1500 ml of $H_2O$. The mixture was refluxed for an additional 3 hr and after cooling extracted with $Et_2O$. The $Et_2O$ phases were treated with 5 *M* NaOH and the red phenolate precipitated. It was collected and then dissolved in $Et_2O$ saturated with HCl. The $Et_2O$ solution was dried ($MgSO_4$) and the solvent evaporated to afford 5.7 g of a solid product. Recrystallization from aqueous EtOH gave 5.25 g (66%) of yellowish phenol: mp 175.5–177° (lit.[6] 177–178.5°); nmr ($Me_2CO$-$d_6$) δ 6.9–7.6 (m, 4, arom protons), 7.9 (m, 2, H-1 and H-9), 8.9 (s, 1, OH).

**2-(4-Methoxy-3-dibenzofuranyloxy)-2-methylpropionic Acid (21).** A solution of 4-methoxy-3-hydroxydibenzofuran (6.5 g, 30 mmol) in 100 ml of dry $Me_2CO$ was stirred at room temperature with 10.1 g (180 mmol) of solid KOH. After 15 min the reaction mixture was cooled in an ice bath, and a solution of 1,1,1-trichloro-2-methyl-2-propanol (8.4 g, 45 mmol), prepared according to Fishburn and Watson,[14] in 50 ml of dry $Me_2CO$ was added dropwise during 45 min. The suspension was kept in the ice bath for 45 min. The reaction mixture was stirred at room temperature for 1 hr, refluxed for 4 hr, and finally stirred overnight at room temperature. The mixture was dissolved in $H_2O$ and the $Me_2CO$ was evaporated. The aqueous solution was acidified with HCl and extracted with $Et_2O$. The $Et_2O$ solution was treated with saturated $NaHCO_3$ solution and the aqueous solution was acidified and extracted with $Et_2O$. After drying ($MgSO_4$) and evaporation of the solvent 8.3 g (91%) of a yellow oil was obtained. The oil crystallized upon standing. Recrystallization from petroleum ether (bp 95–110°)–EtOAc gave crystals with mp 107–111°.

The acids **1**, **3**, **5**, **7**, **13**, **15**, **17**, **19**, and **23** were similarly prepared (Table I). However, in the preparations of **1**, **3**, **5**, and **7** equimolar amounts of 1,1,1-trichloro-2-methyl-2-propanol were used. 1-Hydroxydibenzofuran and 3-hydroxydibenzofuran used as starting materials in the syntheses of the acids **1** and **5** were prepared according to Stjernström[15] and Erdtman, *et al.*,[16] respectively.

**Ethyl 2-(1-Dibenzofuranyloxy)-2-methylpropionate (2).** 2-(1-Dibenzofuranyloxy)-2-methylpropionic acid (**1**, 6.7 g, 25 mmol) was dissolved in 250 ml of EtOH saturated with HCl gas and the mixture was refluxed for 4 hr. The solvent was evaporated and the dark oil was dissolved in $Et_2O$. The $Et_2O$ solution was washed with saturated $NaHCO_3$ solution, dried ($MgSO_4$), and evaporated to yield 6.6 g (88%) of a dark oil. The oil was distilled *in vacuo* to give 5.4 g (72%) of a slightly yellow oil, bp 156–158° (0.06 mm). After chromatography on a column of 300 g of $SiO_2$ (Merck, activity grade 2–3, 0.2–0.5 mm) with benzene as eluent, 4.3 g (58%) of a crystalline product was obtained: mp 74–77°; nmr ($CDCl_3$) δ 1.2 (t, 3, $J$ = 7, $OCH_2CH_3$), 1.8 [s, 6, $C(CH_3)_2$], 4.25 (q, 2, $J$ = 7, $OCH_2CH_3$), 6.6 (m, 1, H-2), 7.1–7.6 (m, 5, arom protons), 8.2 (m, 1, H-9).

The esters **4**, **6**, **8**, **14**, **16**, **18**, **20**, **22**, and **24** were similarly prepared; however, chromatography was unnecessary in these preparations (Table I).

**Ethyl 4-Dibenzofuranyloxyacetate (12).** Sodium (0.58 g, 25 mmol) was added to 200 ml of super-dry EtOH. When the reaction had ceased 4.6 g (25 mmol) of 4-hydroxydibenzofuran and 5.0 g (30 mmol) of ethyl bromoacetate were added. The reaction mixture was refluxed for 16 hr. The solvent was then evaporated and the remaining solid was dissolved in 150 ml of $H_2O$ and 150 ml of $Et_2O$. The ethereal layer was washed with NaOH solution, dried ($MgSO_4$), and evaporated to give 3.3 g of crude product. Distillation gave 2.65 g (39%) of a colorless oil, bp 238–241° (1.0 mm). The oil crystallized upon cooling, mp 50–51.5°.

**Ethyl 2-(4-dibenzofuranyloxy)propionate (10)** was prepared from 4-hydroxydibenzofuran and ethyl 2-bromopropionate in analogy with **12**. The product was distilled to give a colorless oil in 43% yield, bp 170–173° (0.2 mm), which crystallized upon cooling, mp 38.5–40°.

