hydrazide structure as opposed to the N-aminosuccinimide structure. We find that XVIII, when dissolved in excess sodium hydroxide and back-titrated potentiometrically with hydrochloric acid, gives a neutralization equivalent of 172 (theory for monobasic acid $C_8H_{12}O_2N_2$, 168). The direct titration of XVIII with sodium hydroxide is difficult since XVIII combines rather slowly with the base and our titrimetric data indicate that XVIII undergoes hydrolysis to give the salt of XX under the conditions of the titration. Since either XVIIIa or XVIIIb might be expected to undergo ready hydrolysis to XX, the titration is also unable to distinguish between the two likely structures for XVIII.

Oxidation of XIV with Lead Tetraacetate.—The anhydride XIV (5.0 g., 0.0182 mole) was dissolved in 200 ml. of hot pyridine (which had been dried over KOH pellets and freshly distilled). Lead tetraacetate (9.0 g., 0.020 mole) was added and the pyridine solution was maintained at 70–80° for 1 hour. During the first 5 minutes of reaction there was a vigorous evolution of carbon dioxide. The solvent was removed *in vacuo* with aid of a steam-bath. The dark brown residue was acidified with hydrochloric acid and extracted with ether three times. The ethereal extracts were combined, washed with water twice and dried over anhydrous $MgSO_4$. After removal of the ether, 1.5 g. of crystalline product was obtained. This was sublimed at 0.025 mm. at a bath temperature of 170° to give 1.45 g. (39.5% yield) of crystalline sublimate. After two recrystallizations from chlorobenzene the product XIII had m.p. 166.9–168.7°.

Anal. Found: C, 70.89, 70.92; H, 4.80, 4.95. Calcd. for $C_{12}H_{10}O_3$: C, 71.28; H, 4.98.

A sample of the maleic anhydride addition product of cyclooctatetraene was prepared[13] by heating maleic anhydride with cyclooctatetraene under a nitrogen atmosphere at a bath temperature of 180° for 30 min. The product after sublimation *in vacuo* and recrystallization from chlorobenzene had m.p. 167.3–168.7° and gave no depression of m.p. when mixed with the product from the lead tetraacetate oxidation. Likewise the two products had identical infrared spectra.

A higher yield was obtained from reaction of 2.40 g. (0.00875 mole) of the anhydride XIV with 5.8 g. (0.013 mole) of lead tetraacetate in 85 ml. of pyridine maintained at 50–60° for 1 hour. The reaction mixture after standing overnight was worked up as previously. After one sublimation *in vacuo*, the product amounted to 1.00 g. (56% yield) of material, m.p. 166.6–168.3°. A similar oxidation with lead tetraacetate in which 2 moles of lead tetraacetate was used per mole of anhydride gave only 34% yield of XIII.

Oxidation of the tetracarboxylic acid of XIV with lead tetraacetate was hindered, at least in part, by the low solubility of the acid in pyridine. Tetracarboxylic acid (5.0 g., 0.0161 mole) did not dissolve very completely in 150 ml. of boiling pyridine. To the mixture at 95–100° was added 10.0 g. (0.023 mole) of lead tetraacetate and the reaction mixture was kept at this temperature for 1 hour. The reaction was worked up as with the anhydride. From the ether extracts 0.50 g. of crude product was obtained. After one recrystallization from methanol the product amounted to 0.35 g. of material, m.p. 160–161.5°. Another recrystallization from methanol gave a product XIII of m.p. 167.1–167.9° which was identical with the maleic anhydride adduct of cyclooctatetraene (mixed m.p. and infrared spectral comparisons).

Bromolactone of XI.—The dihydro derivative[14] of the maleic anhydride adduct of cyclooctatetraene (XI) (2.00 g., 0.0098 mole, m.p. 142.5–143.5°) was dissolved in a warm solution of 3 g. of KOH in 50 ml. of water and the solution was filtered to remove a trace of suspended material. The solution was neutralized to a phenolphthalein end-point with hydrochloric acid and bromine was added dropwise with shaking. The color of bromine disappeared rapidly during addition of 0.45 ml. (8.8 mmoles), but a permanent yellow color remained after addition of 0.05 ml. (1.0 mmole) more bromine. Acidification gave a precipitate which after separation by filtration and recrystallization from 95% ethanol amounted to 2.50 g. (85% yield) of bromolactonic acid, m.p. 243.3–243.8° dec.

Anal. Found: C, 47.73, 47.81; H, 4.52, 4.40; Br, 26.61. Calcd. for $C_{12}H_{13}O_4Br$: C, 47.86; H, 4.35; Br, 26.54.

