in vacuum. The residue was dissolved in ether, extracted with a sodium carbonate solution, washed with water, dried over sodium sulfate and evaporated to dryness in a vacuum. The oily residue was distilled. The product was collected at 131–132° at 0.8–0.9 mm. and weighed 3.4 g. (71%). It was a clear, colorless oil, λ_{max} 271, 277 and 281 mμ (ε 399, 331 and 355), λinfl. 263 mμ (ε 252), $n^{20}D$ 1.5663, m.p. 17° (as determined by the plateau in the warming curve). Badger, *et al.*,[13] give only b.p. 106–110° (air-bath temperature) (0.3 mm.).

Anal. Calcd. for $C_{15}H_{20}$: C, 89.93; H, 10.06. Found: C, 90.05; H, 10.25.

9-Methyl-*s*-octahydroanthracene (V).—This was prepared essentially according to the procedure of Badger, *et al.*,[10] from *s*-octahydroanthracene. The latter was prepared by the reduction[17] of purified anthracene in absolute ethanol using Raney nickel at 900 p.s.i. and 145°. The intermediate **9-chloromethyl-*s*-octahydroanthracene** melted at 90–91°, λ_{max} 292 mμ (ε 1780), λinfl. 286–288 mμ (ε 1760). Badger, *et al.*,[10] gave m.p. 91–92°. It was dehalogenated by hydrogenation in ethanol at room temperature and atmospheric pressure using 10% by weight of 5% palladium-on-charcoal. One molecular equivalent of hydrogen was absorbed in 30 minutes. The product consisted of lustrous flakes, m.p. 50.5–51.5°, λ_{max} 274, 279 and 283 mμ (ε 664, 548 and 720), λ infl. 265 mμ (ε 350). Badger, *et al.*,[10] give m.p. 52°.

(17) (a) H. I. Waterman, J. J. Leendertse and A. C. Cranedonk. *Rec. trav. chim.*, **58**, 83 (1939); (b) G. Schroeter, *Ber.*, **57**, 2003 (1924).

BETHESDA 14, MARYLAND

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A New Synthesis of 2-Phosphoryl-D-glyceric Acid

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A definitive synthesis of 2-phosphoryl-D-glyceric acid is described which yields the pure compound in quantity as the crystalline trisodium salt. Although the rotation of this synthetic material is in disagreement with that of previously reported preparations of 2-phosphoryl-D-glyceric acid, chemical and enzymatic studies have given conclusive proof of the identity and purity of this preparation.

2-Phosphoryl-D-glyceric acid (2PGA), the glycolytic intermediate between 3-phosphoryl-D-glyceric acid (3PGA) and phosphoryl enolpyruvic acid (PEPA), was first isolated by Meyerhof and Kiessling from yeast,[1] and was characterized as a crystalline barium salt whose analysis indicated the empirical formula $C_3H_5O_7PBa \cdot 1^1/_2 H_2O$. When dissolved in 1 *N* hydrochloric acid, the substance showed $[\alpha]D$ +24.3°, the concentration being calculated on the basis of the free acid. Meyerhof and Kiessling[1] also described the preparation of unnatural 2-phosphoryl-L-glyceric acid from synthetic 2-phosphoryl-DL-glyceric acid[2] by treatment with a muscle extract which metabolized the natural substance. The L-isomer remaining was isolated as a crystalline barium salt, $C_3H_5O_7PBa \cdot 3H_2O$, and under the same conditions described above showed $[\alpha]D$ −23.6°.

A second synthesis of 2-phosphoryl-D-glyceric acid has been described by Neuberg, who treated methyl D-glycerate with ethyl metaphosphate.[3] From the mixture of phosphorylated compounds he isolated, by a prolonged fractionation of metal salts, a small yield of 2-phosphoryl-D-glyceric acid as the barium salt (no analysis), which was converted to a silver salt, $C_3H_4O_7PAg_3$. The barium salt in 1 *N* acid showed $[\alpha]D$ +23.2°.