**2-(4-Dibenzofuranyloxy)propionic acid (9) and 4-dibenzofuranyloxyacetic acid (11)** were obtained by alkaline hydrolysis of **10** and **12**, respectively.

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## References

(1) L. A. Carlson, C. Hedbom, E. Helgstrand, A. Misiorny, B. Sjöberg, N. E. Stjernström, and G. Westin, *Acta Pharm. Suecica*, **9**, 411 (1972).
(2) J. L. Beaumont, L. A. Carlson, G. R. Cooper, Z. Fejfar, D. S. Fredrickson, and T. Strasser, *Bull. W. H. O.*, **43**, 891 (1970).
(3) R. I. Levy, S. H. Quarfordt, W. V. Brown, H. R. Sloan, and D. S. Fredrickson, *Advan. Exp. Med. Biol.*, **4**, 377 (1969).
(4) H. Gilman and D. L. Esmay, *J. Amer. Chem. Soc.*, **76**, 5787 (1954).
(5) N. M. Cullinane and H. J. H. Padfield, *J. Chem. Soc.*, 1131 (1935).
(6) S. Shibata, S. Natori, and Y. Sumi, *J. Pharm. Soc. Jap.*, **72**, 1333 (1952); *Chem. Abstr.*, **47**, 3923 (1953).
(7) H. Gilman, L. C. Cheney, and H. B. Willis, *J. Amer. Chem. Soc.*, **61**, 951 (1939).
(8) L. A. Carlson, S. O. Fröberg, and E. R. Nye, *Gerontologia*, **14**, 65 (1968).
(9) W. D. Block, K. C. Jarret, Jr., and J. B. Levine, *Clin. Chem.*, **12**, 681 (1966).
(10) G. Kessler and H. Lederer in "Automation in Analytical Chemistry," L. T. Skeggs, Ed., Mediad, New York, N. Y., 1965, p 341.
(11) K. Oita, R. G. Johnson, and H. Gilman, *J. Org. Chem.*, **20**, 657 (1955).
(12) H. Gilman and R. V. Young, *J. Amer. Chem. Soc.*, **57**, 1121 (1935).
(13) W. Borsche and W. Bothe, *Ber.*, **41**, 1940 (1908).
(14) A. G. Fishburn and H. B. Watson, *J. Amer. Pharm. Ass.*, **23**, 491 (1939).
(15) N. E. Stjernström, *Acta Chem. Scand.*, **16**, 553 (1962).
(16) H. Erdtman, F. Haglid, and N. E. Stjernström, *ibid.*, **15**, 1761 (1961).

# Synthesis of Phosphonic Acid Isosteres of 2-Phospho-, 3-Phospho-, and 2,3-Diphosphoglyceric Acid

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Phosphonic acid isosteres of 2-phosphoglyceric acid, 3-phosphoglyceric acid, and 2,3-diphosphoglyceric acid were prepared using the Arbuzov reaction and the Michaelis–Becker modification, followed by vigorous acid or base hydrolysis of the precursor esters. The small, highly charged molecules were tested *in vitro* on human red cell suspensions in physiological buffer for their effects on the oxygen-dissociation curve. None of the compounds exhibited a right or left curve shift in this assay when compared to controls.

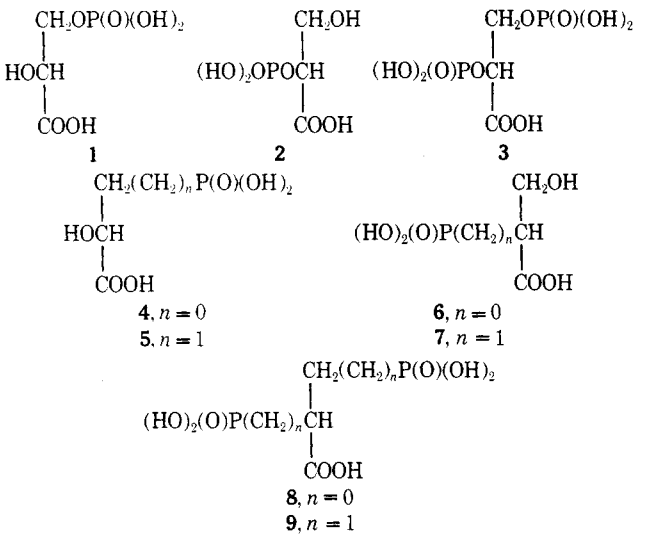
Recent reports have shown that the affinity of oxygen for hemoglobin can be altered by 2,3-diphosphoglyceric acid,[1,2] the predominant organic phosphate in the red blood cell. Our interest in improving performance of oxygen transport mechanisms *via* the 2,3-diphosphoglyceric acid–hemoglobin interaction has encouraged investigations directed at the utilization of drug-induced shifts[3] in the hemoglobin–oxygen dissociation curve. The ultimate objective was a compound which would alter the dissociation pressure and shift the curve to the right by *ca.* 3–5 mm at 50% saturation.[4] If a compound can induce a right shift of this magnitude, it will impart a greater "unload-

