

and then a solution of 3-chloro-N,N-diethylpropylamine (made from 20.5 g., 0.11 mole, of the corresponding hydrochloride) in 200 ml. of anhydrous toluene was added over 30 min. Stirring and heating were continued for 1.5 hr. longer, and then the mixture was concentrated *in vacuo* and poured into a large volume of ice water. The product was isolated by extraction with methylene chloride and recrystallization from hexane (Table I).

Method B. [Via 7-Chloro-1-(3-chloropropyl)-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-one (II)].—To a solution of 6.75 g. (0.025 mole) of I in 40 ml. of anhydrous N,N-dimethylformamide was added 1.4 g. (0.026 mole) of sodium methoxide, and the mixture was stirred and heated on the steam bath for 30 min. After being cooled to 30°, it was treated with 2.66 ml. (0.0275 mole) of 1-bromo-3-chloropropane and stirred at 20° for 66 hr. The solvent was evaporated *in vacuo*, and the residue was extracted with methylene chloride. The crude product was purified by filtration of an ether solution through Woelm neutral alumina activity I. Crystallization from hexane-ether gave II as colorless rods, m.p. 87–90° (86%).

Anal. Calcd. for C₁₈H₁₈Cl₂N₂O: C, 62.26; H, 4.64. Found: C, 62.13; H, 4.74.

A mixture of 10 g. (0.0288 mole) of II, 4.37 g. (0.0288 mole) of NaI, 5.9 ml. (0.0574 mole) of diethylamine, and 50 ml. of 2-butanone was stirred and refluxed for 18 hr. After evaporation *in vacuo*, the residue was partitioned between methylene chloride and water, and the organic layer was extracted with dilute HCl. The aqueous acid layer was made basic with NaOH solution, and the product was isolated by extraction with methylene chloride. Recrystallization from hexane gave 29, identical with material prepared by method A.

7-Chloro-1-(3-chloropropyl)-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one. **Method B.**—Alkylation of 7-chloro-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one with 1-bromo-3-chloropropane, as described for the synthesis of II, and isolation of the product in the same manner gave colorless prisms, m.p. 86–89° (48%).

Anal. Calcd. for C₁₈H₁₅Cl₂FN₂O: C, 59.19; H, 4.14. Found: C, 59.27; H, 4.31.

7-Chloro-4-(2-diethylaminoethyl)-5-(2-fluorophenyl)-1,3,4,5-tetrahydro-1-methyl-2H-1,4-benzodiazepin-2-one Hydrochloride (41). **Method C.**—A solution of 2 g. (6.5 mmoles) of 7-chloro-5-(2-fluorophenyl)-1,3,4,5-tetrahydro-1-methyl-2H-1,4-benzodiazepin-2-one (IV) in 10 ml. of N,N-dimethylformamide was treated with a solution of 5.3 g. (34.8 mmoles) of 2-chloro-N,N-diethylamine in 10 ml. of anhydrous toluene and 1 g. (66 mmoles) of NaI. The mixture was stirred for 16 hr. at 50°, and then it was evaporated *in vacuo*. The residue was dissolved in 75 ml. of methylene chloride, washed with water, dried (MgSO₄), and filtered over 10 g. of Woelm activity I neutral alumina. Evaporation of the eluates and conversion of the residue to the hydrochloride gave colorless prisms (Table II).

7-Chloro-1-(2-diethylaminoethyl)-5-(2-fluorophenyl)-1,3,4,5-tetrahydro-2H-1,4-benzodiazepin-2-one (40). **Method D.**—A solution of 26.4 g. (68.4 mmoles) of 7-chloro-1-(2-diethylaminoethyl)-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one (12) in 150 ml. of glacial acetic acid was hydrogenated, at atmospheric pressure and temperature, over a prerduced platinum catalyst (prepared from 1.5 g. of PtO₂). When absorption of hydrogen had ceased (1 equiv.), the catalyst was removed by filtration, and the product was isolated in the usual manner. Recrystallization from ether-petroleum ether gave 40 (Table II).

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Further Syntheses in the Study of Structure-Activity Relationships of Neuropharmacologically Active Amino Acids

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N-Methylation of cysteine and homocysteine sulfinic acids did not increase the excitatory action of these substances on neurones within the cat central nervous system. This result was similar to that previously obtained with the sulfonic acid analogs and was again in marked contrast to the unique potency-increasing effect of N-methylation of D-aspartic acid. N-Phenyl-DL-aspartic acid and the methyl esters of ethanolamine, choline, and serine phosphates displayed little or no neuropharmacological action under the test conditions employed.