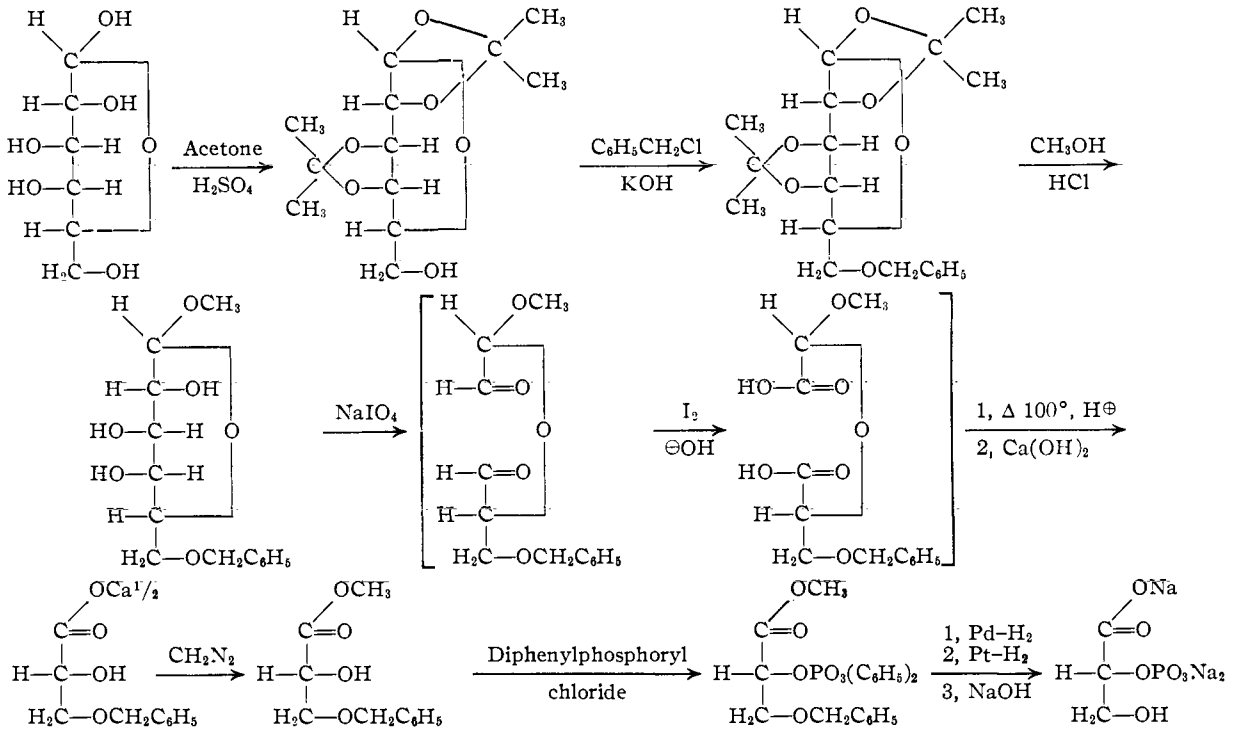
We are reporting in this paper the first definitive synthesis of 2-phosphoryl-D-glyceric acid, which affords the natural isomer in quantity in an optically pure form as the easily crystallized trisodium salt (pentahydrate). Since the rotation which we have observed for our preparation, $[\alpha]D$ +13° (1 *N* hydrochloric acid), is in disagreement with that reported by previous workers, we will outline the synthesis and characterization of the compound in detail.

Our preparation of 2-phosphoryl-D-glyceric acid was carried out by phosphorylation of methyl 3-*O*-benzyl D-glycerate. This intermediate was prepared from D-galactose by (1) acetonation to 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose; (2) benzylation of this compound to give 1,2:3,4-di-*O*-isopropylidene-6-*O*-benzyl D-galactopyranose; (3) conversion of the benzylated compound to methyl 6-*O*-benzyl α-D-galactopyranoside with methanolic hydrogen chloride; (4) cleavage of this compound with periodate, followed by oxidation of the dialdehyde to the dicarboxylic acid, which was hydrolyzed to glyoxylic acid and 3-*O*-benzyl D-glyceric acid; (5) isolation of 3-*O*-benzyl D-glyceric acid as the crystalline calcium salt; and (6) conversion of the calcium salt to methyl 3-*O*-benzyl D-glycerate with diazomethane. The optical purity of the 3-*O*-benzyl D-glyceric acid and its methyl ester were established by debenzylation to give D-glyceric acid with the recorded specific rotation.

Phosphorylation of methyl 3-*O*-benzyl D-glycerate[4] was carried out with diphenylphosphoryl chloride. The blocking groups were removed from the methyl 3-*O*-benzyl-2-diphenylphosphoryl D-glycerate by treatment first with hydrogen and palladium, followed by hydrogen and platinum. The resulting methyl 2-phosphoryl-D-glycerate was saponified, and the acid then crystallized as the trisodium salt from water by the addition of methanol. The over-all yield from D-galactose was about 20%.

Characterization of this synthetic material has been effected as follows: Elemental analyses on the hydrate and on the anhydrous material are in excellent agreement with the calculated values. Dephosphorylation with acid according to Kiessling and Schuster,[4a] or with intestinal phosphatase gave D-glyceric acid with the recorded rotation, thus es-

(1) O. Meyerhof and W. Kiessling, *Biochem. Z.*, **276**, 239 (1935).
(2) W. Kiessling, *Ber.*, **68**, 243 (1935).
(3) C. Neuberg, *Arch. Biochem.*, **3**, 105 (1943).
(4) We have also used this substance as a starting material for a definitive synthesis of 3-phosphoryl-D-glyceric acid.
(4a) W. Kiessling and P. Schuster, *Ber.*, **71**, 123 (1938).



tablishing the optical purity of the glyceric acid portion. The optical rotation of our 2-phosphoryl-D-glyceric acid in the presence of molybdate is $[\alpha]_D$ +5°. The rotation of natural 3-phosphoryl-D-glyceric acid in molybdate ($[\alpha]_D$ −745°)[4b] is a unique and identifying characteristic of this latter substance. When our 2-phosphoryl-D-glyceric acid was heated at 100° in 1 *N* hydrochloric acid for 30 minutes (conditions which are known to cause phosphate migration),[5] the rotation in molybdate changed to $[\alpha]_D$ −600°. This indicates 80.5% conversion of our compound into 3-phosphoryl-D-glyceric acid at equilibrium. When natural 3-phosphoryl-D-glyceric acid was treated with acid in the same way, the rotation changed to −610°. This equilibrium value (3PGA/2PGA = 4.1) reached from both sides by acid-catalyzed phosphate migration, corresponds roughly to that found for the enzymatic equilibrium established between these two compounds. This ratio was also calculated from the rotation of the acid solution, the equilibrium mixture having a specific rotation of −9.0°, and the ratio 3PGA/2PGA being 4.0.