†The authors dedicate this manuscript to Dr. Alfred Burger who has been a friend, colleague, and advisor for many years.

ing" or alternatively a fixed delivery at a higher partial pressure of oxygen at the capillary level. Such a compound could be useful for treatment of ischemic conditions such as angina, myocardial infarction, stroke, and peripheral vascular disease.[5,6]

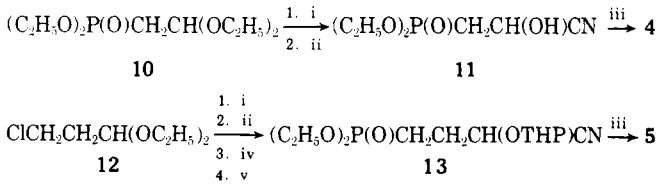
It appeared desirable to synthesize small, highly charged molecules akin to 2,3-diphosphoglyceric acid which incorporated phosphonate (P-C) bonds instead of phosphate (P-O-C) linkages. These compounds might mimic the role of 2,3-diphosphoglyceric acid as a hemoglobin cofactor or alternatively influence the enzymatic reactions in red cell metabolism which exert control over cellular concentrations of organic phosphates. Phosphonic acid derivatives of glyceric acids could also be useful for study of specific enzymatic steps in the major pathways of carbohydrate metabolism in mature red blood cells.[7,8] At the outset, we wished to determine the effect of phosphonic isosteres of 3-phosphoglyceric acid (**1**, 3-PG), 2-phosphoglyceric acid (**2**, 2-PG), and 2,3-diphosphoglyceric acid (**3**, 2,3-DPG) on the oxygen-hemoglobin dissociation curve.

Our immediate goals were phosphonic derivatives of **1–3** in which the oxygen atom of phosphate esters was replaced by a carbon to phosphorus bond (compounds **4**, **6**, and **8**) or a methylene group was substituted for the oxygen atom (compounds **5**, **7**, and **9**).‡




Syntheses of the two phosphonate derivatives **4** and **5** of 3-PG are shown in Scheme I. Compound **4** was prepared from diethyl 2,2-diethoxyethane-1-phosphonate (**10**) which is readily available[9] from the Arbuzov coupling of triethyl phosphite and 1-bromo-2,2-diethoxyethane. Hydrolysis of **10** to the aldehyde, conversion to the cyanohydrin **11**, and acid hydrolysis led to 3-phosphonolactic acid (**4**) isolated as the trilithium salt. We found the lithium salts to be most satisfactory for the characterization of most of the

**Scheme I**



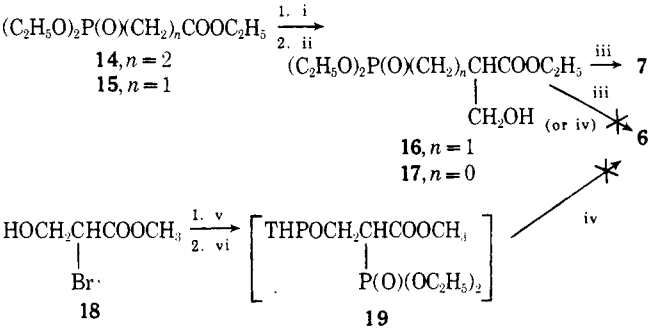

reagents: i = aqueous $H_2SO_4$, ii = $NaHSO_3$–NaCN, iii = concentrated HCl at reflux, iv = dihydropyran–TsOH, v = $P(OC_2H_5)_3$ at 150°

‡Compounds 4–9 are racemic.

phosphonic acids reported in this paper. For the synthesis of **5**, the phosphonyl intermediate **13** was prepared from the halogenated THP-protected cyanohydrin which was obtained from **12**. Attempted stepwise hydrolysis of **13** with dilute HCl followed by vigorous reaction with lithium hydroxide did not give a clean product. Direct hydrolysis of **13** with hot concentrated HCl afforded a 25% yield of 3-carboxy-3-hydroxypropane-1-phosphonic acid (**5**).

Conversion of ethyl 2-diethylphosphonopropionate (**14**) to the $\alpha$-hydroxymethylene derivative **16** is illustrated in Scheme II. Hydrolysis of **16** with hot lithium hydroxide gave 2-carboxy-3-hydroxypropane-1-phosphonic acid (**7**), a phosphonic isostere of 2-PG. In an attempt to prepare **6** from **15** using the route successful for **7** only phosphonoacetic acid was isolated from the complex reaction mixtures after either acid or basic hydrolysis of crude **17**. An alternate route to **6** was tried starting with the bromohydrin **18** but hydrolysis of the crude **19** did not afford **6**.