The bromolactonic acid was dissolved in tetrahydrofuran and a solution of diazomethane in ethyl ether was added until a pale yellow color persisted. The solvents were removed by evaporation and the resulting crystalline residue was recrystallized twice from methanol to give methyl ester XXI, m.p. 166.9–167.9°.

Anal. Found: C, 49.45, 49.63; H, 4.80, 4.96; Br, 25.25. Calcd. for $C_{13}H_{15}O_4Br$: C, 49.54; H, 4.80; Br, 25.35.

Spectra.—Infrared spectra were run with samples in potassium bromide disks on a Perkin–Elmer model 21 infrared spectrometer with rock-salt prisms. Ultraviolet spectra were determined on a Beckman model DK-1 quartz spectrophotometer. The nuclear magnetic resonance absorption spectrum was determined on a Varian frequency R-F unit model V4310C with frequency of 40 megacycles and a field of 10,000 gauss. The tetramethyl ester of XIV (0.1 g.) was dissolved in 0.3 ml. of $DCCl_3$ and the chemical shifts were determined relative to $HCCl_3$ which was contained within an inner concentric tube. For calculations of chemical shifts relative to water, chloroform was taken to have a chemical shift of 96 cycles/sec. at 40 Mc.

Acknowledgment.—We are indebted to Dr. Leon Mandell for the nuclear magnetic resonance spectrum.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, THE UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA]

The Synthesis and Properties of D-*glycero*-Tetrulose 1-Phosphate and 4-Phosphate (D-Erythrulose 1-Phosphate and 4-Phosphate)

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The synthesis of two phosphate monoesters of a keto-tetrose is described. These are D-*glycero*-tetrulose 1-phosphate and D-*glycero*-tetrulose 4-phosphate, the common names being D-erythrulose 1-phosphate and D-erythrulose 4-phosphate. The compounds were obtained as stable dimethyl ketals, which by mild acid hydrolysis yield the free ketoses. The degradation of the tetrulose phosphates by acid and alkali followed the pattern previously observed for the triose phosphates. D-*glycero*-Tetrulose 1-phosphate acts as a substrate for the enzyme *glycerol phosphate dehydrogenase* and is reduced to D-erythritol 1-phosphate. This product is consistent with the specificity of the action of the enzyme on its normal substrate dihydroxyacetone phosphate.

At present, information concerning the possible metabolic role of keto-tetroses is meager. L-Glycero-tetrulose (L-erythrulose) has been shown to be formed and cleaved in systems involving the enzyme transketolase,[1] and it is also metabolized by rat-liver slices.[2] Glycero-tetrulose 1-phosphate has been obtained by the action of a

(1) B. L. Horecker, P. Z. Smyrniotis and H. Klenow, *J. Biol. Chem.*, **205**, 661 (1953); J. Hickman and G. Ashwell, *ibid.*, **234**, 758 (1959); F. Dickens and D. H. Williams, *Nature*, **178**, 1349 (1956); A. G. Datta and E. Racker, *Arch. Biochem. Biophys.*, **82**, 489 (1959).

(2) R. D. Batt, F. Dickens and D. H. Williams, *Biochem. J.*, **74**, 10P (1960).

rat-liver enzyme, "phosphoketotetrose aldolase" on a mixture of dihydroxyacetone phosphate and formaldehyde.[3] Tetrulose phosphates might well be intermediates in the metabolism of tetritols, since it has been established that the first step in the utilization of erythritol by *Propionibacterium pentosaceum*[4] involves the phosphorylation of the tetritol to give L-erythritol 1-phosphate.

Biochemical studies on the tetrulose phosphates would, of course, be facilitated by the availability of pure samples of the different compounds. Aside from the enzymatic preparation of glycero-tetrulose 1-phosphate referred to above,[3] only one other synthesis of a tetrulose phosphate has been reported,[5] and in that case the product was admittedly impure and incompletely characterized.

We have perfected syntheses of D-glycero-tetrulose 1-phosphate and 4-phosphate, and this paper is a report of the methods used and of the properties of the products obtained. The same methods can be used to prepare the L-isomers, and with some extension we expect to obtain the glycero-tetrulose 1,4-diphosphates. These further investigations are now receiving our attention.