The discrepancy between the rotations in molybdate for our preparation (+5°) and for the Meyerhof and Kiessling preparation (−68°)[1] is probably due to a contaminant in their material of about 10% 3-phosphoryl-D-glyceric acid. This would not be surprising in view of the very similar properties shown by these two compounds, and the obvious difficulties associated with their separation. It is unfortunate that rotations in molybdate have not been given for either of the previous synthetic 2-phosphorylglyceric acids described.[1,3] The problem of phosphate migration is now readily appreciated.[6] Although Kiessling used mild conditions in most of his preparations, one step in the recrystallization of trisilver 2-phosphoryl-DL-glycerate involved redissolving the salt in hot dilute nitric acid.[2] It is now apparent that such treatment could bring about considerable phosphate migration. We have studied the rate of phosphate migration of our 2-phosphoryl-D-glyceric acid, the results of which are shown in the figure. The substance was stable for at least 2 hours in 0.25 *N* hydrochloric acid at 50°, but at higher temperatures migration occurred, and was very rapid in 1.0 *N* acid at 100°.

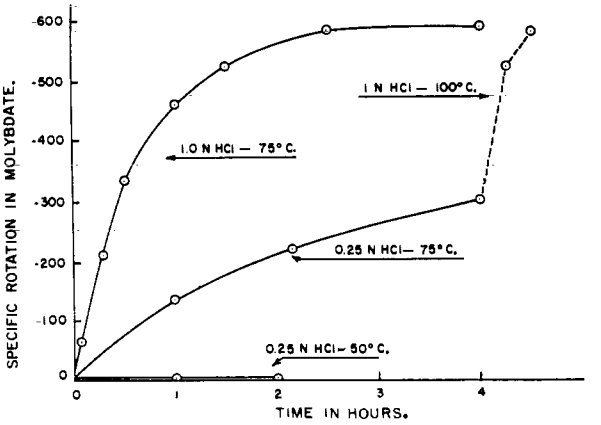


Fig. 1.—Acid-catalyzed phosphate migration of 2-phosphoryl-D-glyceric acid to 3-phosphoryl-D-glyceric acid.

That Kiessling[2] observed identical rates for acid-catalyzed phosphate hydrolysis of 2-phosphoryl-D-glyceric acid and 3-phosphoryl-D-glyceric acid is readily explained if hydrolysis occurs *via* a common

(4b) O. Meyerhof and W. Schulz, *Biochem. Z.*, **297**, 60 (1938). This is the highest value reported. If the true value is nearer −725° the ratio would be 4.8 instead of 4.1.

(5) E. Baer and M. Kates, *J. Biol. Chem.*, **185**, 615 (1950).

(6) M. C. Bailley, *Compt. rend.*, **206**, 1902 (1938); E. Chargaff, *J. Biol. Chem.*, **144**, 455 (1942).

intermediate resulting from prior phosphate migration. As shown in the hydrolysis curve of 2,3-diphosphoryl-D-glyceric acid, where phosphate migration can occur initially only to the carboxyl group, two different rates were observed.[2]

Acid-base titration of our preparation indicates the presence of one mole each of primary phosphate (pK 1.8), carboxyl (pK 3.63), and secondary phosphate (pK 6.64). The pK's are very similar to those of natural 3-phosphoryl-D-glyceric acid and synthetic 2-phosphoryl-DL-glyceric acid determined under the same conditions.

We have further characterized our preparation by the following preliminary enzymatic experiments carried out with purified enzyme preparations. When incubated with phosphorylglyceric acid mutase and a catalytic amount of 2,3-diphosphoryl-D-glyceric acid, the 2-phosphoryl-D-glyceric acid was converted rapidly to 3-phosphoryl-D-glyceric acid. Acid isomerized 2PGA (200 mg.) was treated with barium acetate, and the crystalline barium 3-phosphoryl-D-glyceric acid isolated (150 mg.) amounted to 90% of that present in the equilibrium mixture. Enolase brought about the expected change to phosphoryl enolpyruvate, the constant (PEPA/2PGA) of 2.4 being found.[7] After equilibration of 200 mg. of 2PGA with enolase, 87% of the PEPA present at equilibrium was isolated from the reaction as the crystalline silver barium salt (160 mg.). When incubated in the presence of enolase coupled with phosphopyruvate transphosphorylase, adenosine diphosphate, lactic dehydrogenase and reduced diphosphopyridine nucleotide, the compound was converted quantitatively to lactic acid.

The total of this evidence indicates that we have obtained the first preparation of pure 2-phosphoryl-D-glyceric acid. The elemental analyses given for previous preparations have never been satisfactory, the appending of a varying amount of water of crystallization (which was never analyzed for itself) being necessary to bring correspondence between the calculated and observed values. The higher rotation (+24.3°) reported by Meyerhof and Kiessling for the isolated material[1] could have been due to an impurity of α-D-glucose 1,6-diphosphate ($[\alpha]_D$ +70°). However, the excellent agreement between their value and those reported for the synthetic preparations, by Kiessling (−23.6°, unnatural form)[1] and Neuberg (+23°)[3] tends to rule this out.

The very useful method for estimating 3-phosphoryl-D-glyceric acid (which is based on the rotation of this substance in molybdate solution) has, in the past, required a correction for the rotation of the 2-phosphoryl-D-glyceric acid in the same solution.[8] It is now apparent that this correction is not necessary, since pure 2-phosphoryl-D-glyceric acid has an insignificant optical activity in molybdate.

The lower rotation we have found for 2-phosphoryl-D-glyceric acid in acid solution likewise necessitates revision in previous calculations of enzymatic equilibria based on optical rotation measurements. Thus, Meyerhof and Oesper[9] observed that 3-phosphoryl-D-glyceric acid and phosphorylglyceric acid mutase equilibrated to a final specific rotation of −8.6°. From this they calculated the conversion to 2-phosphoryl-D-glyceric acid to be 15%. If these same data are recalculated using our value $[\alpha]_D$ +13.0°, the percentage 2-phosphoryl-D-glyceric acid is 21.5, and the ratio becomes 78.5/21.5 or 3.65, in good agreement with the ratio we have found for acid-catalyzed equilibration.

