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

Phosphorylated Sugars. I. A Synthesis of β-D-Ribofuranose 1-Phosphate

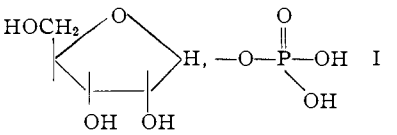
BY R. S. WRIGHT AND H. G. KHORANA

RECEIVED SEPTEMBER 12, 1955

D-Ribofuranose 1-phosphate has been synthesized by the treatment of 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl 1-bromide with triethylammonium dibenzyl phosphate, followed by hydrogenation and alkaline treatment of the product to remove, respectively, the benzyl and the benzoyl groups. The synthetic sample was inactive as a substrate for the fish muscle purine nucleoside phosphorylase. On the basis of enzymatic as well as chemical evidence it has been concluded that the configuration of the synthetic sample is β, in contrast with the α configuration of enzymatically prepared samples of ribose 1-phosphate. D-Ribopyranose 1-phosphate has also been synthesized by standard procedures.

It was first shown by Kalckar[1] that a mechanism similar to the phosphorolysis of polysaccharides operated in the enzymatic cleavage of the N–glycosyl bond in certain purine ribonucleosides. A highly acid-labile aldopentose phosphate, concluded to be ribose 1-phosphate, was isolated as a product of the action of the mammalian "nucleoside phosphorylase" on inosine. Subsequent studies[2] reported from different laboratories have demonstrated the widespread occurrence of nucleoside phosphorylases, which have been classified, according to their substrate specificity, as purine ribonucleoside (or deoxyribonucleoside) phosphorylases and pyrimidine nucleoside (or deoxyribonucleoside) phosphorylases. While other mechanisms of enzymatic degradation and synthesis of nucleosides are known, phosphorolysis is probably of considerable significance in the nucleotide metabolism.[3]

The evidence in favor of structure I (D-ribofuranose 1-phosphate) for the product of the phosphorolysis of ribonucleosides may be summarized briefly. The ester, which is non-reducing, is very much more acid-labile than the known ribose 2-, -3- or -5-monophosphates[4] and on hydrolysis liberates equivalent amounts of the reducing sugar (D-ribose) and inorganic phosphate.[1] Certain well-defined purine and pyrimidine D-ribofuranosides may be synthesized from this ester and the heterocyclic bases in the presence of appropriate phosphorylases.[1,5,6] The furanose structure for the lactol ring is further supported by the observation[7] that a synthetic sample of ribopyranose 1-phosphate[8] was much more stable to acid than the enzymatic sample. The configuration of the phosphate group at C_1 in structure I has so far not been established although speculations in favor of both, α[10] and β,[11] configurations have been advanced.



I

No chemical synthesis of ribofuranose 1-phosphate or any other pento- or hexofuranose 1-phosphate has been accomplished so far. The present series of investigations has been undertaken to make available synthetically some selected members of this group, so that their chemical properties and suitability as substrates for the various nucleoside phosphorylases may be studied. It is hoped that studies of this kind will help gain an understanding of the mechanism of action of the nucleoside phosphorylases and also provide information concerning their specificity with respect to the nature of the sugar component in the substrates. In this communication we wish to present the first phase of our work which has led to the synthesis of β-D-ribofuranose 1-phosphate and has furnished direct chemical as well as enzymatic evidence that the configuration of the phosphate residue in samples of ribose 1-phosphate prepared by the use of two phosphorylases of different origin must be α. A preliminary account of this work has already appeared.[12]

A comprehensive review of the methods which have previously been used for the synthesis of sugar phosphates has been provided by Leloir.[11a] It can be seen from this account that all the syntheses of sugar 1-phosphates reported have utilized suitably protected sugar derivatives in which the hemiacetal hydroxyl is replaced by a halide group. These halides are brought into reaction with metal salts of phosphoric acid or more often phosphate esters such as dibenzyl and diphenyl phosphates. After hydrogenolysis of the benzyl or phenyl groups, the sugar hydroxyls are uncovered under mildly alkaline conditions. An analogous route to the synthesis of D-ribofuranose 1-phosphate using a well-characterized D-ribofuranose derivative appeared feasible although it was expected that the problem

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

(2) Excellent reviews of the biosynthesis of nucleosides and nucleotides are available. (a) H. M. Kalckar, "The Enzymes of Nucleoside Metabolism," in L. Zechmeister, "Progress in the Chemistry of Organic Natural Products," Vol. IX, Springer Verlag, Vienna, 1952, p. 363; (b) G. Schmidt, "Enzymes Attacking Nucleic Acids" in the "The Nucleic Acids," Academic Press, Inc., New York, N. Y., Vol. I, 1955, p. 555; (c) F. Shlenk, "Biosynthesis of Nucleosides and Nucleotides," ref. 2b, Vol. II, 1955, p. 309.

(3) See, for example: H. M. Kalckar, *Biochim. Biophys. Acta*, **12**, 250 (1953).

(4) For references on these ribose phosphates see J. X. Khym, D. G. Doherty and W. E. Cohn, THIS JOURNAL, **76**, 5523 (1954).

(5) M. Friedkin, *ibid.*, **74**, 112 (1952); M. Friedkin and D. Roberts, *J. Biol. Chem.*, **207**, 257 (1954).