The steps employed in our synthetic approach are outlined in the reaction scheme. Brief comments follow. 1,2-5,6-Tetra-O-benzoyl-D-mannitol (III) was prepared in 60% yield from D-mannitol (I) by a modification of published procedures.[6] Oxidation of III to 2,3-di-O-benzoyl-D-glycerose (IV) was done efficiently by a mixture of lead tetraoxide in glacial acetic,[7] eliminating the necessity for preparing lead tetraacetate. The preparation of the acid chloride (VI) and coupling with diazomethane to give the diazoketone (VII) parallel the reactions carried out by Iwadare[8] on 2,3-isopropylidene-D-glyceric acid. The hydrolysis of the diazo-compound (VII) to 3,4-di-O-benzoyl-D-glycero-tetrulose (VIII) went surprisingly well, and the product seemed to be perfectly stable under the conditions required for the hydrolysis Ketalation of VIII was accomplished smoothly using methanolic hydrogen chloride. It has been shown previously that ketalation of such compounds can be done only when one of the hydroxyl groups adjacent to the ketone is unsubstituted.[9] Subsequent steps lead in a straightforward manner to D-glycero-tetrulose 1-phosphate dimethyl ketal (XI).

Synthesis of the 4-phosphate required deacylation of IX or XIII and the selective tritylation of the D-glycero-tetrulose dimethyl ketal (XIV). Apparently, steric hindrance owing to the dimethyl ketal is sufficient to direct substitution to the 4-position, exclusively. By benzoylation, detritylation and phosphorylation, one comes eventually to D-glycero-tetrulose 4-phosphate dimethyl ketal (XVIII).

The dimethyl ketals of D-glycero-tetrulose 1-phosphate (XI) and 4-phosphate (XVIII) were characterized by elemental analysis, and by the facts that the 1-phosphate (XI) having an adjacent glycol group consumed one mole of sodium periodate while the 4-phosphate (XVIII) did not react with this reagent. The two had very similar chromatographic properties but differed in optical rotation, infrared spectra and degree of hydration of the crystalline cyclohexylamine salts.

The free glycero-tetrulose phosphates were obtained from the ketals by mild acid hydrolysis. In solution, they are reasonably stable, but no attempt was made to isolate crystalline salts. A notable difference between the 1-phosphate and the 4-phosphate is in their degradation by alkali. D-Glycero-tetrulose 1-phosphate gave a quantitative yield of inorganic phosphate on treatment with 1 *N* sodium hydroxide for 20 minutes. This is comparable to the result with dihydroxyacetone phosphate.[10] The 4-phosphate, however, released only about 50% of its phosphate as inorganic phosphate. This is reminiscent of the result obtained when erythrose 4-phosphate is treated similarly,[11] and it is not surprising since the tetrulose phosphate and tetrose phosphate should be rapidly interconverted under these conditions. Presumably some organic phosphate compound is formed that cannot undergo the phosphate elimination reaction typical of glyceraldehyde and dihydroxyacetone phosphates.

The tetrulose phosphates showed the sensitivity to acid hydrolysis expected of such compounds. The half-times for hydrolysis in 1 *N* hydrochloric acid at 100° were 7 minutes for the 1-phosphate and 15 minutes for the 4-phosphate.

The ability of D-glycero-tetrulose 1-phosphate to act as a substrate for glycerol phosphate dehydrogenase was determined, since it can be considered to be an analog of the natural substrate dihydroxyacetone phosphate. The latter is reduced to D-glycerol 1-phosphate (L-glycerol 3-phosphate) in the presence of reduced diphosphopyridine nucleotide. The tetrulose phosphate was reduced surprisingly well, at a rate about 10% that of the natural substrate. The product of the reduction was shown to be D-erythritol 1-phosphate (L-erythritol 4-phosphate); and thus, it is apparent that the enzyme follows the same steric pattern with this analog as it does with dihydroxyacetone phosphate.

Experimental

3,4-O-Isopropylidene-1,2:5,6-tetra-O-benzoyl-D-mannitol (II).—To a suspension of 55 g. (0.3 mole) of D-mannitol in 200 ml. of pyridine was added from a dropping funnel a total of about 90 ml. (0.75 mole) of redistilled benzoyl chloride during a period of about 30 minutes with mechanical stirring. The temperature increased during the reaction and solution of the D-mannitol was complete before the bulky precipitate of pyridine hydrochloride was formed. A cold water-bath was used during the later stage of the reaction to keep the temperature around 60–70°. The reaction mixture was left at room temperature for 2 hr., then it was poured into 2 liters of water with stirring. The solidified product was filtered off, ground in a mortar with water, filtered again and washed free of pyridine.

(3) P. C. Charalampous, *J. Biol. Chem.*, **211**, 249 (1954).

(4) J. K. Shetter, *J. Am. Chem. Soc.*, **78**, 3722 (1956).

(5) P. A. J. Gorin, L. Hough and J. K. N. Jones, *J. Chem. Soc.*, 2699 (1955).

(6) E. Fischer, *Ber.* **48**, 266 (1915); P. Brigl and H. Grüner, *ibid.*, **66**, 931 (1933).

(7) L. Vargha, *Nature*, **162**, 927 (1948).