The equilibrium constants calculated from the enzymatic studies presented in this paper are of a preliminary nature, and are offered only as confirmation of the identity of our preparation of 2-phosphoryl-D-glyceric acid. We do not suggest that they should replace the presently accepted (although controversial) values, but it is obvious that the way is open for a fruitful reinvestigation of this problem.

Experimental

1,2:3,4-Di-*O*-isopropylidene-D-galactopyranose.[10]—D-Galactose (100 g.) was stirred for 18 hours with 8 l. of commercial grade acetone containing 80 ml. of concentrated sulfuric acid, during which time most of the sugar dissolved. The vessel and contents were then cooled in ice-water, and the acid was neutralized by passing ammonia gas through the solution. The precipitated salt was removed by filtration, and the filtrate was concentrated to a sirup which was dissolved in 1 l. of chloroform and washed once with 100 ml. of water. The chloroform layer was separated and concentrated to a sirup, which was distilled giving a major fraction at 0.1–0.2 mm. and 110–120°. The yield of the diacetone compound was 100–110 g. (70%).

1,2:3,4-Di-*O*-isopropylidene-6-*O*-Benzyl-D-galactopyranose.—Di-*O*-isopropylidene-D-galactose (110 g.) was stirred with 100 ml. of toluene, 100 ml. of benzyl chloride and 150 g. of powdered potassium hydroxide at 100° for 5 hours. After the mixture had cooled to room temperature, 250 ml. of ice water was added, and the stirring was continued until the salts dissolved. The organic layer was separated and washed three times with water.[11] It was finally concentrated to a sirup and distilled. The fraction with b.p. 130–160° (0.01 mm.) was collected, and weighed 140 g. (95%).

Anal. Calcd. for $C_{19}H_{26}O_6$: acetone, 33.4. Found: acetone 33.5.

Methyl 6-*O*-Benzyl-α-D-galactopyranoside.—Di-*O*-isopropylidene-6-*O*-benzyl-D-galactopyranose (140 g.) was dissolved in 500 ml. of dry methanol containing 2% hydrogen chloride gas, and the solution was refluxed for 3 hours, during which deacetonation and glycoside formation occurred. The cooled solution was stirred with silver oxide (35 g.) until neutral, and the silver chloride formed was removed by filtration through Filter-cel. The alcoholic filtrate was treated with hydrogen sulfide to remove a trace of silver ion, and again filtered through Filter-cel. The filtrate was then concentrated *in vacuo* to dryness, during which the product crystallized spontaneously. It was redissolved in 150–200 ml. of hot absolute ethanol, and the solution was left overnight at 5° to crystallize. The product was collected by filtration, and washed free of color on the funnel with a little cold absolute ethanol. The crystals were dissolved in 200 ml. of absolute ethanol, the solution was treated with a small amount of decolorizing charcoal, filtered, and allowed to cool. Crystallization gave 40 g. of almost pure methyl 6-*O*-benzyl-α-D-galactopyranoside, m.p. 142–144°. The ethanolic mother liquor from above was concentrated *in vacuo* to a thick sirup, treated again with methanolic hy-

(7) O. Warburg and W. Christian, *Biochem. Z.*, **310**, 384 (1941).

(8) For example, E. W. Sutherland, T. Posternak and C. F. Cori, *J. Biol. Chem.*, **181**, 153 (1949).

(9) O. Meyerhof and P. Oesper, *ibid.*, **179**, 1371 (1949).

(10) H. Ohle and G. Berend, *Ber.*, **58**, 2585 (1925).

(11) Vigorous shaking in the separatory funnel should be avoided because of the tendency for emulsions to form. Once formed, the emulsion may be broken by adding a solution of 10% potassium chloride.

drogen chloride as above, and the reaction was worked up in the same manner. A second crop of 30 g. of the galactoside was thus obtained. A third treatment of the mother liquor yielded an additional 10 g. The total yield of methyl 6-*O*-benzyl-α-D-galactopyranoside was 80 g. (70%).

For analysis, the product was recrystallized several times from absolute ethanol, and dried at 50° and 0.1 mm. over phosphorus pentoxide. It showed $[\alpha]_D$ +113° (*c* 3, water), and m.p. 144–145°.

Anal. Calcd. for $C_{14}H_{20}O_6$ (284): C, 59.2; H, 7.1. Found: C, 59.2; H, 6.9.

The pure substance consumed two moles of periodate per mole, and on catalytic debenzylation with hydrogen and palladium was converted to methyl α-D-galactopyranoside with m.p. 102–105° and $[\alpha]_D$ +180° (*c* 2, water). Methyl α-D-galactopyranoside is reported to melt at 110° and show $[\alpha]_D$ +179° (water).

Calcium 3-*O*-Benzyl-D-glycerate.—To a cooled solution of 40 g. of sodium metaperiodate in 300 ml. of water was added 20 g. of methyl 6-*O*-benzyl-α-D-galactopyranoside. The galactoside dissolved readily, and the solution was left overnight at room temperature. It was then extracted four times with 400-ml. portions of ethyl ether, and the combined ether extract (without drying)[12] was poured into a 2-l. flask containing 100 ml. of water, and the mixture was concentrated (bath 35°) to remove the ether. The remaining water solution of the dialdehyde showed $[\alpha]_D$ +82.5°, and the dialdehyde had an oxidation equivalent of 64.4 (theory 63.0).

