subsided the mixture was heated at 40° for 1.5 hours. The excess of benzoyl chloride was decomposed by the addition of ice chips and the mixture extracted with chloroform. The chloroform solution was washed, first with water, then with ice-cold potassium bisulfate solution, next with sodium bicarbonate solution and finally with water. The dried solution was evaporated *in vacuo* and the residue dissolved in 200 ml. of hot 95% ethyl alcohol. The solution deposited on cooling 7.9 g. (50%) of crystalline material with m.p. 98–101°. Recrystallization of this product from ethyl alcohol and then benzene–light petroleum ether gave 7.6 g. of methyl 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside (m.p. 100–101.5°) $[\alpha]^{21}D$ −19.1° (*c* 2.05, chloroform). *Anal.* Calcd. for $C_{27}H_{24}O_8$: C, 68.06; H, 5.08. Found: C, 68.02; H, 4.93.

Ness and Fletcher[19] found m.p. 101–103° and $[\alpha]^{20}D$ −19.5° in chloroform (*c* 2.67) for this substance.

Alkaline hydrolysis of the above product gave methyl α-D-arabinofuranoside which was crystallized at low temperature from a small volume of ethyl acetate. It had m.p. 50° and $[\alpha]^{22}D$ +125.2° (*c* 1.47, water). Montgomery and Hudson[17] quote 65–67°, $[\alpha]D$ +123°; Augestad and Berner[18] give m.p. 52° and $[\alpha]^{20}D$ +128°.

D-Arabinofuranose 1-Phosphate. (a) **From 1,2,3,5-Tetra-*O*-acetyl-D-arabinofuranose.**—To a solution of 1 g. of 1,2,3,5-tetra-*O*-acetyl-D-arabinofuranose in 5 ml. of dry dichloromethane were added 3 ml. of 32% hydrogen bromide in acetic acid and 0.2 of acetic anhydride and the solution kept at room temperature for one hour. The solvent and the excess of reagent were evaporated *in vacuo* and the last traces removed by codistillation (three times) with toluene *in vacuo*. The residual bromide was dissolved in 5 ml. of dry benzene and to the solution was added a benzene solution of one equivalent of triethylammonium dibenzyl phosphate. After one hour at room temperature the crystalline triethylammonium bromide (0.46 g., 81%) was filtered off and washed with dry benzene. The combined filtrate was concentrated under reduced pressure and at room temperature to a gum which was taken up in anhydrous methyl alcohol. The solution was filtered and hydrogenated at 0° in the presence of palladium on charcoal catalyst (0.5 g. of 10%). After the uptake of hydrogen had ceased (156 ml. absorbed; theoretical, 132 ml.) the catalyst was removed and the solution diluted (25% by volume) with water. It was then brought to and maintained at *p*H 11.3 with 1 *N* sodium hydroxide solution for three hours. Some sodium phosphate which separated was removed and the solution then treated with an excess of pyridinium IR-120 resin. After the removal of the resin, the solution was concentrated *in vacuo* to a small volume (about 5 ml.) with the occasional addition of a few drops of pyridine to maintain approximately neutral *p*H. Saturated aqueous barium hydroxide was added to *p*H 10.5 and the resulting precipitate collected by centrifugation. It was extracted with three 5-ml. portions of water and the combined extract treated with 1.5 volumes of ethyl alcohol. After being kept for one hour at 0°, the precipitate was collected by centrifugation, washed first with 60% ethyl alcohol, then ethyl alcohol and finally ether. Two further reprecipitations of the product from aqueous solution with ethyl alcohol and final washes as above gave 0.511 g. of the amorphous barium D-arabinofuranose 1-phosphate; $[\alpha]^{23}D$ +6.4° (*c* 1.7, water). A sample was dried at 100° for 2 hours *in vacuo*. *Anal.* Calcd. for $C_5H_9O_8P \cdot Ba \cdot 1H_2O$: arabinose, 39.1; P, 8.08. Found: arabinose,[34] 38.7; P,[35] 8.1. On paper chromatograms in isopropyl alcohol–ammonia–water (7–1–2, v./v.)[36] it travelled as a single spot with R_f 0.10.