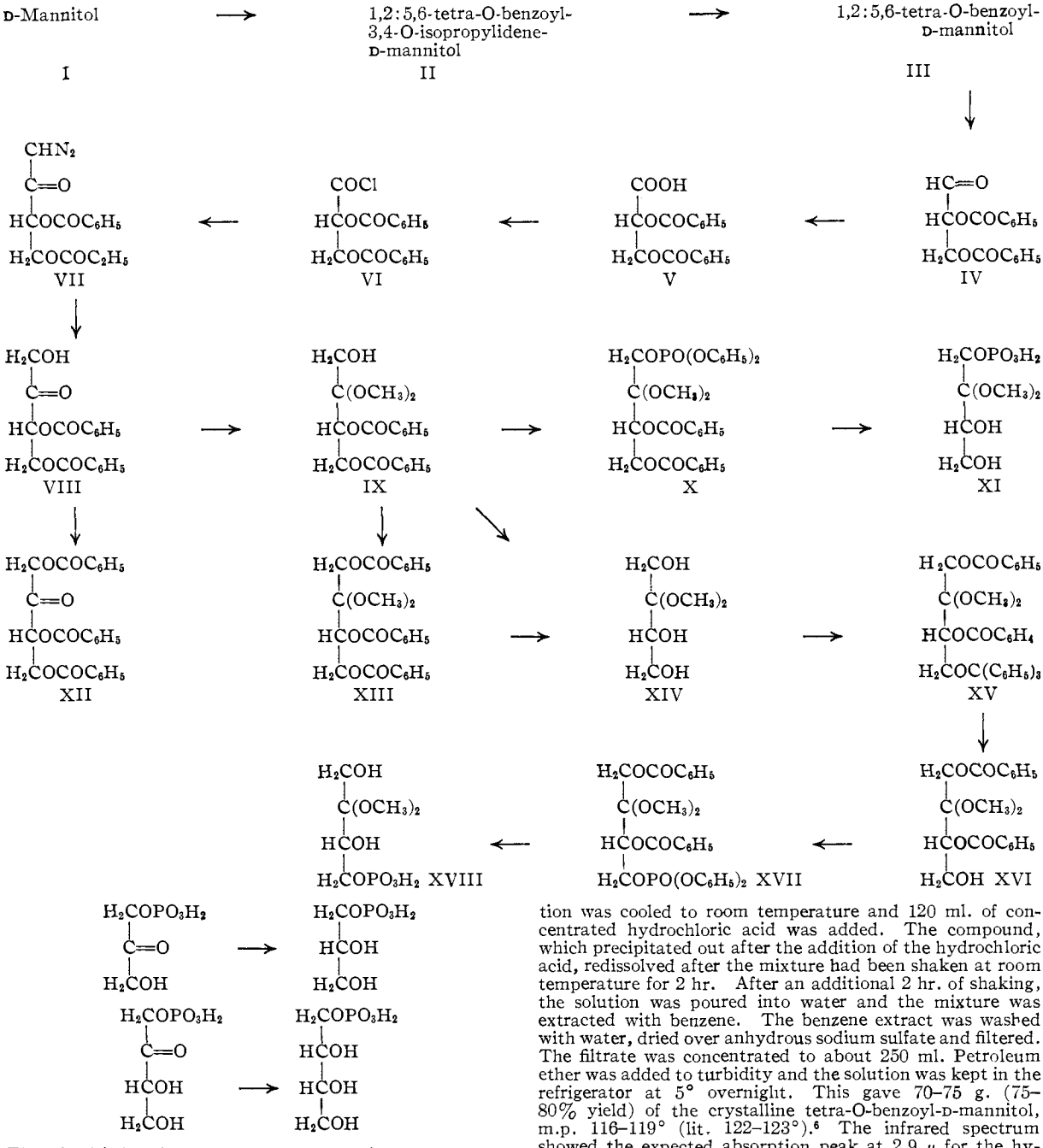
(8) K. Iwadare, *Bull. Chem. Soc. Japan*, **14**, 131 (1939).

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

(10) C. E. Ballou and H. O. L. Fischer, *J. Am. Chem. Soc.*, **78**, 1659 (1956).

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

SCHEME FOR THE SYNTHESES OF D-GLYCERO-TETRULOSE PHOSPHATES



The air dried weight was 128 g., and the product consisted of a mixture of dibenzoyl and tribenzoylmannitol.