The dialdehyde solution (from 20 g. of methyl 6-*O*-benzyl-α-D-galactopyranoside) was diluted to about 400 ml. with water. To this was added a solution prepared from 56 g. of iodine[13] and 70 g. of potassium iodide in 50 ml. of water. The iodine solution was followed immediately by a buffer prepared from 65 g. of potassium carbonate, 48 g. of potassium bicarbonate and 500 ml. of water. The mixture was swirled to ensure good mixing, and left at room temperature in the dark for 2 hours. A sirup which separated at first went into solution as the reaction proceeded. This was greatly facilitated by occasionally shaking the mixture. After 2 hours, when the oxidation was complete, the vessel was placed in a large pan to prevent loss from foaming, and 142 ml. of 10 *N* sulfuric acid was cautiously added (gas evolution). The excess iodine was reduced by adding solid sodium thiosulfate (40–50 g.), and the clear solution was filtered through cotton to remove a small amount of a dark oil. It was then extracted four times with 1-l. portions of ethyl ether.[14] The ether extract, mixed with 100 ml. of water, was concentrated *in vacuo* (bath 50°) to remove the ether. The resulting water solution of the sirupy dicarboxylic acid showed $[\alpha]_D$ +14.6°.

The water solution of the dicarboxylic acid was heated on a steam-bath for 2 hours. During this time the rotation decreased to zero as the acetal structure was hydrolyzed. The cooled solution was extracted four times with 100-ml. portions of ethyl ether, and the dried ether extract (sodium sulfate) was concentrated *in vacuo* (bath 50°) to a sirup. The sirup was dissolved in 50 ml. of water and the solution again concentrated to remove a small amount of formic acid. The resulting sirup (12 g.) was dissolved in 120 ml. of water, and 3.0 g. of powdered calcium hydroxide was added. The mixture was swirled and slowly warmed over a bunsen burner to hasten formation of the glyceric acid calcium salt. The mixture was then heated to boiling to dissolve the salt, and was filtered rapidly while hot from some insoluble brown material. On cooling, the filtrate deposited crystals of calcium 3-*O*-benzyl-D-glycerate. After several hours at 5° this was collected and recrystallized from hot water (110 ml.). The pure material separated as long needles and when air-dried weighed 9.5 g. (60%). It contained one molecule of water of crystallization.

Anal. Calcd. for $C_{10}H_{11}O_4Ca_{1/2}\cdot H_2O$ (233): Ca, 8.60. Found: Ca, 8.75.

(12) If the dialdehyde is dehydrated, it changes to a water-insoluble oil that does not yield the desired 3-*O*-benzyl-D-glyceric acid.

(13) The use of bromine as the oxidizing agent was unsuccessful since some benzyl cleavage and halogen addition occurred, and the glyceric acid derivative could not be obtained pure.

(14) The water layer may become colored during this extraction due to formation of free iodine. If so, more solid sodium thiosulfate should be added.

For complete analysis the compound was dried for 2 hours at 100° and 0.01 mm. over phosphorus pentoxide. The dried (anhydrous) material melted at 215–220° with slight decomposition, and showed $[\alpha]^{22}_D$ +20° (*c* 0.5, water).

Anal. Calcd. for $C_{10}H_{11}O_4Ca_{1/2}$ (215): C, 55.8; H, 5.1; Ca, 9.3. Found: C, 55.6; H, 5.0; Ca, 9.15.

Catalytic debenzylation with hydrogen and palladium gave calcium D-glycerate with $[\alpha]^{23}_D$ +11.8°. The reported rotation is $[\alpha]_D$ +12.9°.

Methyl 3-*O*-Benzyl-D-glycerate.—Nine grams of pure calcium 3-*O*-benzyl-D-glycerate was dissolved in 50 ml. of 1 *N* hydrochloric acid, and the solution was extracted four times with 50-ml. portions of ether. The combined ether extract, which contained about 8.0 g. of 3-*O*-benzyl-D-glyceric acid, was dried over sodium sulfate and filtered from the salt. The dry ether filtrate was treated with an ether solution containing about 2.0 g. of diazomethane. A slight excess of diazomethane was present as indicated by the permanent yellow color. After 30 minutes, the solution was concentrated *in vacuo* to a sirup (8.5 g.), which was stripped of traces of solvent in a high vacuum at 50°. The methyl 3-*O*-benzyl-D-glycerate showed $[\alpha]_D$ −1.31° (in substance), and was used in the phosphorylation step without further purification.

Some of the ester was distilled at 0.1 mm.; the middle fraction, b.p. 95–100°, had d^{25}_4 1.160 and showed $[\alpha]_D$ −1.45° (in substance).

Anal. Calcd. for $C_{11}H_{14}O_4$ (210): C, 62.9; H, 6.7; OCH_3, 14.75. Found: C, 62.6; H, 6.6; OCH_3, 14.76.

A sample of methyl 3-*O*-benzyl-D-glycerate was reduced with lithium aluminum hydride to give a benzylglycerol that consumed 1 mole of periodate per 188 g. The molecular weight of *O*-benzylglycerol is 182. Debenzylation of the *O*-benzylglycerol gave the expected glycerol, isolated as the tri-*O*-benzoate, m.p. 74–76°. The m.p. was not depressed when the substance was mixed with authentic glycerol tri-*O*-benzoate.

Phosphorylation of Methyl 3-*O*-Benzyl-D-glycerate.[15]—A solution of 8.0 g. of methyl 3-*O*-benzyl-D-glycerate in 40 ml. of dry pyridine was cooled to 5° in ice-water. From a dropping funnel, attached in such a way as to exclude moisture, 10.8 g. of diphenylphosphoryl chloride was added dropwise over a 10-minute interval. Crystals of pyridine hydrochloride began to separate during this time; the funnel was rinsed with 10 ml. of pyridine, and the reaction vessel was stoppered and left overnight at 5–10°.