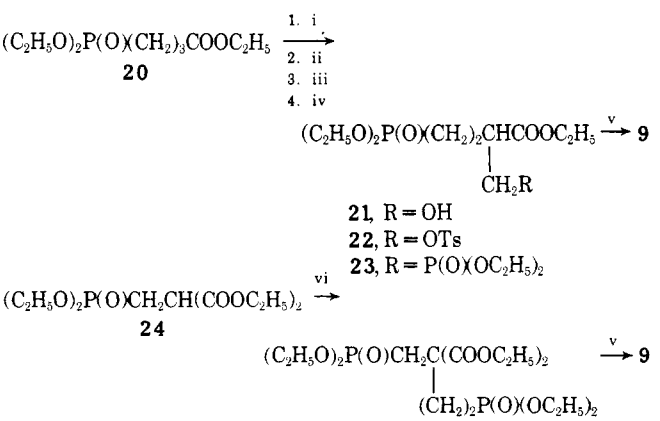
**Scheme II**




reagents: i = Na, $HCOOC_2H_5$, ii = $NaBH_4$, iii = hot LiOH, iv = 6 *N* HCl at 100°, v = dihydropyran–$POCl_3$, vi = $P(OC_2H_5)_3$ at 150°

During the course of this work, the synthesis of 2,3-diphosphonopropionic acid (**8**) was reported;[10] we prepared a sample by the published procedures but purified the pentalithium salt. Compound **9**, the bishomo derivative of **8**, was synthesized by the two routes shown in Scheme III. Ethyl 3-diethylphosphonobutyrate (**20**) was formylated[11] and reduced to the alcohol **21** in 35% overall yield. The tosylate **22** was displaced by the diethyl phosphite anion to give **23** which was hydrolyzed with 6 *N* HCl to give 2-carboxybutane-1,4-diphosphonic acid (**9**). Compound **9** was isolated both as the free acid and the dibarium salt. The second route to **9** utilized the alkylation of diethyl diethylphosphonomethyl-

**Scheme III**




reagents: i = Na, $HCOOC_2H_5$, ii = $NaBH_4$, iii = TosCl, pyridine, iv = NaH, $HP(O)(OC_2H_5)_2$, v = 6 *N* HCl at 110°, vi = Na, $Br(CH_2)_2P(O)(OC_2H_5)_2$, EtOH at 80°

malonate[11] with diethyl 2-bromoethylphosphonate to afford **25**. Vigorous acid hydrolysis of **25** gave **9**.

The phosphonic acids were evaluated *in vitro* for their effects on the oxygen dissociation curve of human red cell suspensions in physiological buffer.[3] The compounds were inactive at $10^{-3}$ *M* in this assay;[3] *i.e.*, they did not alter the dissociation curve (right or left) within experimental error. A sample of **9** did not produce a shift§ in a cell-free, human hemoglobin solution.[3,12] These compounds were also tested for antibiotic activity against several strains of gram-positive and gram-negative bacteria[13] but showed no activity at 200 μg/ml.

## Experimental Section

Elemental analyses were performed by the Analytical and Chemistry Department of Smith Kline & French Laboratories. Where analyses are indicated by symbols of elements, analytical results for those elements were within ±0.4% of the theoretical values. Solutions were dried over $MgSO_4$. The ir and nmr of the intermediate compounds agreed with the structures. Nmr of compounds **4**, **5**, and **7**–**9** were determined on a 90-MHz Perkin-Elmer R-32 spectrometer and chemical shifts are reported in parts per million upfield from HOD in 20% DCl–$D_2O$.

**Diethyl 2-Cyano-2-hydroxyethane-1-phosphonate (11).** A solution of 15.3 g (0.06 mol) of diethyl 2,2-diethoxyethane-1-phosphonate (**10**)[9] in 30 ml of dioxane, 50 ml of $H_2O$, and 2.5 ml of concentrated $H_2SO_4$ was heated at 60° for 75 min. The solution was cooled to 0° and a mixture of 6.9 g (0.15 mol) of $NaHSO_3$ in 5 ml of $H_2O$ was added. After 10 min a solution of 8.9 g (0.18 mol) of NaCN in 25 ml of $H_2O$ was added over 20 min to the suspension with intermittent, careful addition of 6 *N* $H_2SO_4$ to maintain pH at 7.5. Stirring was continued at 0° for 1 hr, ice was added, and the white suspension was extracted with 5% MeOH in diethyl ether (four times). The ethereal extracts were washed with saturated $NaHCO_3$ solution and brine. The dried concentrate (4.8 g, 37%) was passed through a short column of Florisil with $CHCl_3$ to give **11** as a mobile liquid. Tlc on silica gel G with 5% MeOH in EtOAc gave an $R_f$ of 0.53. *Anal.* ($C_7H_{14}NO_4P$) C, H, N.