(29) H. H. Schlubach and V. Prochownick, *Ber.*, **63**, 2298 (1930). See also R. K. Ness, H. G. Fletcher, Jr., and C. S. Hudson, THIS JOURNAL, **73**, 3742 (1951).

(30) H. Zinner, *Ber.*, **83**, 153 (1950).

(31) This gave only a very low yield of the pyranosyl bromide, while the crystalline α-tetra-*O*-acetyl-D-arabinopyranose gave a high yield of the bromide.

(32) R. E. Deriaz, W. G. Overend, M. Stacey, E. G. Teece and L. F. Wiggins, *J. Chem. Soc.*, 1879 (1949).

(33) H. L. A. Tarr, *Federation Proc.*, **15**, 369 (1956). We are grateful to Dr. Tarr for the enzymatic test.

(34) D. H. Brown, *Arch. Biochem. Biophys.*, **11**, 269 (1946).

(35) O. H. Lowry and J. A. Lopez, *J. Biol. Chem.*, **162**, 421 (1946).

(36) D. M. Brown and A. R. Todd, *J. Chem. Soc.*, 2040 (1952).

(b) **From Methyl 2,3,5-Tri-*O*-benzoyl-α-D-arabinofuranoside.**—Three grams of methyl 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside was dissolved in 7.5 ml. of dry dichloromethane and the solution treated with 7.5 ml. of 32% hydrogen bromide in acetic acid and 0.5 ml. of acetic anhydride. The resulting solution was allowed to stand at room temperature for 2 hours and was then concentrated *in vacuo* and the last traces of the reagents removed by codistillation with toluene (three 15-ml. portions). Phosphorylation of the resulting bromide and workup in the manner described above gave 1.3 g. of barium D-arabinofuranose 1-phosphate, $[\alpha]^{20}D$ −5.7° (*c* 0.97, water).

2,3,4-Tri-*O*-acetyl-β-D-arabinopyranosyl Bromide.[23]—Four grams of D-arabinose was dissolved in 15 ml. of boiling pyridine and the solution rapidly cooled. Twenty-five ml. of acetic anhydride was added with further cooling at 0°. After standing overnight at 0°, the solution was poured into ice-water with stirring and the resulting solution extracted several times with chloroform. The chloroform solution was washed with water, then with sodium bicarbonate solution and dried over sodium sulfate. Evaporation *in vacuo* and removal of the residual pyridine with toluene gave a gum which was dissolved in 30 ml. of 32% hydrogen bromide in acetic acid and 0.3 ml. of acetic anhydride. Evaporation after one hour and codistillation with toluene gave a partly crystalline residue. The crystalline material was separated from the oil by an ether wash and was recrystallized from a mixture of benzene and petroleum ether (b.p. 65–110°) to give 4.2 g. (46%) of 2,3,4-tri-*O*-acetyl-β-D-arabinopyranosyl bromide, m.p., 137°, Gehrke and Aichner[23] quote 139°; $[\alpha]^{20}D$ −290.7° (*c* 3.65, chloroform).

2,3,4-Tri-*O*-acetyl-β-L-arabinopyranosyl Bromide.—This was prepared by the method described above and had m.p. 137°, $[\alpha]^{20}D$ +287° (*c* 3.49. chloroform). Deriaz, *et al.*,[32] quote m.p. 138°, $[\alpha]^{20}D$ +282° (*c* 3.67, chloroform).