(6) H. L. A. Tarr, *Federation Proc.*, **14**, 291 (1955), and unpublished.

(7) Ref. 3, p. 257.

(8) Since the anomeric configurations of the enzymatically prepared ribose 1-phosphate and the synthetic ribopyranose 1-phosphate have not been defined, the reported inactivity of the latter, as a substrate for the inosine phosphorylase,[9] which could be ascribed to different configurations of the two samples, does not constitute evidence in favor of the furanose structure of the enzymatic sample.

(9) H. M. Kalckar, *Biochim. Biophys. Acta*, **4**, 232 (1950).

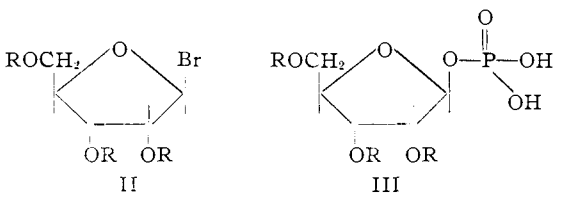
(10) H. M. Kalckar and H. Klenow, *Ann. Rev. Biochem.*, **23**, 527 (1954).

(11) (a) L. F. Leloir, "Sugar Phosphates" in L. Zechmeister, "Progress in the Chemistry of Organic Natural Products," Vol. VIII, Springer Verlag, Vienna, 1951, p. 47; (b) G. W. Overend and M. Stacey, "The Nucleic Acids," Vol. I, Academic Press Inc., New York, N. Y., 1955, p. 9.

(12) R. S. Wright and H. G. Khorana, THIS JOURNAL, **77**, 3423 (1955).

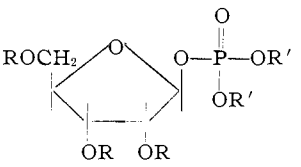
of synthesis would be complicated by the extreme lability of this ester and its derivatives. 2,3,5-Tri-*O*-benzoyl-β-D-ribose, whose preparation from D-ribose in good yield has recently been described by Ness, Diehl and Fletcher,[13] was chosen as the starting material.[14] Since the final step in the projected synthesis involved alkaline treatment, the reported stability[2a] of enzymatically prepared samples of ribose 1-phosphate to alkali was confirmed at the outset of these experiments. (This stability is discussed later.)

2,3,5-Tri-*O*-benzoyl-β-D-ribose was converted to the corresponding ribofuranosyl 1-bromide[15] (II) to which the β-configuration has been assigned by Ness and Fletcher.[16] The reaction of this bromide with monosilver phosphate in an inert solvent at low temperature was studied first. Although some reaction appeared to occur, as was shown by the appearance of a fast-travelling phosphorus-containing material (presumably III) on paper chromatograms, no water-soluble barium salt of an organic phosphate could be isolated after removal of the benzoyl groups.



II III

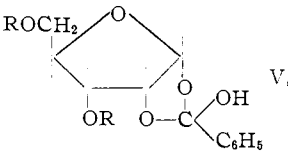
II–IV, R = benzoyl



IV, R′ = Benzyl

The use of silver dibenzyl phosphate was next investigated. A large number of experiments were carried out at low temperature, employing chloroform and dichloromethane as the medium. Reaction again occurred as shown by the formation of silver bromide. After 3 hours the insoluble salts were removed and the reaction mixtures hydrogenated at low temperature in aqueous methyl alcohol in the presence of freshly prepared palladium catalyst. Paper chromatography of the neutralized hydrogenation product showed again the presence of an appreciable amount of a fast-travelling labile phosphate III but a large proportion of inorganic phosphate also was formed. Debenzoylation under alkaline conditions was again followed by paper chromatographically and the total phosphates were then precipitated as barium salts. Extraction of this precipitate with water gave a variable but always low yield (2–5%) of the water-soluble barium salt of ribose 1-phosphate, the characterization of which is described below.

The formation of large amounts of inorganic phosphate[17] in the above experiments required the decomposition either of IV or of III, during and after hydrogenation. The breakdown of the neutral ester, IV, which was expected to be extremely labile, was considered to play an important part. To reduce losses a much shorter reaction period appeared advisable and advantage was taken of the high solubility in benzene of triethylammonium dibenzyl phosphate. When a cooled benzene solution of the latter was added to a precooled solution of freshly prepared II, the reaction was rapid and triethylamine hydrobromide soon separated. Our initial attempts to isolate the product IV of the reaction only confirmed our view regarding the great lability of this substance. Attempts at crystallization of the oily product led to the isolation of a small amount of a substance with m.p. 142–143° which did not contain phosphorus. From the mother liquors dibenzyl phosphate could be extracted with sodium hydrogen carbonate. The identification of the substance with m.p. 142–143° as V was conclusively established through direct comparison (m.p., mixed m.p. and infrared spectra) with a sample of this substance prepared by the method of Ness and Fletcher.[16] The extreme lability of IV[18] emphasized the necessity of working in completely anhydrous media, and it appeared that direct hydrogenation of a short period reaction run at low temperature was desirable in order to secure some stabilization of the ester through the creation of a phosphoryl dissociation.[19] This view was borne out by experiment and after complete hydrogenation in anhydrous methyl alcohol followed by removal of the benzoyl groups, a considerably improved yield (20%) of ribose 1-phosphate was obtained.