γ -Aminobutyric acid and glutamic acid have strong actions on a variety of nervous and muscular tissues.^{1a} The former substance depresses neuronal and muscular activity while the latter substance is an excitant, causing repetitive firing of neurones in vertebrate and invertebrate animals, and muscular contraction in invertebrates. Structure-activity relationships have been extensively investigated, leading to the conclusion that depressant activity is a consequence of one anionic and one cationic group separated by a distance corresponding to two or three carbon atoms, whereas excitant action is due to these same structural features together with the presence of a second anionic group, which is optimally attached α with respect to the carbon

atom bearing the positively charged group.²⁻⁶ The most active depressant found in these studies was 3-aminopropane-1-sulfonic acid,³⁻⁴ while the strongest excitants were N-methyl-D-aspartic acid and D-homocysteic acid.³⁻⁵ Details of the syntheses of a variety of related amino acids have been reported.⁶

The present extension of these studies was undertaken for a dual purpose. First, it was desired to compare the effects of N-methylation of cysteinesulfinic acid and of homocysteinesulfinic acid with those observed on N-methylation of aspartic, cysteic, glutamic, and homocysteic acids. Second, it was desired to

(1) (a) D. R. Curtis and J. C. Watkins, in preparation; (b) D. R. Curtis in "Physical Techniques in Biological Research," Vol. 5, W. L. Nastuk, Ed., Academic Press Inc., New York, N. Y., 1964, p. 144.

(2) D. R. Curtis and J. C. Watkins, *J. Neurochem.*, **6**, 117 (1960).

(3) D. R. Curtis, J. W. Phillis, and J. C. Watkins, *Brit. J. Pharmacol.*, **16**, 262 (1961).

(4) D. R. Curtis and J. C. Watkins, *Nature*, **191**, 1010 (1961).

(5) D. R. Curtis and J. C. Watkins, *J. Physiol.*, **166**, 1 (1963).

(6) J. C. Watkins, *J. Med. Pharm. Chem.*, **5**, 1187 (1962).

TABLE I

Compd.	Optical form	Structure	Neuropharmacological action and potency ^a
Glutamic acid (I)	l.	$\text{HO}_2\text{CCH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{CO}_2\text{H}$	+++
Cysteinesulfinic acid (II)	l.	$\text{HO}_2\text{S}-\text{CH}_2\text{CH}(\text{NH}_2)\text{CO}_2\text{H}$	+++
Cysteinesulfinic acid (III)	dl.	$\text{HO}_2\text{S}-\text{CH}_2\text{CH}(\text{NH}_2)\text{CO}_2\text{H}$	+++
N-Methylcysteinesulfinic acid (IV)	dl.	$\text{HO}_2\text{S}-\text{CH}_2\text{CH}(\text{NHCH}_3)\text{CO}_2\text{H}$	++(+)
N-Methylcysteinesulfinic acid (V)	l.	$\text{HO}_2\text{S}-\text{CH}_2\text{CH}(\text{NHCH}_3)\text{CO}_2\text{H}$	+
2-Amino-4-sulfinobutyric acid (VI)	dl.	$\text{HO}_2\text{S}-\text{CH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{CO}_2\text{H}$	+++
2-Methylamino-4-sulfinobutyric acid (VII)	dl.	$\text{HO}_2\text{S}-\text{CH}_2\text{CH}_2\text{CH}(\text{NHCH}_3)\text{CO}_2\text{H}$	++
N-Phenylaspartic acid (VIII)	dl.	$\text{HO}_2\text{C}-\text{CH}_2\text{CH}(\text{NHPh})\text{CO}_2\text{H}$	0
Ethanolamine methyl phosphate (IX)		$\text{CH}_3\text{OP}(\text{OH})(\text{OCH}_2\text{CH}_2\text{NH}_2)$	0
Choline methyl phosphate (X)		$\text{CH}_3\text{OP}(\text{O}^-)(\text{OCH}_2\text{CH}_2\text{N}^+(\text{CH}_3)_3)$	0
Serine methyl phosphate ^b (XI)	dl.	$\text{CH}_3\text{OP}(\text{OH})(\text{OCH}_2\text{CH}(\text{NH}_2)\text{CO}_2\text{H})$	0 to ++
Ethanolamine phosphate (XII)		$\text{HOP}(\text{OH})(\text{OCH}_2\text{CH}_2\text{NH}_2)$	0
Choline phosphate (XIII)		$\text{HOP}(\text{O}^-)(\text{OCH}_2\text{CH}_2\text{N}^+(\text{CH}_3)_3)$	0
Serine phosphate (XIV)		$\text{HOP}(\text{OH})(\text{OCH}_2\text{CH}(\text{NH}_2)\text{CO}_2\text{H})$	0