The above product was acetonated by shaking it for 36 hr. in 1.5 liters of acetone containing 5 ml. of concentrated sulfuric acid and 60 g. of anhydrous copper sulfate. The solution was then neutralized with ammonia gas and filtered to remove the ammonium sulfate. The filtrate was concentrated to a dry sirup, 144 g., which was taken into 200 ml. of pyridine and further benzoylated with 60 ml. of benzoyl chloride (0.5 mole). The crude product, precipitated by pouring the reaction mixture into water, was collected, washed free of pyridine and crystallized from 1.5 liters of methanol. This gave a total yield of 145 g. (75%) in two crops, m.p. 117–119° (lit. 122–123°).[6] About 5–8 g. of D-mannitol hexabenzoate separated from the second crop, m.p. 144–148° (lit. 149°).

1,2:5,6-Tetra-O-benzoyl-D-mannitol (III).—One hundred grams of II in 1 liter of glacial acetic acid was heated on a steam-bath until all of the solid had dissolved. The solution was cooled to room temperature and 120 ml. of concentrated hydrochloric acid was added. The compound, which precipitated out after the addition of the hydrochloric acid, redissolved after the mixture had been shaken at room temperature for 2 hr. After an additional 2 hr. of shaking, the solution was poured into water and the mixture was extracted with benzene. The benzene extract was washed with water, dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated to about 250 ml. Petroleum ether was added to turbidity and the solution was kept in the refrigerator at 5° overnight. This gave 70–75 g. (75–80% yield) of the crystalline tetra-O-benzoyl-D-mannitol, m.p. 116–119° (lit. 122–123°).[6] The infrared spectrum showed the expected absorption peak at 2.9 μ for the hydroxyl groups.

2,3-Di-O-benzoyl-D-glyceraldehyde (IV).—A solution of 60 g. (0.1 mole) of the tetra-O-benzoyl-D-mannitol (III) in 500 ml. of glacial acetic acid was oxidized with 75 g. (0.11 mole) of lead tetraoxide which was added in several portions with mechanical stirring.[7] The solution became slightly warm and solution of the red lead was complete within an hour. After stirring for another 3 hr., the excess oxidizing agent was destroyed with ethylene glycol and the solution was poured into water and extracted with benzene. The benzene layer was washed with water, dried over sodium sulfate and evaporated to dryness. The sirup, 58 g., solidified on standing overnight at room temperature. Attempted crystallization from ether–petroleum ether gave a recovery of only one-third of the material, m.p. 79–80° (reported m.p. 80°).[12] The crude product was used in the subsequent reactions.

(12) P. Brigl and H. Gruner, *Ber.*, **66**, 933 (1933).

2,3-Di-O-benzoyl-D-glyceric Acid (V).—The crude di-O-benzoyl-D-glyceraldehyde, 52 g., in 300 ml. of ethyl acetate was refluxed for 3 hr. with 100 ml. of 4 *M* peroxypropionic acid.[13] The solution was concentrated to a sirup which was taken into benzene and washed with water. After the removal of the solvent, the sirup was crystallized from toluene–petroleum ether, giving 36 g. (66% yield), m.p. 80–83°. Recrystallization from the same solvents raised the m.p. to 88–89°, $[\alpha]_D$ −27.0° (chloroform).

Anal. Calcd. for $C_{17}H_{14}O_6$(314): C, 65.0; H, 4.50. Found: C, 65.0; H, 4.67.

1-Deoxy-1-diazo-3,4-di-O-benzoyl-D-glycero-tetrulose (VII).—Twenty-two grams of the di-O-benzoyl-D-glyceric acid in 220 ml. of dry benzene was refluxed with 20 ml. of thionyl chloride (freshly distilled) for 3 hr. The solution was left at room temperature overnight, then evaporated to dryness with the repeated addition of dry benzene. The sirup VI, 23.5 g., was dissolved in 100 ml. of anhydrous ether and poured with rapid stirring into a solution of 7.4 g. of diazomethane in 440 ml. of ether that was cooled in a Dry Ice–acetone bath to about −20°. The reaction was complete within a few minutes. The solution was filtered and evaporated to about 150 ml. The product VII crystallized readily. Petroleum ether, 150 ml., was added and the mixture was kept at 4° overnight. The light yellow crystals which were filtered off weighed 11.8 g. (50% yield) and had m.p. 98–101°. An analytical sample was obtained by purifying the compound on a Magnesol–Celite column by elution with benzene, followed by recrystallization from ether–petroleum ether, m.p. 105–106°, $[\alpha]_D$ −3.1° (chloroform).

Anal. Calcd. for $C_{18}H_{14}O_5N_2$ (338): C, 63.9; H, 4.17; N, 8.28. Found: C, 64.2; H, 4.48; N, 8.22.

The infrared spectrum showed the characteristic absorption peak at 4.73 μ for the diazo-group.