V, R = Benzoyl

This material after purification by reprecipitation from water–ethyl alcohol mixtures was free from inorganic phosphate[20] and was non-reducing.[21] Paper chromatography in several solvent systems showed this material to be homogeneous and identical in its behavior with an enzymatically prepared

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

(14) It is worthy of note that the usefulness of this derivative for the synthesis of ribonucleosides has been demonstrated very recently by the elegant work of Baker and co-workers (H. M. Kissman, C. Pidacks and B. R. Baker, THIS JOURNAL, **77**, 18 (1955)).

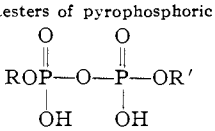
(15) Reference 13, footnote 19.

(16) R. K. Ness and H. G. Fletcher, THIS JOURNAL, **76**, 1663 (1954).

(17) Because of the very limited solubility of silver dibenzyl phosphate in the solvents employed it is extremely unlikely that the inorganic phosphate arose from the hydrogenation of unreacted material.

(18) The manner of its decomposition thus appears to be similar to that of the hydrolysis of II studied by Ness and Fletcher (ref. 16).

(19) An analogy may be drawn between the labile ester, IV, and the fully substituted pyrophosphates which also are usually very labile. Conversion of the tetraesters of pyrophosphoric acid to the diesters



confers enhanced stability on the pyrophosphate linkage (*cf.* S. M. H. Christie, G. W. Kenner and A. R. Todd, *J. Chem. Soc.*, 46 (1954)).

(20) Determined by the method of O. H. Lowry and J. A. Lopez, *J. Biol. Chem.*, **162**, 421 (1946).

(21) M. Macleod and R. Robison, *Biochem. J.*, **23**, 517 (1929).

sample of ribose 1-phosphate.[22] The analytical data of the synthetic sample were in agreement with the values required for an aldopentose monophosphate. On very gentle acidic hydrolysis, the sample liberated inorganic phosphate and ribose. The latter was identified by paper chromatography in three solvent systems all of which differentiated between authentic D-ribose and D-arabinose.[23]

The route employed and the properties of the synthetic ester left little doubt concerning the structure of this product as D-ribofuranose 1-phosphate. It was, however, considered desirable to obtain further support in favor of the furanose structure through direct comparison of this product with a sample of D-ribopyranose 1-phosphate, prepared by standard procedures. The latter ester has been briefly mentioned in the literature, but, as far as we are aware, no details of the synthesis of this substance have been recorded. In our work, we employed the previously characterized 2,3,4-tri-*O*-acetyl-D-ribopyranosyl 1-bromide.[24] This was brought into reaction with silver dibenzyl phosphate and the protecting groups were removed by successive hydrogenation and alkaline treatment as before. D-Ribopyranose 1-phosphate was isolated as the highly crystalline barium salt (tetrahydrate) in a satisfactory yield (54%). As recorded previously[7] this ester was found to be much more stable to acid than the synthetic as well as the enzymatically prepared samples of ribofuranose 1-phosphates (see hydrolysis curves, Fig. 1).

Enzymatic tests on the synthetic and the natural samples of ribofuranose-1-phosphate were kindly carried out by Dr. H. L. A. Tarr. These experiments which will be reported upon in detail by him elsewhere, showed that the synthetic sample was inactive as a substrate for the fish muscle nucleoside phosphorylase. This inactivity of the synthetic product was the first evidence which indicated that the natural and the synthetic samples differed in their anomeric configurations. The results of the extensive studies which have been carried out on various polysaccharide phosphorylases support this conclusion. Thus the synthetic β-D-glucose 1-phosphate[25] is inactive as a substrate for the muscle[26] as well as sucrose[27] phosphorylases and α-D-glucose 1-phosphate[28] is not a substrate for the maltose phosphorylase.[29]

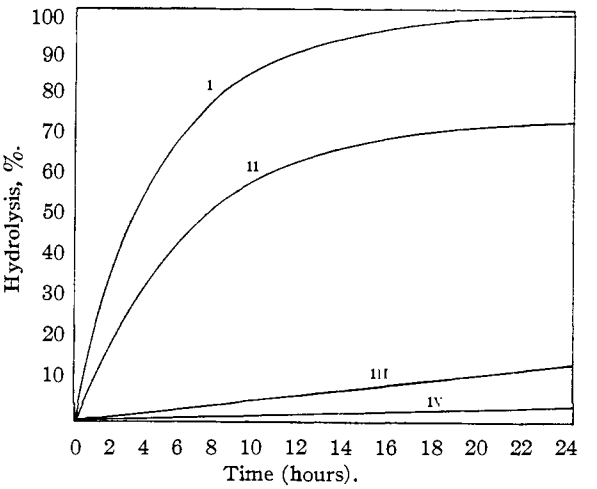
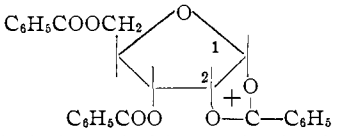


Fig. 1.—Hydrolysis of ribose phosphates in 0.01 *N* HCl at 20°: curve I, ribofuranose α-1-phosphate; II, ribofuranose β-1-phosphate; III, ribopyranose 1-phosphate; IV, product X of reaction of ribose α-1-phosphate with DCC.