**3-Phosphonolactic Acid (4).** A mixture of 2.4 g (0.016 mol) of **11** and 40 ml of concentrated HCl was refluxed for 18 hr. The mixture was concentrated on a rotary evaporator to a semisolid and diluted with acetone and a small amount of cream-colored solid was separated. The filtrate was concentrated to 2.3 g of a dark yellow oil, 5 ml of $H_2O$ was added, and a saturated solution of LiOH (under toluene) was added under a $N_2$ atmosphere to give a pH 8.5. Excess acetone was added and the white solid was collected and crystallized (twice) from $H_2O$–EtOH–acetone. From each recrystallization, a small amount of insoluble material was filtered off. The white, powdery trilithium salt of **4** (0.87 g, 44%) had mp >400°. Tlc on Avicel plates‡ with *n*-BuOH–HOAc–$H_2O$ (5:3:1) gave an $R_f$ of 0.48; ir (KBr) 6.26 (broad), 7.05, 9.1–9.4 (broad doublet), and 10.0 μ; nmr (20% DCl in $D_2O$) 3.34 (m, 1) and 5.04 ppm (m, 2). The analytical sample was dried at 156° for 72 hr and 1 mm. *Anal.* ($C_3H_4Li_3O_6P \cdot 0.5H_2O$) C, H, P.

**Diethyl 3-Cyano-3-(2-tetrahydropyranyloxy)propane-1-phosphonate (13).** A mixture of 47.4 g (0.285 mol) of **12**,[15] 300 ml of $H_2O$, and 3 ml of concentrated $H_2SO_4$ was stirred at 25° for 2 hr. After about 1.5 hr the two-phase mixture became homogeneous. The solution was cooled to 0° and a suspension of 21.1 g (0.31 mol) of $NaHSO_3$ in 35 ml of $H_2O$ was added and stirring was continued until solution was complete. Then a solution of 21.1 g (0.43 mol) of NaCN in 100 ml of $H_2O$ was added over 25 min at 5–10°, and stirring was continued for an additional 1.5 hr. After thorough extraction with ether, the combined extract was washed with $NaHCO_3$ solution and brine, dried, and concentrated to yield 31.8 g (94%) of crude cyanohydrin. Tlc on silica gel G with cyclohexane–EtOAc (3:1) showed one major spot at an $R_f$ of 0.61.

The crude cyanohydrin (8.4 g, 0.07 mol) and 17.6 g (0.21 mol) of dihydropyran in 100 ml of dry $C_6H_6$ was stirred with 150 mg of *p*-TosOH·$H_2O$ for 3 hr at room temperature. Excess, saturated $NaHCO_3$ solution was added and the $C_6H_6$ layer was separated, dried, and concentrated to give the crude tetrahydropyran derivative. This was mixed with 17 ml of triethyl phosphite and refluxed in an oil bath for 18 hr. Excess triethyl phosphite was removed at 20 mm and 50°, and the residue was chromatographed on 500 g of Florisil using $CHCl_3$ as eluent and collecting 400-ml aliquots. Fractions 7–16 gave 3.1 g (15%) of homogeneous **13** as a colorless liquid. Tlc on silica gel G with EtOAc gave an $R_f$ of 0.44. *Anal.* ($C_{13}H_{24}NO_5P$) C, H, P.

**3-Carboxy-3-hydroxypropane-1-phosphonic Acid (5).** A mixture of 1.5 g (0.005 mol) of **13** and 25 ml of concentrated HCl was refluxed (oil bath temperature at 135°) for 18 hr. The reaction darkened almost immediately. Charcoal was added to the slightly cooled mixture, the black solid was separated, and the yellow filtrate was concentrated to a small volume and treated with a saturated LiOH solution in an inert atmosphere as described above. Acetone was added and a solid separated. The solid was dissolved in a minimum amount of $H_2O$, the cloudy solution was filtered, and the filtrate was diluted with EtOH to give a gelatinous solid. This solid was removed and more EtOH was added to the filtrate to afford 0.75 g of the crude lithium salt. This precipitation procedure was repeated twice to give 250 mg (25%) of the white, powdery trilithium salt of **5**, mp >400°. Tlc on Avicel with acetone–$NH_4OH$–$H_2O$ (60:10:30) gave an $R_f$ of 0.52; ir (KBr) 6.27 (broad), 6.8–7.1 (weak–broad), 9.17, 9.50, and 10.0 μ; nmr (20% DCl in $D_2O$) 2.52 (m, 1) and 4.85 ppm (m, 4). The analytical sample was dried at 156° for 16 hr at 1 mm. *Anal.* ($C_4H_6Li_3O_6P$) C, H, P.

**Ethyl 2-(Diethylphosphono)-1-hydroxymethylpropionate (16).** Using the method of Kreutzkamp,[11] ethyl 2-diethylphosphonopropionate (**14**, 36 g, 0.15 mol) was converted to the α-formyl derivative with ethyl formate (13.3 g, 0.18 mol) and sodium (3.5 g, 0.15 g-atom) to give 24.7 g (40%) of ethyl 2-(diethylphosphono)-1-formylpropionate after $CHCl_3$ extraction of the acidified aqueous extract.