D-Arabinopyranose 1-Phosphate.—Three grams of the 2,3,4-tri-*O*-acetyl-β-D-arabinopyranosyl bromide was taken through the standard phosphorylation procedure described above for the preparation of the furanose 1-phosphate; $[\alpha]^{24}D$ −44.3° (*c* 2.12, water). *Anal.* Calcd. for $C_5H_9O_8P \cdot Ba \cdot 1H_2O$: arabinose, 39.1; P, 8.08. Found: arabinose, 38.8; P, 8.1. The dicyclohexylammonium salt was prepared by passing an aqueous solution of the barium salt (500 mg.) through cyclohexylammonium IR-120 resin. The effluent and washings were concentrated to dryness *in vacuo* and the residual solid dissolved in hot methyl alcohol. Addition of ether to turbidity gave the crystalline dicyclohexylammonium salt which was recrystallized in the same manner; yield 350 mg. *Anal.* Calcd. for $C_{17}H_{37}O_8N_2P$: C, 47.6; H, 8.70; N, 6.54. Found: C, 47.2; H, 9.0; N, 6.7; $[\alpha]^{21}D$ −39.1° (*c* 2.07, water).

L-Arabinopyranose 1-Phosphate.—This was prepared as the barium salt from 2,3,4-tri-*O*-acetyl-β-L-arabinopyranosyl bromide exactly as above, $[\alpha]^{24}D$ +48.2° (*c* 2.09, water). The crystalline dicyclohexylammonium salt prepared from the barium salt had $[\alpha]^{21}D$ +40.4° (*c* 2.11, water). *Anal.* Calcd. for $C_{17}H_{37}O_8N_2P$: C, 47.6; H, 8.70; N, 6.54. Found: C, 47.8; H, 9.0; N, 6.7.

A sample of crystalline dicyclohexylammonium L-arabinopyranose 1-phosphate also was prepared from 1,2,3,4-tetra-*O*-benzoyl-α-L-arabinopyranose.[25] It had $[\alpha]^{23}D$ +45° (*c* 1.19, water).

D-Arabinofuranose and D-Arabinopyranose 1-Phosphates by Direct Acetylation of D-Arabinose. A. Base-Catalyzed.—In a series of experiments, 0.5-g. portions of D-arabinose were dissolved in boiling pyridine and the cooled solutions acetylated at various carefully controlled temperatures by the slow addition of acetic anhydride.

B. **Acid-catalyzed Acetylation.**[37]—One gram of D-arabinose was suspended in 5 ml. of acetic acid and 0.1 ml. of perchloric acid (72%) at 60° and a mixture of 7 ml. of acetic anhydride and 7 ml. of acetic acid run in slowly (2 hours) with stirring. The D-arabinose dissolved and the solution was kept at 60° for a further two hours and then at room temperature overnight. In all cases, the reaction mixtures were worked up by pouring into water and extracting the tetraacetates into chloroform. These were then converted to the corresponding bromides and phosphorylated by procedures already described. The resulting mixtures of the barium arabinofuranose and arabinopyranose 1-phosphates, which averaged in yield about 35% of theoretical, were converted to the pyridinium salts and separated by descending paper chromatography for three days in the isopropyl alcohol–ammonia system using sheets of Whatman No. 3 MM paper (200 mg. of the mixed barium salts per sheet). The separated bands were eluted with water and the esters converted to the barium salts. The amounts of isomeric phosphates recovered from a paper sheet to which 200 mg. of the original mixture had been applied are listed in Table I.

TABLE I

	Conditions of acetylation	Arabinofuranose 1-phosphate (mg.)	Arabinopyranose 1-phosphate (mg.)
(1)	0° in pyridine	59	75
(2)	20° in pyridine	79	91
(3)	80° in pyridine	128	30
(4)	60° in perchloric acid	130	18

L-Arabinofuranose 1-Phosphate.—This was prepared by the acetylation of L-arabinose at room temperature and subsequent phosphorylation. From three grams of the pentose 2.17 g. of the mixture of barium salts of the furanose and pyranose 1-phosphates was obtained. Five hundred mg. of this mixture yielded, after paper chromatographic separation as described above, 201 mg. of the L-arabinofuranose 1-phosphate, $[\alpha]^{23}D$ +16.9° (*c* 0.98, water).

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(37) Modification of the procedure of Deriaz, *et al.* (*cf.* 32).