The β-configuration for the synthetic ribose 1-phosphate appeared likely on the basis of the existing knowledge concerning the synthesis of nucleosides using acylfuranosyl 1-halides and heavy metal salts of purines and pyrimidines. It has already been generalized by Baker and co-workers[30] that in these syntheses nucleosides having a C_1-C_2 *trans* configuration are obtained, regardless of the relative configuration at C_1-C_2 of the original halo sugar. The β-configuration having been assumed for the synthetic sample it followed from the above that the enzymatically prepared sample had the α configuration. Strong support for these assignments was provided by the optical rotations[31] of the two samples. The cyclohexylamine salt of an enzymatically prepared sample of ribose 1-phosphate is dextrorotatory[32] [$[\alpha]^{24}D$ +53° (*c* 0.0519, in water)] whereas the barium salt of our synthetic sample is levorotatory [$[\alpha]^{23}D$ −9.28° (*c* 4.52 in water)]. Verification of these assignments could perhaps have been obtained by following the changes in the optical rotation upon periodate oxidation of the above samples and of authentic α-D-glucopyranose 1-phosphate. The latter substance and α-D-ribofuranose 1-phosphate should both give rise to the hypothetical dialdehyde (VII).[33] However, a direct ap-

(22) This sample was kindly furnished by Dr. H. L. A. Tarr and was prepared by the phosphorolysis of guanosine using the fish muscle nucleoside phosphorylase (ref. 6).

(23) In the replacement of bromide by dibenzylphosphate ion (II → IV) the cation VI is probably an intermediate (see *e.g.*, R. U. Lemieux, *Advances in Carbohydrate Chem.*, **9**, 1 (1954) and ref. 30). Although highly improbable, the formation of D-arabinose 2-phosphate



VI

through the backside attack of dibenzylphosphate ion on VI at C_2 was considered.

(24) (a) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **92**, 109 (1931); (b) for other references on this substance see R. W. Jeanloz and H. G. Fletcher, *Advances in Carbohydrate Chem.*, **6**, 135 (1951) and ref. 9(b).

(25) (a) L. Zervas, *Naturwiss.*, **27**, 317 (1939); (b) M. L. Wolfrom, C. S. Smith, D. E. Pletcher and A. E. Brown, THIS JOURNAL, **64**, 23 (1942).

(26) C. F. Cori, G. T. Cori and A. A. Green, *J. Biol. Chem.*, **151**, 39 (1943).

(27) M. Doudoroff, *ibid.*, **151**, 351 (1943).

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

(29) C. Fittig and M. Doudoroff, *ibid.*, **199**, 153 (1952).

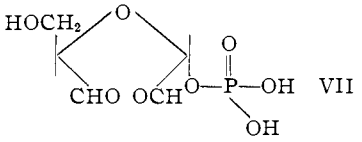
(30) B. R. Baker, J. P. Joseph, R. E. Schaub and J. H. Williams, *J. Org. Chem.*, **19**, 1786 (1954); B. R. Baker and R. E. Schaub, THIS JOURNAL, **77**, 2396 (1955).

(31) C. S. Hudson, *ibid.*, **31**, 66 (1909). The assignment of anomeric configurations to glucose-1-phosphates on the basis of optical rotations has been made by M. L. Wolfrom, *et al.* (ref. 25b).

(32) We are grateful to Drs. D. H. Hayes and H. M. Kalckar for a generous sample of the crystalline dicyclohexylammonium ribose 1-phosphate and for communicating the optical rotation of this sample.

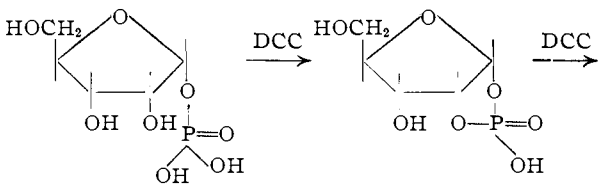
(33) E. L. Jackson, "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 341. See also, J. Davoll, B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 833 (1946), for the use of this technique in the correlation of the configurations of the natural and synthetic nucleosides.

proach was suggested by the work of Dekker and Khorana[34] on the reactions of phosphate esters

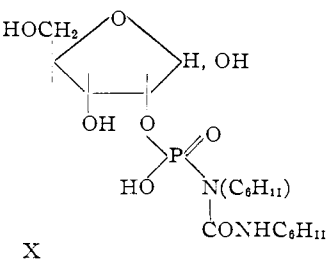


VII

bearing an adjacent *cis*-hydroxyl function with dicyclohexylcarbodiimide (DCC). It was established that these esters react to give, first, the cyclic phosphates (*e.g.*, IX) and that the latter react further to form the N-phosphorylureas (*e.g.*, X). This reaction sequence may be followed readily by paper chromatography in suitable solvent systems, the mobilities of the reaction products following the order X > IX > VIII. It is clear that, because of the more or less planar nature of the furanose ring, of the two anomeric ribofuranose 1-phosphates, only the α phosphate is able to form a five-membered cyclic ring and subsequently give rise to X.



VIII IX



X

A study of the reaction of the pyridine salts of the natural and synthetic samples of ribofuranose 1-phosphate with DCC showed the formation of IX and X from the natural material only, thus showing that the latter has the α-configuration. The synthetic ribose 1-phosphate, for which the β-configuration becomes confirmed, would be expected to undergo the much slower conversion to the corresponding pyrophosphate,[35] and, under the conditions of this experiment, remained largely uneffected. The phosphoryl urea, X, was found to be much more stable than either of the ribofuranose 1-phosphates (see Fig. 1). Its great stability supports the structure X assigned to it. On acidic treatment it would be expected[34] to form first ribose-2,3-cyclic phosphate, which would immediately hydrolyze to a mixture of the relatively stable ribose 2- and 3-phosphates.