3,4-Di-O-benzoyl-D-glycero-tetrulose (VIII).—The diazo-compound, VII, 8.0 g. in 60 ml. of dioxane and 20 ml. of 3 *N* sulfuric acid, was heated to about 70° for 30 minutes. Hydrolysis of the diazo-compound was complete as indicated by the evolution of a quantitative amount of nitrogen gas. The solution was cooled, poured into water and extracted with benzene. The benzene extract was washed with water, dried over sodium sulfate and evaporated to dryness. The sirupy product VIII weighed 7.8 g. and showed $[\alpha]_D$ −32.2° (chloroform).[14]

1,3,4-Tri-O-benzoyl-D-glycero-tetrulose (XII).—This compound was obtained in 40% yield by the benzoylation of VIII in pyridine at 0°. It had m.p. 118–119° after crystallization from ethanol and $[\alpha]_D$ 14.2° (chloroform).

Anal. Calcd. for $C_{25}H_{20}O_7$ (432): C, 69.4; H, 4.65. Found: C, 69.1; H, 4.68.

3,4-Di-O-benzoyl-D-glycero-tetrulose Dimethyl Ketal (IX).—The sirupy product VIII, 7.2 g., was refluxed overnight in a mixture of 20 ml. of trimethyl orthoformate and 20 ml. of dry methanol containing 1% hydrogen chloride. The solution was cooled, poured into water containing 0.5 ml. of concentrated ammonia and extracted with benzene. The benzene solution was washed with water, dried over sodium sulfate and evaporated to a dry sirup, wt. 7.8 g., $[\alpha]_D$ 58° (chloroform). The methoxy content was found to be 14.0% (theor. 16.9%).

1,3,4-Tri-O-benzoyl-D-glycero-tetrulose Dimethyl Ketal (XII).—A portion of the above ketalated product (IX), 0.35 g., was benzoylated in pyridine at 0° to give a 45% yield (0.20 g.) of a crystalline product, m.p. 80–83°. Recrystallization from methanol raised the m.p. to 88–90°, $[\alpha]_D$ 126.6° (chloroform).

Anal. Calcd. for $C_{27}H_{26}O_8$ (478): C, 67.7; H, 5.47; CH_3O, 13.0. Found: C, 68.0; H, 5.64; CH_3O, 14.0.

D-glycero-Tetrulose 1-Phosphate Dimethyl Ketal (XI).—Three grams of the ketalated sirup IX in 15 ml. of dry pyridine was phosphorylated with 3 ml. of diphenyl phosphorochloridate at 0°. The reaction mixture was then left at room temperature for 3 hr. After destroying the excess reagent with water, the solution was poured into water and extracted with benzene. The benzene extract was washed with dilute hydrochloric acid, sodium bicarbonate and water, dried over sodium sulfate and evaporated to a dry sirup that weighed 4.0 g. This was hydrogenated in 200 ml. of abs. ethanol with 1.0 g. of platinum oxide catalyst. The hydrogen uptake was 2000 ml. in 16 hr. (calcd. 2330 ml.). The catalyst was removed by centrifugation. To the alcoholic solution was added 110 ml. of 0.35 *N* barium hydroxide, and the solution was left at room temperature for 6 hr. Alcohol was removed under reduced pressure and an amount of cyclohexylamine sulfate, 5.2 g. in 100 ml. of water, equivalent to the barium hydroxide, was added to the aqueous solution. The precipitate of barium sulfate was removed by centrifugation and the supernatant was evaporated to dryness. The solid residue was extracted with acetone, filtered off and washed with acetone on a funnel. It weighed 3.0 g. Fractional crystallization from aqueous acetone gave 1.2 g. (33% yield) of XI as the dicyclohexylamine salt in two crops, m.p. 160–165°, $[\alpha]_D$ 13.3 °(water).

Anal. Calcd. for $C_{18}H_{41}O_5PN_2$ (444): C, 48.6; H, 9.29; P, 6.97; N, 6.30; CH_3O, 14.0. Found: C, 48.3; H, 9.21; P, 6.78; N, 6.10; CH_3O, 13.4.