One ml. of water was added to hydrolyze the excess phosphorylating reagent, and the solution was left for 30 minutes. It was then concentrated *in vacuo* (bath 50°) at a water aspirator to remove most of the pyridine. The residue was taken up in 100 ml. of chloroform and washed with 100 ml. each of water, cold 1 *N* hydrochloric acid, cold 1 *N* potassium bicarbonate, and finally with water again. The chloroform layer was dried over anhydrous sodium sulfate, then filtered and concentrated *in vacuo* (bath 50°) to a thick sirup that weighed 16.0 g. (95%). This methyl 3-*O*-benzyl-2-diphenylphosphoryl-D-glycerate was used in the following reactions without further purification.

Anal. Calcd. for $C_{23}H_{23}O_7P$ (442): C, 62.4; H, 5.2; OCH_3, 7.0; P, 7.0. Found: C, 62.1; H, 4.9; OCH_3, 7.4; P, 7.7.

Trisodium 2-Phosphoryl-D-glycerate.—The success of this unblocking step depends on the use of a platinum catalyst active enough to remove the phenyl groups in 1.5 hours or less, since a slow reaction (3–5 hours) resulted in considerable phosphate migration (as much as 5%), and a product that was difficult to crystallize. Therefore, it is recommended that the catalysts be prepared fresh and tested on a small-scale reduction of about 0.5 g. of the intermediate prior to larger preparative runs. Once started, the unblocking reactions should be carried through to the saponification step in 2.5 to 3 hours.

Five grams of 5% palladium chloride on carbon[16] was reduced in 100 ml. of 95% ethanol by shaking with hydrogen until uptake was complete (about 150 ml.). The catalyst was washed free of acid with 95% ethanol by a process of repeated suspension and centrifugation, 4–5 washings being required. The washed catalyst was then resuspended in

(15) E. G. Ball, *Biochem. Preps.*, **2**, 40 (1952).

(16) H. Gilman and A. H. Blatt, *Org. Syntheses*, **26**, 77 (1946). Other active palladium catalysts should serve as well.

100 ml. of absolute ethanol and 5.0 g. of methyl 3-*O*-benzyl-2-diphenylphosphoryl-D-glycerate was added. The mixture was shaken with hydrogen at room temperature and atmospheric pressure. The hydrogen uptake was complete in 10–15 minutes, and amounted to 280 ml., or that required for debenzylation of the compound.

The palladium catalyst was then removed by centrifugation, and the solution returned to the hydrogenation chamber. One gram of freshly prepared platinum oxide[17] was added along with about 1 g. of acid-washed charcoal to prevent clumping of the catalyst that otherwise occurs. The mixture was shaken vigorously with hydrogen until gas uptake ceased (2800 ml. in one hour). This amounts to the hydrogen required for removal of the phenyl groups and saturation of the aromatic rings. The platinum catalyst was removed by centrifugation and the alcohol solution was immediately mixed with 25 ml. of 1 *N* sodium hydroxide. The turbid solution was concentrated *in vacuo* (bath 40°) to a sirup which was redissolved in 10 ml. of water. An additional 10 ml. of 1 *N* sodium hydroxide was added, and the solution was left at room temperature for 30 minutes to complete saponification of the methyl ester.

The solution, which now contained trisodium 2-phosphoryl-D-glycerate, was mixed with a little Filter-cel and filtered with suction through No. 50 Whatman paper.[18] About 10 ml. of water was used to rinse the vessel and wash the solid on the funnel. To the combined water filtrate was added methanol to turbidity (about 50 ml.). The flask and contents were left at room temperature to crystallize. First crystals (long needles, or sometimes shiny plates) may not appear for several hours, and their appearance may be hastened by rubbing the inside wall of the container with a glass rod, or by seeding. Crystallization was completed at 5° overnight. The product was best collected by centrifugation, and was washed free of water with methanol; otherwise it may redissolve in traces of water left behind as the methanol evaporates. The final washing was with absolute ether, and the product was left to dry in air. The yield was 2.0–2.5 g. Recrystallization was accomplished by redissolving the product in 15 ml. of water, filtering (with Filter-cel) if cloudy, and adding methanol to turbidity. The product always crystallized the second time as long needles (5–10 mm.) in rosettes, and after staying overnight at 5°, was collected by centrifugation, washed with methanol twice, then ether, and allowed to dry in air.

The trisodium 2-phosphoryl-D-glycerate thus obtained showed $[\alpha]^{22}D$ +3.6° (*c* 2, water), and the free acid $[\alpha]^{22}D$ +12.9° (*c* 1.8 free acid, 1 *N* hydrochloric acid). The analysis corresponded to a pentahydrate.

Anal. Calcd. for $C_3H_4O_7PNa_3 \cdot 5H_2O$ (342): C, 10.5; H, 4.1; P, 9.1; residue (as Na_3PO_4), 48.0; H_2O, 26.3. Found: C, 10.2; H, 3.8; P, 9.2; residue, 48.0; H_2O, 26.0 (wt. lost on drying at 80° and 0.01 mm.).

When dried at 80° and 0.01 mm. for 6 hours, the compound lost all of the water of hydration, and on re-equilibration in a moist atmosphere it regained the exact weight lost by drying. Analysis of the anhydrous compound follows.