The configuration of deoxyribose 1-phosphate, obtained from the phosphorolysis of the different deoxyribosides remains uncertain.[36] If it is assumed that the same enzyme is involved in the phosphorolysis of nucleosides and deoxynucleosides then it may tentatively be suggested that the mechanism of phosphorolysis in the two series is analogous and that the configuration of deoxyribose 1-phosphate is also α.

A detailed discussion of the mechanisms of the action of the various nucleoside phosphorylases is reserved until further studies can be reported, but a few observations may be made here. So far, two samples of ribose 1-phosphate, obtained by the phosphorolysis of nucleosides using enzymes of fish[22] and mammalian origin,[32] have been examined and both have been found to possess the α-configuration. It is therefore clear that at least these two phosphorylases bring about an inversion of configuration during the cleavage of purine (pyrimidine)-β-D-ribofuranosides. Stereochemically, their action is similar to that of maltose phosphorylase[29] and, as suggested by Koshland[37] for the latter, proceeds probably by a single displacement mechanism. In contrast, the muscle[26] and sucrose[27] phosphorylases catalyze reactions which proceed with retention of configuration. Further, the inability of "thymidine phosphorylase" of mammalian origin to catalyze the exchange between ^{32}P-labeled inorganic phosphate and deoxyribose 1-phosphate has been recorded by Friedkin and Roberts.[38] Maltose phosphorylase, similarly, is unable to bring about an exchange between ^{32}P-labeled inorganic phosphate and its substrate, β-D-glucose 1-phosphate.[29] It should be noted, however, that this inability is not necessarily a characteristic of the enzymes which bring about an inversion in configuration.[39]

Attention is drawn to the great stability of ribose 1-phosphate to alkali. Both the synthetic and the enzymatic samples were found to be completely stable to 0.5 *N* sodium hydroxide solution at 80° for 1 hour. This stability is very much higher than that of the isomeric 2-, 3- and 5-phosphates, all of which would be expected to be alkali-labile by virtue of the free "aldehyde" group in them.

Experimental

Materials.—The D-ribose was a commercially available sample; m.p. 84–85.5° (reported m.p. 86–87)[9b]; $[\alpha]^{23}D$ −19°; *c* 8.53, in water. Monosilver phosphate was prepared from silver acetate and aqueous phosphoric acid using equimolecular preparations. The product was precipitated by the addition of ethyl alcohol, dried and stored in the dark before use. Silver dibenzylphosphate was prepared by the method of Reithel.[40]

Attempted Preparation of Ribose 1-Phosphate Using Monosilver Phosphate.—2,3,5-Tri-*O*-benzoyl-β-D-ribose (1 mmole) was converted to the corresponding 1-bromide (II) according to Ness, *et al.*,[13,15] the excess of reactants being removed by codistillation with toluene. It was dissolved in chloroform (10 ml.), finely powdered monosilver phosphate (2 g., *ca.* 8 mmoles) added and the mixture shaken in the presence of glass beads at 0° in the dark for 2 hr. (periods of from 0.5–5 hr. have been tried). The insoluble salts were then separated by centrifugation and the solvent removed *in vacuo*. The residue was taken up in aqueous ethyl alcohol and neutralized with sodium hydroxide solution. Paper chromatography in the solvent system A (see below) revealed the presence of large amounts of inorganic

(34) C. A. Dekker and H. G. Khorana, THIS JOURNAL, **76**, 3522 (1954); see also G. M. Tener and H. G. Khorana, *ibid.*, **77**, 5349 (1955).

(35) H. G. Khorana, *ibid.*, **76**, 3517 (1954).

(36) Obviously neither oxidation with periodic acid nor the cyclization test herein described can be applied to the solution of this problem.

(37) For recent discussions of the mechanisms of the action of phosphorylases see D. E. Koshland, Jr., in "The Mechanism of Enzyme Action," John Hopkins Press, Baltimore, Md., 1954, p. 608, and H. M. Kalckar, p. 675.

(38) M. Friedkin and D. Roberts, *J. Biol. Chem.*, **207**, 245 (1954).

(39) M. Cohn and G. T. Cori, *ibid.*, **176**, 89 (1948).

(40) J. F. Reithel, THIS JOURNAL, **67**, 1056 (1945).

phosphate and a faster-travelling labile phosphate (R_f 0.76). After removal of the benzoyl groups and precipitation of the barium salts, as described below, no water-soluble barium salt could be isolated.