A mixture of 3.53 g (0.093 mol) of $NaBH_4$ in 70 ml of EtOH–THF–$H_2O$ (1:1:1) was cooled to 0° and a solution of 24.7 g (0.093 mol) of the above crude formyl derivative in 40 ml of the same solvent mixture was added dropwise over 15 min. The mixture was then stirred an additional 20 min, neutralized with glacial HOAc, and concentrated *in vacuo* at 30°, and the residue was extracted well with $CHCl_3$. The combined extracts were washed with 5% $NaHCO_3$ solution and brine, dried, and concentrated to about 20 g of syrup. This was chromatographed on 800 g of Florisil eluting with 2 l. of $CHCl_3$ followed by 2 l. of 5% EtOH in $CHCl_3$. The homogeneous fractions (from 5% EtOH in $CHCl_3$) afforded 15.4 g (62%) of the colorless, syrupy **16**. Tlc on silica gel G with 4% MeOH in EtOAc gave an $R_f$ of 0.46. *Anal.* ($C_{10}H_{21}O_6P$) C, H, P.

**2-Carboxy-3-hydroxypropane-1-phosphonic Acid (7).** Into a pressure tube were placed 0.53 g (2 mmol) of compound **16**, 0.192 g (8 mmol) of LiOH, and 1.5 ml of $H_2O$. The mixture was heated for 18 hr at 135°. Hot $H_2O$ (10 ml) was added and heating was continued for 48 hr at 135°. A tan precipitate was filtered (under $N_2$) and washed with a small amount of $H_2O$. The combined filtrate was acidified with water-washed IR 120 ($H^+$) resin. The resin was separated and a freshly prepared, filtered solution of saturated LiOH (under toluene) was added to pH 8. The mixture was concentrated to one-half volume, enough absolute EtOH was added to give a small amount of precipitate, and the solid was filtered under $N_2$. Further cooling of the filtrate gave 0.17 g of the crude lithium salt. Recrystallization from $H_2O$–EtOH gave 0.1 g (24%), mp >400°, of the trilithium salt of **7**. Tlc on cellulose with acetone–$NH_4OH$–$H_2O$ (60:10:30) gave an $R_f$ of 0.3;[14] ir (KBr) 6.35 (broad), 7.02, 7.55 (weak), 9.03, 9.43, and 10.0 μ; nmr (20% DCl in $D_2O$) 3.50 (d, 2), 4.41 (m, 1), and 5.20 ppm (m, 2). The analytical sample was dried at 156° for 18 hr at 1 mm. *Anal.* ($C_4H_6Li_3O_6P \cdot H_2O$) C, H, P. The *p*-anisidine salt was prepared by dissolving a portion of the IR 120 ($H^+$) filtrate above in *t*-BuOH and adding an ethereal solution of *p*-anisidine to pH 8. The white solid was crystallized from aqueous EtOH–ether to give the *p*-anisidine salt of **7**, mp 168–170°. *Anal.* ($C_4H_9O_6P \cdot C_7H_9N \cdot 0.5H_2O$) C, H, N.

**Ethyl 3-Diethylphosphono-1-hydroxymethylbutyrate (21).** Sodium (3.2 g, 0.14 g-atom) was suspended in 200 ml of dry ether and 0.5 ml of absolute EtOH was added. A mixture of ethyl 3-diethylphosphonobutyrate (**20**,[16] 35.2 g, 0.14 mol) and distilled ethyl formate (12.4 g, 0.168 mol) was added to the cooled (0°) suspension over 25 min. The cooling bath was removed before the addition was completed and stirring was continued at room temperature for 22 hr. The dark orange mixture was diluted with 300 ml of iced $H_2O$ and the orange aqueous phase was separated. The ether layer was extracted with $H_2O$ (2 × 75 ml) and the extracts were added to the original aqueous phase. The combined aqueous

§Baer and Robinson (ref 10) have cited that there is no difference in binding of either D-2,3-diphosphoglyceric acid or its optical antipode to hemoglobin.

‡Phosphorus was detected on Avicel plates by the Bandurski and Axelrod method (ref 14a) and on silica by spraying with the Dittmer reagent (ref 14b) and heating to 110°.

phase was backwashed with ether, the aqueous extracts were acidified with dilute HCl to pH 2.5 and extracted with $CHCl_3$ (4 × 150 ml), and the combined $CHCl_3$ extracts were washed with brine, dried, and concentrated to 39.2 g (71%) of crude formyl derivative. The ethereal washes afforded a 16% recovery of the starting material **20**.

The crude formyl derivative (27.8 g, 0.1 mol) was dissolved in 40 ml of EtOH–$H_2O$–THF (1:1:1) and the solution was added dropwise at 5° to a solution of $NaBH_4$ (3.74 g, 0.1 mol) in 120 ml of the same solvent mixture. The mixture was stirred for 30 min, neutralized with HOAc, and evaporated at 30° *in vacuo*. The residual aqueous solution was diluted with brine and extracted with $CHCl_3$ (5 × 200 ml), and the organic layer was washed with $NaHCO_3$ solution and brine. The dried residual yellow liquid (18.0 g) was chromatographed on 400 g of Florisil (400-ml fractions) eluting with $CHCl_3$ (1.6 l.), 1% EtOH in $CHCl_3$ (2.0 l), and 2% EtOH in $CHCl_3$. Most of the homogeneous, oily **21** came off in the 2% EtOH fractions. Tlc on silica gel G gave an $R_f$ of 0.43 with 5% MeOH in EtOAc. *Anal.* ($C_{11}H_{23}O_6P$) C, H, P.