This compound consumed 0.8 mole equivalent of sodium periodate as determined spectrophotometrically at 260 mμ.[15]

Preparation of Free D-glycero-Tetrulose 1-Phosphate.—A solution of 5.20 mg. of the D-glycero-tetrulose 1-phosphate dimethyl ketal dicyclohexylamine salt (XI) in 0.5 ml. of water was treated with Dowex 50-(H) resin. The mixture was swirled for 1 minute and filtered. The filtrate was made up to 1 ml. with washings and kept at 40° for 4 hr., when hydrolysis of the ketal was complete as revealed from the paper chromatogram which showed a single component with a slower R_f than the ketal derivative. The phosphate was hydrolyzed in 1 *N* hydrochloric acid at 100° with a half-time of 7 minutes. The solution was 0.0117 *M* in D-glycero-tetrulose 1-phosphate by calculation, 0.0115 *M* in alkali labile phosphate (*N* sodium hydroxide for 18 minutes at room temperature). It assayed 0.0108 *M* in D-glycero tetrulose 1-phosphate with the enzyme L-α-glycerophosphate dehydrogenase, the yield of the "biologically active" product being 92% on the basis of the amount of DPNH oxidized.[16] The rate of reduction was found to be 11% of that for dihydroxyacetone phosphate under the same conditions.

Identification of the Tetritol from the Enzymatic Reduction.—To the remaining solution from the above assay, 0.8 ml. (5.20 mg. of XI per ml.), was added 14 mg. of reduced diphosphopyridine nucleotide, 1 ml. of *M* diethanolamine buffer solution (*p*H 7.5). The solution was diluted to 5 ml., and 0.4 ml. of the enzyme stock solution (L-α-glycerol phosphate dehydrogenase, 1480 units per ml.) was added. After 18 hr. at room temperature, the solution was diluted to 20 ml. and treated first with 0.3 g. of Darco G-60 to remove the nucleotide, then with Dowex-50(H). The resulting solution was brought to *p*H 9.5 with sodium hydroxide and dephosphorylated with intestinal phosphatase at 40° for 24 hr. The solution was brought to *p*H 7 with Dowex-50(H), then treated with mixed-bed resin to remove the inorganic salts and the filtrate was evaporated to dryness. The tetritol was identified as erythritol by electrophoresis with boric acid–borate buffer solution (*p*H 6.0) at 1800 v. and 20 ma.

D-glycero-Tetrulose 4-Phosphate Dimethyl Ketal (XVIII).—One gram of the ketalated sirup (IX) was debenzoylated in 5 ml. of dry methanol with the addition of 1.5 ml. of 0.4 *N* barium methoxide. The solution was left at room temperature for 8 hr., then evaporated to dryness. The sirupy product was tritylated with 0.89 g. of trityl chloride in 5 ml. of dry pyridine. After 2 days at 4°, 1 ml. of benzoyl chloride was added and the reaction mixture was left at room temperature for 3 hr. The excess reagent was then destroyed with a few drops of water. The pyridine solution was poured into water and the mixture was extracted with benzene. The benzene extract was washed with dilute hydrochloric acid, sodium bicarbonate and water, dried over sodium sulfate and evaporated to a dry sirup that

(13) R. Barker and D. L. MacDonald, *J. Am. Chem. Soc.*, **82**, 2301 (1960).

(14) We have been able to hydrolyze the analogous diazo compound in the pentulose series to give 3,4,5-tri-O-benzoyl-D-erythro-pentulose as a crystalline well-defined product. The ketalation step has however, given anomalous results which have to this time precluded the synthesis of the pentulose phosphates by this procedure.

(15) J. S. Dixon and D. Lipkin, *Anal. Chem.*, **26**, 1092 (1954).

(16) G. Beisenherz, T. Bücher and K. H. Garbade, "Methods in Enzymology," Vol. I, Academic Press, Inc., New York, N. Y., 1955, p. 391.

weighed 1.9 g. This was hydrogenated in 150 ml. of methanol with a 5% palladium chloride on carbon catalyst. The hydrogen uptake was 110 ml. (calcd. 67 ml.) in 5 hr. After the removal of the catalyst, the solution was concentrated to about 15 ml. Triphenylmethane, 0.5 g., was filtered off and the filtrate was evaporated to a dry sirup which was taken into 10 ml. of pyridine and phosphorylated with 1 ml. of diphenyl phosphorochloridate at 0° for 5 hr. The sirupy product, isolated as for the previous phosphorylation, was hydrogenated in 200 ml. of methanol with 0.8 g. of platinum oxide catalyst. The hydrogen uptake was 850 ml. (calcd. 900 ml.) in 20 hr. After the removal of the catalyst, 40 ml. of 0.345 *N* barium hydroxide was added to the alcoholic solution, and the solution was left at room temperature for 6 hr. Alcohol was removed under reduced pressure and the barium ion was precipitated by the addition of 1.9 g. of cyclohexylamine sulfate in 40 ml. of water. After the removal of the barium sulfate by centrifugation, the solution was evaporated to dryness. The residue was triturated with acetone and filtered. The solid weighed 0.7 g. Crystallization from aqueous acetone gave 0.15 g. (13% yield) of the tetrulose 4-phosphate (XVIII), m.p. 165–170° and $[\alpha]_D$ 21.5° (water).