Preparation of Ribose 1-Phosphate Using Triethylammonium Dibenzylphosphate.—Freshly prepared II (from 1 g. of 2,3,5-tri-*O*-benzoylribose) was treated in benzene solution at 5° with one equivalent of triethylammonium dibenzyl phosphate (triethylamine, 0.220 g., dibenzyl phosphate, 0.601 g.). After one-half hour, the triethylamine hydrobromide which had separated was removed by centrifugation and the solution concentrated *in vacuo* at room temperature. The residual sirup was dissolved in anhydrous methyl alcohol (50 ml.) and hydrogenated at 0° in the presence of freshly prepared 15% palladium on charcoal catalyst[41] (0.5 g.) until the uptake of hydrogen ceased (in *ca.* 1 hour; 102 ml. absorbed). The solution was then filtered free from catalyst, diluted with water (15 ml.) and brought to and maintained at *p*H 11.3 with dilute sodium hydroxide solution. Debenzoylation was complete after *ca.* 3 hours at room temperature and the solution was filtered,[42] brought to *p*H 8.5 with dilute hydrochloric acid and concentrated *in vacuo* to 5 ml. The barium salts were precipitated by the addition of 2 *M* barium acetate solution (2 ml.) and ethyl alcohol (5 ml.). The precipitate was collected by centrifugation and washed with 60% aqueous ethyl alcohol, ethyl alcohol and finally ether. The air-dried material was extracted with four 5-ml. portions of water at 35° and the combined extracts concentrated *in vacuo* to 3 ml. and separated from a small amount of insoluble material by centrifugation. Ethyl alcohol (1.5 vol.) was then added to the clear solution and the precipitate after being kept at 0° for 1 hour was collected and washed in the manner described above. After two reprecipitations the completely water-soluble product was dried over phosphorus pentoxide for 18 hours in a high vacuum; yield 0.165 g. (20%). *Anal.* Calcd. for $C_5H_9O_8PBa \cdot 1H_2O$: ribose, 39.1; P, 8.07. Found: ribose,[43] 38.8; P,[44] 7.9.

Isolation of 3,5-Di-*O*-benzoyl-1,2-*O*-(1-hydroxybenzylidene)-D-ribose (V).—In one experiment, II was brought into reaction with triethylammonium dibenzyl phosphate as described above and the oily product obtained after removal of benzene taken up in a mixture of benzene and petroleum ether (20–40°). On prolonged standing a small amount of crystalline material separated which after repeated crystallization had a constant m.p. of 143°; yield 0.12 g. No depression in the melting point of this substance was observed on admixture with a sample of V prepared by the method of Ness and Fletcher (ref. 16, p. 1665). The two samples had identical infrared spectra.

Ribopyranose 1-Phosphate.—2,3,4-Tri-*O*-acetyl-D-ribopyranosyl 1-bromide (prepared from 1 g. of 1,2,3,4-tetra-*O*-acetyl-β-D-ribopyranose according to the method of Levene and Tipson[24]) was dissolved in chloroform (10 ml.) and shaken in the dark for two hours with silver dibenzyl phosphate (1.7 g.) in the presence of some glass beads. The precipitated silver bromide and the excess of silver dibenzyl phosphate were removed by centrifugation and washed with chloroform. The combined solution and washings were concentrated *in vacuo* at room temperature and the residual sirup was hydrogenated in ethyl alcohol (50 ml.) in the presence of freshly prepared 15% palladium on charcoal catalyst[41] (0.5 g.). Hydrogen uptake was complete in 2 hours and the solution was then filtered from the catalyst and concentrated *in vacuo* to 5 ml. Water (20 ml.) was then added and the solution brought to and maintained at *p*H 11.5 by the addition of sodium hydroxide solution. After 2 hours at room temperature the solution was neutralized to *p*H 8.5 with dilute hydrochloric acid and concentrated *in vacuo* to 10 ml.; 3 ml. of 2 *M* barium acetate solution was then added and the small amount of precipitate which appeared was removed after one hour by centrifugation. On the addition of ethyl alcohol (1.5 vol.) to the supernatant a heavy precipitate formed which was collected by centrifugation and washed with 60% aqueous ethyl alcohol, ethyl alcohol and finally ether. On the addition of ethyl alcohol to its aqueous solution the product crystallized and was further purified by recrystallization from hot water; yield 0.74 g. (54%). *Anal.* Calcd. for $C_5H_9O_8PBa \cdot 4H_2O$: C, 13.72; H, 3.92; P, 7.1. Found: C, 13.80; H, 4.20; P, 7.4, $[\alpha]^{28}D$ −47.1° (*c* 2.081, in 5% acetic acid) (the sample was found to be stable in this solvent at room temperature for at least two days).

Paper Chromatography of Ribose Phosphates.—Spots were applied in duplicate and the chromatograms sprayed separately for phosphorus[45] and for ribose.[46] The behavior of the various ribose phosphates on being sprayed for phosphate detection was suggestive of their relative acid-labilities. Thus the ribofuranose 1-phosphates appeared immediately as yellow spots, characteristic of inorganic phosphate, and ribopyranose 1-phosphate appeared as a yellow spot after brief heating at 80°. Ribose 2-, -3-,[47] and -5-phosphates[47] were "stable" under these conditions and appeared as blue spots only after irradiation under an ultraviolet lamp. The R_f's of the various substances are listed in Table I.