Anal. Calcd. for $C_3H_4O_7PNa_3$ (252): C, 14.3; H, 1.6; P, 12.3; residue (as Na_3PO_4), 65.0; Na, 27.4. Found: C, 14.5; H, 1.9; P, 12.0; residue, 64.5; Na, 27.1.

The anhydrous material showed $[\alpha]^{21}D$ +13.0° (*c* 2.4 free acid, 1 *N* hydrochloric acid), (64.4 mg. of the trisodium salt in 2 ml. of 1 *N* acid). The rotation in neutral 25% ammonium molybdate solution was $[\alpha]^{22}D$ +5° (concentration in terms of the free acid). For the material isolated from yeast, Meyerhof and Kiessling[1] reported $[\alpha]D$ +24.3° (concentration in terms of the free acid, 1 *N* hydrochloric acid), and in 25% ammonium molybdate $[\alpha]D$ −68° (concentration in terms of the free acid).

Although previous workers have obtained a crystalline acid barium salt of 2-phosphoryl-D-glyceric acid,[19] we have only succeeded in preparing amorphous products. When dissolved in 1 *N* hydrochloric acid the amorphous barium salt showed $[\alpha]D$ +14° (concentration in terms of the free acid).

(17) H. Gilman and A. H. Blatt, *ibid.*, Coll. Vol. I, 463 (1941).

(18) The solution should be water clear. A slight yellow coloration, resulting from a slow reduction, may portend difficulty in crystallizing the product.

(19) Kiessling was unable to crystallize the acid barium salt of 2-phosphoryl-DL-glyceric acid.

Acid Hydrolysis of 2-Phosphoryl-D-glyceric Acid.—A sample of the acid was heated in 1 *N* hydrochloric acid in a sealed tube at 125° for 18 hours. To the acid solution was added an excess of barium acetate and sodium hydroxide equivalent to the acid. The precipitated barium phosphate was removed, and the rotation of the supernatant determined. The observed $[\alpha]D$ +10.9° compares well with $[\alpha]D$ +12° reported for barium D-glycerate.

Acid-catalyzed Phosphate Migration.—Trisodium 2-phosphoryl-D-glycerate was dissolved in hydrochloric acid to give an 0.8% solution of the desired acidity. This solution was then heated in a water-bath and 0.5-ml. aliquots were taken periodically, added to 0.5 ml. of sodium hydroxide solution equivalent to the acid. One ml. of 25% ammonium molybdate solution was then added, and the rotation of the mixture was determined in a 1-dcm. tube. The results are shown in Fig. 1. No inorganic phosphate was formed during the reaction.

Isolation of 3-Phosphoryl-D-glyceric Acid from Isomerized 2-Phosphoryl-D-glyceric Acid.—About 200 mg. of trisodium 2-phosphoryl-D-glycerate was dissolved in 5 ml. of 1 *N* hydrochloric acid, and the solution was heated at 100° for 1 hour. To the hot solution was then added 40% barium acetate dropwise until the solution became turbid (about 2 ml.). On rubbing the inner wall of the test-tube with a glass rod, the acid barium salt of 3-phosphoryl-D-glycerate crystallized. After completion of crystallization at 5° for 4 hours, the salt was collected by centrifugation washed twice with 5-ml. portions of 50% ethanol, once with absolute ethanol, and dried *in vacuo* at 80° and 0.01 mm. for 1 hour. The yield was 150 mg., or 90% of the amount present in the equilibrium mixture.

A solution of 45.2 mg. of the salt in 2.0 ml. of 1 *N* hydrochloric acid showed αD −0.17° (1 dcm.), or $[\alpha]D$ −14.3° (*c* 1.19 free acid, 1 *N* hydrochloric acid). The reported value for the free acid is $[\alpha]D$ −14.5°.

A mixture of 0.5 ml. of the above acid solution, 0.1 ml. of 1 *N* sulfuric acid, 0.5 ml. of 1 *N* sodium hydroxide and 1.0 ml. of 30% ammonium molybdate was centrifuged to remove the barium sulfate. The clear supernatant showed αD −2.09° (1 dcm.), or $[\alpha]D$ −740° (*c* 0.283 free acid, 15% ammonium molybdate). The highest reported value for 3-phosphoryl-D-glyceric acid in neutral molybdate is −745°.

Action of Enolase.—A purified, but not crystalline, enolase was incubated with the 2-phosphoryl-D-glyceric acid at *p*H 7 and 5° in presence of 3×10^{-4} *M* magnesium sulfate. The ultraviolet absorption of the solution increased rapidly and came to equilibrium at a value of 2.5 (PEPA/2PGA) calculated from a molar extinction coefficient for phosphoryl enolpyruvate of 1.73×10^3. From the ratio of easily hydrolyzable phosphate to total phosphate, the equilibrium value 2.3 was found.

When the action of enolase was coupled with that of phosphopyruvate transphosphorylase and lactic dehydrogenase in the presence of adenosine diphosphate and reduced diphosphopyridine nucleotide, a solution made up to contain 3.36 μM./ml. of 2-phosphoryl-D-glyceric acid assayed 3.17 μM./ml. (95%) as represented by the oxidation of reduced diphosphopyridine nucleotide.