**2-Carbethoxy-1,4-bis(diethylphosphono)butane (23).** The alcohol **21** (4.0 g, 0.014 mol) was dissolved in anhydrous pyridine (25 ml), the solution was cooled in ice–$H_2O$ and 5.42 g (0.028 mol) of tosyl chloride was added. The mixture was stirred at 0° for 2 hr and at 25° for 3 hr. Ether (300 ml) and 100 ml of iced 3 *N* HCl were added. The layers were separated, the aqueous layer was extracted well with ether, and the combined ethereal solution was washed quickly with iced dilute HCl, cold $H_2O$, iced $NaHCO_3$ solution, and $H_2O$ again. The dried solution gave 6.0 g of a yellow oil. Tlc showed all of the alcohol was converted to the tosylate **22**, $R_f$ 0.66 in the system used for **21**. This crude tosylate was azeotroped several times with dry $C_6H_6$ and used in the displacement reaction with sodium diethyl phosphite.

To a suspension of 2.6 g (0.07 mol) of 55% NaH in mineral oil in 100 ml of dry dioxane (from 5A molecular sieves) there was added at reflux (strong evolution of $H_2$) 13.8 (0.1 mol) of distilled diethyl phosphite. After about 15 min the almost clear solution was treated with a solution of the crude tosylate **22** (0.014 mol) in 50 ml of dioxane. The mixture was refluxed (oil bath at 110°) for 18 hr. Sodium tosylate precipitated in a short time. The solvent was evaporated at $H_2O$ aspirator pressure and then at 10 mm at 50°. The residue was partitioned between $H_2O$ and ether, the aqueous layer was extracted with ether, and the combined ethereal solution was washed with brine, dried, and evaporated. The crude product (7.5 g) was chromatographed on 200 g of Florisil (200-ml fractions) with an EtOH in $CHCl_3$ gradient. Elution with 600 ml each of $CHCl_3$, 1% EtOH in $CHCl_3$, then from 2 to 6% EtOH in $CHCl_3$, gave 1.4 g (25%) of pale yellow, oily **23** which came over in the 4–5% EtOH in $CHCl_3$ fractions. Tlc on silica gel G gave an $R_f$ of 0.31 with 5% MeOH in $CHCl_3$. *Anal.* ($C_{15}H_{32}O_8P_2$) C, H, P.

**2-Carboxybutane-1,4-diphosphonic Acid (9). Method A.** A solution of 3.5 g (8.7 mmol) of **23** in 50 ml of 6 *N* HCl was heated at reflux (oil bath at 110°) for 18 hr. The solution was concentrated *in vacuo* and repeatedly azeotroped with *t*-BuOH to afford a brownish residue (3.2 g). An aliquot (1.6 g) was dissolved in $H_2O$, filtered, and covered with toluene and $N_2$ was bubbled into the mixture. The acidic solution was adjusted to pH 10.5 with a filtered solution of saturated $Ba(OH)_2$ which was kept under toluene. The buff solid was collected (under $N_2$), redissolved in 3 *N* HCl, and reconverted to the barium salt as described above. The white dibarium salt of 9 (312 mg) was dried at 100° and 1 mm: mp >300°; ir (KBr) 6.47 (broad), 7.04, 7.59 (weak), 9.2–9.7 (broad), and 10.6 μ; nmr (20% DCl in $D_2O$) 4.24 (m, 1) 4.77 (m, 2), and 5.10 ppm (m, 4). *Anal.* ($C_5H_8Ba_2O_8P \cdot 6H_2O$) C, H, P. The remaining crude product (1.6 g) was purified on Avicel plates (20 × 20 cm × 1 mm). About 200 mg was applied (in $H_2O$) to each of eight plates and developed with acetone–$NH_4OH$–$H_2O$ (60:10:30). The major material ascended about 3 cm and the heart of the zone was scraped off, slurried with $H_2O$, and heated with excess water-washed IR 120 ($H^+$) resin at 50° for 5 hr. The free phosphonic acid **9** was obtained by filtering, washing the filter cake well with $H_2O$, and lyophilizing the filtrate. After drying at 100° and 1 mm the colorless glassy **9** (287 mg) was stored in a dessicator over KOH. *Anal.* ($C_5H_{12}O_8P_2 \cdot 0.5H_2O$) C, H, P.

**Method B.** Hydrolysis of **25** with 6 *N* HCl at 110° for 18 hr gave a major product which was identical (tlc) with **9**, obtained from **23**.