TABLE I

	Substance	R_f's in solvent systems[a] A	B	C	D
1	Ribose	0.45	0.52	0.59	0.60
2	2,3,5-Tri-*O*-benzoyl-β-D-ribofuranose 1-phosphate	.76	.80	.62	..
3	2,3,4-Tri-*O*-acetyl-D-ribopyranose 1-phosphate	.61	..	..	..
4	Ribofuranose 1-phosphate (natural as well as synthetic)	.36	.15	.46	.23
5	Ribopyranose 1-phosphate	.27	..	..	.21

[a] Solvent systems: A, *n*-butyl alcohol–acetic acid–water (2-2-1, v./v.).[48] Chromatograms were run at 0°. B, isopropyl alcohol–ammonia–water (70-10-20, v./v.).[49] C, isopropyl alcohol–1% ammonium sulfate solution (2-1, v./v.).[50] D, ethyl alcohol–1 *M* ammonium acetate (7.5-3, v./v.).[51]

The identification of the sugar obtained on acidic hydrolysis of the natural and synthetic ribofuranose 1-phosphates as ribose was confirmed by paper chromatography in the following solvent systems, all of which are known to differentiate between D-ribose and D-arabinose. Standards were run side by side: 1, *n*-butyl alcohol–acetic acid–water (4-1-5, v./v.)[52]; 2, methyl ethyl ketone–1% ammonia,[52] and 3, *n*-butyl alcohol–water.[53]

Reaction of Ribose Phosphates with Dicyclohexylcarbodiimide. General Procedure.—The barium or cyclohexylamine salt (10 mg.) of the ribose phosphate was dissolved in 0.5 ml. of water and the solution well stirred with the pyridine form of Dowex 50 ion-exchange resin (50–100 mesh; 100 mg.) for five minutes. The resin was removed by filtration and washed with two small portions of water. The combined filtrate was evaporated *in vacuo* at low temperature to a gum which was taken up in 0.1 ml. of 50% aqueous pyridine. A further amount of pyridine (0.5 ml.) and dicyclohexylcarbodiimide (50 mg.) were added and the clear solution allowed to stand at 0°. After suitable periods of time, 0.1-ml. portions of the solution were withdrawn, diluted with water (0.2 ml.) and extracted once with ether. The solutions were then chromatographed on Whatman No. 1 paper, using solvent system D (see above). The following results were obtained.

Enzymatically Prepared Ribose 1-Phosphate.—The two samples[22,32] showed identical behavior. After 1 hour the

(41) R. Mozingo, *Org. Syntheses*, **26**, 78 (1946).

(42) The precipitate formed at this stage is sodium phosphate.

(43) A. H. Brown, *Arch. Biochem. Biophys.*, **11**, 269 (1946).

(44) R. A. Bonnar and E. L. Duggan, *J. Biol. Chem.*, **212**, 697 (1955).

(45) C. S. Hanes and F. A. Isherwood, *Nature*, **164**, 1107 (1949); R. S. Bandurski and B. Axelrod, *J. Biol. Chem.*, **193**, 405 (1951).

(46) S. M. Partridge, *Nature*, **164**, 443 (1949). 0.5 ml. of concd. hydrochloric acid/100 cc. was incorporated in the reagent (ref. 48).

(47) Kind gifts of Dr. W. E. Cohn of Oak Ridge National Laboratory.

(48) H. L. A. Tarr, *Biochem. J.*, **59**, 386 (1955).

(49) Modification of the system devised by R. Markham and J. D. Smith, *ibid.*, **52**, 552 (1952).

(50) N. Anand, V. M. Clark, R. H. Hall and A. R. Todd, *J. Chem. Soc.*, 3665 (1952).

(51) A. C. Paladini and L. F. Leloir, *Biochem. J.*, **51**, 426 (1952).

(52) S. M. Partridge, *ibid.*, **42**, 238 (1948).

(53) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 1702 (1950).

reaction mixtures still contained appreciable amounts of unchanged starting materials (R_f 0.12) but showed a fairly strong spot with R_f 0.42 due presumably to IX with a weak spot of R_f 0.82, attributed to X. After 5 hours, the intensities of the spots corresponding to IX and X had increased at the expense of the slowest-travelling spot. After 19 hours, only traces of the starting material and the cyclic phosphate IX remained, X being the major product of reaction. In these experiments some simple hydrolysis of the starting material also occurred as judged by the appearance of a weak spot corresponding to free D-ribose.

Synthetic[54] Ribofuranose 1-Phosphate.—After 19 hours this substance was largely unchanged, except for some simple hydrolysis to free ribose.

Hydrolysis of Ribose Phosphates.—In 0.1 *N* perchloric and 0.1 *N* hydrochloric acids, no difference in the rates of the hydrolysis of the synthetic and the enzymatic samples of ribofuranose 1-phosphate could be detected. However, in 0.01 *N* hydrochloric acid at 20° the synthetic ester was found to be slightly more stable (curves I and II, Fig. 1). Synthetic ribopyranose 1-phosphate (curve III) was much more stable than either of the above esters. In 0.1 *N* hydrochloric acid at 20° the extent of hydrolysis of this ester was as follows: 32.5% in 2 hours, 50% in 4 hours and 75% in 9 hours. Curve IV shows the relative stability of the product X of reaction (22 hours) of ribose α-1-phosphate (VIII) with D.C.C. Of the total amount of phosphate present in the reaction mixture, 10.8% had appeared as inorganic phosphate[20] and is to be ascribed to the hydrolysis of VIII prior to the formation of X.

The ribofuranose 1-phosphates and ribopyranose 1-phosphate were separately heated in 0.5 *N* sodium hydroxide at 80° for 1 hour in polyethylene tubes. In no case could any inorganic phosphate be detected[20] after this treatment. Paper chromatography, after neutralization of the solutions showed the presence of intact phosphate esters. These neutralized solutions were then treated briefly with acid and the expected free ribose and inorganic phosphate identified by paper chromatography.