Isolation of Phosphoryl Enolpyruvate from 2-Phosphoryl-D-glyceric Acid Equilibrated with Enolase.—A solution of 200 mg. of trisodium 2-phosphoryl-D-glycerate, 1.5 ml. of water, 0.5 ml. of 0.01 *M* magnesium sulfate and 0.2 ml. of enolase solution (5 mg. protein/ml.) was left at room temperature for 1 hour. A solution of 100 mg. of silver nitrate in 2 ml. of water was then added, followed by the dropwise addition of 1 *N* nitric acid (about 2 ml.) to dissolve the precipitate of silver salts. A small amount of suspended matter was removed by centrifugation and discarded. The supernatant was decanted, 2 ml. of 40% barium acetate was added to it, and the mixture was left in the dark overnight at room temperature. The solution was decanted from the crystals of the silver barium salt of phosphoryl enolpyruvate, which were washed by decantation with four 5-ml. portions of 50% ethanol, then transferred to a büchner funnel and washed twice with absolute ethanol. The product was dried *in vacuo* at room temperature over phosphorus pentoxide. The yield was 160 mg., or 87% of that present in the equilibrium mixture.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF THE UNIVERSITY OF ILLINOIS]

The Electrolytic Reduction of Tricyclic α-Aminoketones. Synthesis of Medium Rings Containing Nitrogen. III

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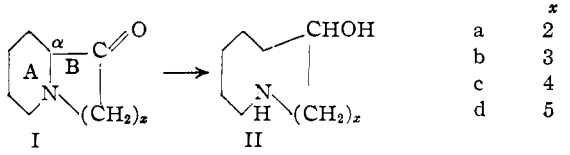
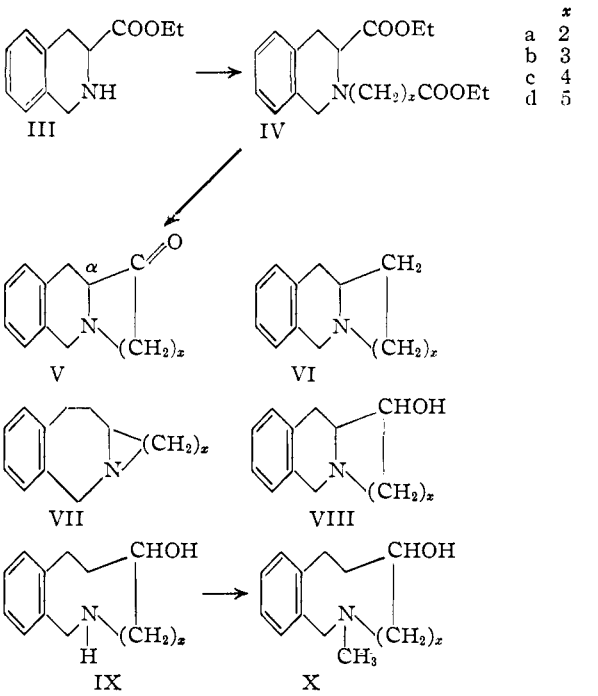
Our study of the electrolytic reduction of α-aminoketones has been extended to linear tricyclic α-aminoketones (V) in which one external ring is benzenoid and the other external ring, sharing a bridgehead nitrogen and containing the ketone group, is of varying ring size: 5, 6, 7 and 8 members. The extent of cleavage of the Cα–N bond and of rearrangement during electrolytic reduction at a lead cathode in 30% sulfuric acid at 60° has been found to be dependent upon the size of the ketone-containing ring. Moreover, when the latter is 7- or 8-membered, the process leads to a medium-size ring compound containing nitrogen and having a benzo grouping fused to the newly formed 11- or 12-membered ring.

The electrolytic reduction of bicyclic α-aminoketones (I), using a lead cathode in 30% sulfuric acid at 60°, is a unique method of making azacycloalkanols (II) of medium ring size.[2,3] It was of interest to determine whether the method would also apply to tricyclic α-aminoketones of the type in which a benzo grouping is fused to the A-ring (nonketonic) of I, especially since the additional benzenoid ring would be expected to induce certain steric restrictions on the conformations available to the *ortho* substituent groups.



The common intermediate in the synthesis of four test compounds of the tricyclic α-aminoketone type was the aminoester, ethyl 1,2,3,4-tetrahydroisoquinoline-3-carboxylate (III), prepared from DL-phenylalanine by the method of Archer.[4] From this compound a series of aminodiesters of general formula IV were made. These diesters were subjected to Dieckmann cyclization reactions in order to obtain the corresponding α-aminoketones V. Closure of the seven- and eight-membered ring ketones (Vc,d) was effected under conditions of high dilution in xylene with potassium *t*-butoxide.[5] The products of the electrolytic reduction of each aminoketone V were separated and identified. In general, it was possible to account for 80–90% of the reduced material.

Three products are likely to be formed by the electrolytic reduction at a lead cathode of the first member of the linear tricyclic α-aminoketone series V, the six-six-five fused ring system, benzo[c]-7-keto-1-azabicyclo[4.3.0]nonane (Va). These products are benzo[c]-1-azabicyclo[4.3.0]nonane (VIa), and benzo[c]-7-hydroxy-1-azabicyclo[4.3.0]nonane (VIIIa) and benzo[c]-7-hydroxyazacyclononane (IXa). The ketone Va was unstable in 30% sulfuric acid, so that the reduction had to be carried out as quickly as possible in order to have any identifiable product result. The only product characterized unequivocally was VIa, which formed a picrate identical with the picrate of authentic benzo[c]-1-azabicyclo[4.3.0]nonane, prepared by the Wolff–Kishner reduction of Va. The residual product was contaminated with unreacted ketone, but infrared analysis indicated the presence of OH/NH. This product (or products) could not be purified for complete identification. In the bicyclic series, by contrast, the six-five fused ring system (Ia)



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(2) N. J. Leonard, S. Swann, Jr., and J. Figueras, Jr., THIS JOURNAL, **74**, 4620 (1952).

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(4) S. Archer, *J. Org. Chem.*, **16**, 430 (1951).

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