**Diethyl Diethylphosphonomethyl-2-diethylphosphonoethylmalonate (25).** Sodium (0.5 g, 0.02 g-atom) was dissolved in 25 ml of absolute EtOH and 5.72 g (0.02 mol) of diethyl diethylphosphonomethylmalonate (**24**)[11] in 5 ml of EtOH was added. After refluxing for 15 min, a solution of 4.9 g (0.02 mol) of diethyl 2-bromoethylphosphonate[17,**] in 8 ml of absolute EtOH was added, and reflux was continued for 8 hr. Sodium bromide began to separate after about 45 min. The cooled reaction was diluted with $H_2O$ and $CHCl_3$. The water-washed $CHCl_3$ was concentrated to 8.0 g of a yellowish syrup which was a mixture of **25** and the two starting materials. Chromatography over 250 g of Florisil (350-ml fractions) with an EtOH–$CHCl_3$ gradient gave 1.2 g (17%) of pale yellow syrupy **25** from the 4–5% EtOH in $CHCl_3$ fractions. Tlc on silica gel G with 5% MeOH in EtOAc gave an $R_f$ of 0.38. *Anal.* ($C_{18}H_{36}O_{10}P_2$) C, H, P.

**2,3-Diphosphonopropionic Acid (8).** Compound 8 was prepared following the procedure of Baer and Robinson.[10] Methyl 2-chlorolactate was prepared from glycerol α-monochlorohydrin in 40% overall yield. Conversion of 17.8 g (0.12 mol) of methyl 2-chlorolactate to methyl 2,3-diethylphosphonopropionate (10.3 g, 24%) was achieved after distillation [bp 160–170° at 0.05 mm (reported bp 134–136° at 0.005 mm)], followed by chromatography over Florisil with $CHCl_3$ elution. *Anal.* Calcd for $C_{12}H_{26}O_8P_2$: P, 17.19. Found: P, 17.39. Using hot concentrated HCl instead of HBr, hydrolysis of methyl 2,3-diethylphosphonopropionate followed by the usual isolation of the lithium salt (procedure described above) gave a 27% yield of the pentalithium salt of **8**: mp >400° (from $H_2O$–acetone); ir (KBr) 6.3 (broad), 7.05, 7.62 (weak), 8.7–9.3 (broad), 9.92 (weak), and 10.5 μ; nmr (20% DCl in $D_2O$) 4.28 (m, 1) and 4.74 ppm (m, 2). Tlc on Avicel with *n*-BuOH–HOAc–$H_2O$ (5:3:1) gave an $R_f$ of 0.18. *Anal.* ($C_3H_3Li_5O_8P_2 \cdot H_2O$) C, H, P, Li. (Li by atomic absorption: calcd, 13.2; found, 12.2.)

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## References

(1) R. Benesch and R. E. Benesch, *Biochem. Biophys. Res. Commun.*, **26**, 162 (1967).
(2) A. Chanutin and R. R. Curnish, *Arch. Biochem. Biophys.*, **121**, 96 (1967).
(3) R. G. Pendleton, D. J. Newman, S. S. Sherman, E. G. Brann, and W. E. Maya, *J. Pharmacol. Exp. Ther.*, **180**, 647 (1972).
(4) (a) P. Bard, "Medical Physiology," C. V. Mosby Co., St. Louis, Mo., 1961, p 593; (b) L. D. Miller, F. A. Oski, J. F. Diaco, H. J. Sugerman, A. J. Gottlieb, D. Davidson, and M. Delivoria-Papadopoulos, *Surgery*, **68**, 187 (1970).
(5) G. J. Brewer and J. W. Eaton, *Science*, **171 (3977)**, 1205 (1971).
(6) S. D. Chappel, J. A. Murray, M. G. Nasser, R. E. Wills, J. D. Torrance, and C. J. M. Lenfant, *N. Engl. J. Med.*, **282**, 1219 (1970).
(7) (a) Z. B. Rose and J. Liebowitz, *J. Biol. Chem.*, **245**, 3232 (1970); (b) D. R. Harkness, W. Thompson, S. Roth, and V. Grayson, *Arch. Biochem. Biophys.*, **138**, 208 (1970).
(8) *Forsvarsmedicin*, **5**, 143 (1969).
(9) N. D. Dawson and A. Burger, *J. Amer. Chem. Soc.*, **74**, 5312 (1952).
(10) E. Baer and R. Robinson, *Can. J. Biochem.*, **49**, 300 (1971).
(11) N. Kreutzkamp and W. Mengel, *Chem. Ber.*, **100**, 709 (1967).
(12) R. Benesch, R. E. Benesch, and C. I. Yu, *Biochemistry*, **8**, 2567 (1969).
(13) T. Jen, B. Dienel, J. Frazee, and J. Weisbach, *J. Med. Chem.*, **15**, 1172 (1972).
(14) (a) R. S. Bandurski and B. Axelrod, *J. Biol. Chem.*, **193**, 405 (1951); (b) J. C. Dittmer and R. L. Lester, *J. Lipid Res.*, **5**, 126 (1964).
(15) J. W. C. Crawford and J. Kenyon, *J. Chem. Soc.*, 396 (1927).
(16) J. Falbe, R. Paatz, and F. Korte, *Chem. Ber.*, **98**, 2312 (1965).
(17) G. M. Kosolapoff, *J. Amer. Chem. Soc.*, **70**, 1971 (1948).

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