(54) Synthetic ribopyranose 1-phosphate has been found to undergo the reaction sequence of the type (VIII → X). The dependence of the process of cyclization in pyranose 1-phosphates upon the conformations of the phosphate and the adjacent hydroxyl groups will be discussed in a forthcoming communication.

Acknowledgments.—This work was carried out under a consolidated grant from the National Research Council of Canada, Ottawa. We are grateful to Dr. G. M. Shrum for his kind encouragement of this work.

VANCOUVER, BRITISH COLUMBIA, CANADA

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX S. A.]

Steroids. LXXIII.[1a] The Direct Oppenauer Oxidation of Steroidal Formate Esters. A New Synthesis of 17α-Hydroxyprogesterone[1b]

BY H. J. RINGOLD, BJARTE LÖKEN, G. ROSENKRANZ AND FRANZ SONDHEIMER

RECEIVED SEPTEMBER 19, 1955

It is shown that steroidal Δ^5-3β-ol formates are oxidized directly by the Oppenauer method to the corresponding Δ^4-3-ketones. Δ^5-Pregnene-3β,17α-diol-20-one 3-formate (IVa) was prepared from 16α,17α-oxido-Δ^5-pregnen-3β-ol-20-one acetate (Ia) by hydrogen bromide addition, hydrogenation over a palladium–calcium carbonate catalyst, saponification and formylation, or more simply from free 16α,17α-oxido-Δ^5-pregnen-3β-ol-20-one (Ib) by hydrogen bromide addition, hydrogenation over a palladium–charcoal catalyst and formylation. Acetylation of IVa at C-17 gave Δ^5-pregnene-3β,17α-diol-20-one 3-formate 17-acetate (IVb), which on Oppenauer oxidation yielded 17α-acetoxyprogesterone (V), saponified to 17α-hydroxyprogesterone (VI). Another example of the formate protection procedure in the androstane series is described, which leads from dehydroisoandrosterone formate (VIIb) to testosterone acetate (IXa) and propionate (IXb).

In the course of an investigation aimed at finding new routes to certain of the adrenal hormones from readily available starting materials, we were faced with the problem of protecting 3β-hydroxy-Δ^5-steroids at C-3 in such a way that operations could be carried out on the side chain, while permitting the necessary Δ^4-3-one system to be formed subsequently in a simple fashion. It was found that this objective could be achieved readily through protection at C-3 by means of the formate esters.

Such Δ^5-3β-ol formates were shown to be stable toward a number of reagents used to modify the side chain, such as acetic anhydride and *p*-toluenesulfonic acid (for C-17 acetylation of 17α-hydroxypregnan-20-one derivatives), bromine, followed successively by sodium iodide and potassium acetate (for introduction of the 21-acetoxy group into 17α-hydroxypregnan-20-ones) and to sodium borohydride in non-alcoholic solvents (for reduction of 17-ketoandrostanes).[2] Moreover, once the necessary reactions have been carried out in the side chain, we found that the Δ^5-3β-ol formate grouping may then be oxidized by the Oppenauer method directly in one step to the required Δ^4-3-one. In the present paper a new and convenient synthesis of 17α-hydroxyprogesterone (VI) by use of the formate protection procedure is described, as well as another application in the androstane series. In the subsequent paper[3] the method is used for syntheses of Reichstein's substance S and related compounds.

The 3-formate (IVa) of Δ^5-pregnene-3β,17α-diol-20-one (IIIb) is a key intermediate both for the synthesis of 17α-hydroxyprogesterone and of substance S, but unfortunately the previously described syntheses of IIIb[4] are either involved or proceed in rather poor yield. Two syntheses of IVa were therefore worked out which employ 16α,17α-oxido-Δ^5-pregnen-3β-ol-20-one (Ib) (obtainable in high yield by alkaline hydrogen peroxide treatment[5] of $\Delta^{5,16}$-pregnadien-3β-ol-20-one acetate, the degradation product of diosgenin) as starting ma-

(1a) Paper LXXII, F. Neumann, O. Mancera, G. Rosenkranz and F. Sondheimer, THIS JOURNAL, **77**, 5676 (1955).

(1b) The major part of the work described in this paper forms the basis of Mexican Patent Application No. 34216 (Aug. 2, 1952), No. 35342 (Jan. 13, 1953) and No. 36949 (Aug. 18, 1953).

(2) Furthermore H. Hirschmann, F. B. Hirschmann and G. L. Farrell (*ibid.*, **75**, 4862 (1953)) have shown that formates of Δ^5-3β-ols are stable toward lead tetraacetate in acetic acid (for introduction of the 21-acetoxy group into pregnan-20-ones).

(3) H. J. Ringold, G. Rosenkranz and F. Sondheimer, *ibid.*, **78**, 820 (1956).

(4) (a) H. G. Fuchs and T. Reichstein, *Helv. Chim. Acta*, **24**, 804 (1941); (b) P. Hegner and T. Reichstein, *ibid.*, **24**, 828 (1941); (c) P. L. Julian, E. W. Meyer and I. Ryden, THIS JOURNAL, **72**, 367 (1950).

(5) P. L. Julian, E. W. Meyer, W. J. Karpel and I. R. Waller *ibid.*, **72**, 5145 (1950).