Anal. Calcd. for $C_{18}H_{41}O_8PN_2 \cdot 2H_2O$ (480): C, 45.0; H, 9.44; P, 6.46; N, 5.84; CH_3O, 13.0. Found: C, 44.5; H, 9.13; P, 6.34; N, 5.59; CH_3O, 12.6.

The presence of water in the crystal was supported by the infrared spectrum, in which the intensity of the hydroxyl absorption was much stronger than that for the tetrulose 1-phosphate.

This compound was chromatographically indistinguishable from the tetrulose 1-phosphate in 2-propanol–ammonia–water (70:10:20). However, it did not consume any periodate as did the 1-phosphate. After treatment of a solution with Dowex 50(H) and hydrolysis at 40° for 4 hr., a paper chromatogram showed a single new component. The R_f value was slower than that of the ketal derivative. Treatment with 1 *N* sodium hydroxide at room temperature for 20 minutes resulted in the release of about 50% of the phosphate as inorganic phosphate. When the tetrulose 4-phosphate was heated at 100° in 1 *N* hydrochloric acid, inorganic phosphate was formed with a half-time of 15 minutes.

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The Beckmann Rearrangement. IX. A Study of Polyphosphoric Acid as a Rearrangement Medium

BY D. E. PEARSON AND R. M. STONE[1]

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The rates of rearrangement of substituted acetophenone oximes were studied in polyphosphoric acid of various phosphorus pentoxide concentrations. The rates were remarkably fast compared to those in sulfuric acid even though the H_0 of polyphosphoric acid is much less than that of sulfuric acid. Also substituents on the acetophenone oxime had little effect in changing the rate (ρ = *ca.* −0.25). A mechanism of rearrangement in polyphosphoric acid *via* an ester is proposed and discussed.

Polyphosphoric acid (PPA) is becoming a reagent of major importance in synthetic organic chemistry without truly being understood. The composition has only recently been made available,[2] and "very little is known about the precise mechanisms of reactions catalyzed by PPA."[3] Since PPA is quite suitable as a medium for the Beckmann rearrangement and since we have had some experience with this rearrangement in sulfuric acid,[4] we decided to study the rearrangement in PPA to compare mechanisms in the two reacting solvents and to increase knowledge about the use of PPA in general. For example, is it best for the synthesis of amides by the Beckmann rearrangement to dissolve the oxime in PPA at 100–130° and maintain for some arbitrary number of hours as is usually done?

We found early a need for a facile method of determining concentrations expressed as percentage phosphorus pentoxide in PPA,[5] but, except for the rather tedious titration of a hydrolyzed sample with alkali, we found none. The specific gravity was particularly insensitive to composition (see Table III) and viscosity the most sensitive (see Table IV). Yet, the latter was the most difficult to determine. The viscosities in the Experimental section extend the work of Griffith and Van Wazer[2] in the range most important for synthetic work. It is indeed remarkable how great the change in viscosity is in the range 81–85% P_2O_5.

The high viscosity of PPA gave rise to two difficulties in the kinetic work in which the rate of rearrangement of acetophenone oximes was followed by spectroscopic determination of the unrearranged ketone concentration: (1) The PPA could not be delivered accurately on a volume basis so that a weight basis was used. (2) The solution of oxime in PPA was very sluggish. Special means of solution was devised. Still a third difficulty arose because of the use of PPA: the rearrangement product, the acetanilide, could not be hydrolyzed by simple dilution and heating of an aliquot. Dilute sulfuric acid had to be added to bring about hydrolysis. The procedure described in the Experimental section overcame these difficulties and yielded the information in Tables I and II. All these rates fitted pseudo first-order rate equations.

The most noteworthy feature of Table II is that the rearrangement of all acetophenone oximes in PPA is some 12–35 times as rapid as in sulfuric acid

(1) Abstracted in major part from Ph.D. Thesis, Vanderbilt University, of R.M.S., University Microfilm, *Dissertation Abstracts*, **20**, (4), 1196 (1959), Ann Arbor, Mich.

(2) For summary see J. R. Van Wazer, "Phosphorus and Its Compounds," Vol. I, Interscience Publishers, Inc., New York, N. Y., 1958, pp. 747 and 770.

(3) F. D. Popp and W. E. McEwen, *Chem. Revs.*, **58**, 321 (1958).

(4) Beckmann Rearrangement. VIII, P. J. McNulty and D. E. Pearson, *J. Am. Chem. Soc.*, **81**, 612 (1959).

(5) In spite of the sensitivity of viscosity to composition, we believe the refractive index will provide the best compromise for a correlation of a physical property with percentage phosphorus pentoxide; see following paper.