^a Electrophoretically tested on cat spinal neurones.^{1a,2} The number of + signs gives an approximate estimate of excitatory potency: 0 = no detectable action. ^b Sample contained about 45% of N-methyl-DL-serine methyl phosphate: see Experimental Section.

obtain and test compounds which had not only the required charge distribution for depressant and excitant amino acid action, but which also resembled the terminal moieties of phospholipids. It has been suggested that the action of the amino acids, and also that of acetylcholine, may be due to the dissociation of phospholipid-protein complexes in cell membranes.⁷ Incidental to this investigation, but reported here for convenience, was the preparation of N-phenyl-DL-aspartic acid which was required for comparison with other N-substituted aspartic acids.

The sulfinic acids were prepared from the appropriate disulfides by a modification⁶ of the general method of Lavine.⁸ They were tested on spinal interneurons and Renshaw cells of the cat by the microelectrophoretic method.^{1b,2} The pharmacological results, summarized in Table I, show that both N-methyl-DL-cysteinesulfinic acid (IV) and the higher homolog, DL-2-methylamino-4-sulfinobutyric acid (VII), are weaker excitants of cat spinal neurones than the parent unmethylated compounds (III and VI, respectively). The fact that N-methyl-L-cysteinesulfinic acid (V) is somewhat weaker than the DL form indicates that the D isomer is the stronger of the two optical forms, both of which, however, would rank only among the "medium-weak" group of amino acid excitants.³ Thus, the effect of N-methylation of the sulfinic acids is very similar to the effect of the same structural modification to the sulfonic acid analogs, cysteic acid and homocysteic acid, and in neither case was there observed the marked increase in potency uniquely resulting from N-methylation of D-aspartic acid.³

N-Phenyl-DL-aspartic acid (VIII) was prepared by the reaction of aniline with monomethyl maleate. The main product isolated was maleanilic acid, but a small amount of the required product was obtained, after hydrolysis of the mother liquor, by the use of ion-exchange chromatography and preparation of the

barium salt, from which the free acid was recovered. The latter was inactive when tested by the microelectrophoretic method, in accord with previous findings^{1a} that bulky groups attached to the nitrogen atom abolish activity in both depressant and excitant amino acids.

The phospho diesters IX and X were prepared by methylation of ethanolamine phosphate and choline phosphate (XII and XIII, respectively) with diazomethane. The mono(tetramethyl)ammonium salt of XII and the hydroxide form of XIII were used because their alcohol solubility allowed the methylation to proceed in homogeneous solution following the addition of the diazomethane in ether. The products were isolated by the use of a column of Dowex 50 (H⁺), which had only a weak retarding effect on the passage of the diesters, but retained more strongly the small amounts of starting materials and by-products, as well as tetramethylammonium ion, where present. The preparation of DL-serine methyl phosphate (XI) from the di(tetramethyl)ammonium salt of DL-serine phosphate was also attempted using a similar procedure, but the product, although substantially the desired ester, also contained a high proportion (about 45%) of N-methylated by-product which was not separated by the column, nor removed by subsequent recrystallization.

When these three phospho diesters were tested by the microelectrophoretic method on cat spinal interneurons and Renshaw cells, only the serine derivative showed any activity, and even in this case the excitant action observed was irregular and not consistently demonstrated. All of the corresponding phospho monoesters were inactive when tested by the same method. These results would be expected on the theory that the amino acids and acetylcholine manifest their actions as a consequence of disturbing the equilibrium of complex formation between protein and phosphatidylethanolamine, -serine, and -choline.⁷ The synthetic phosphorus compounds resemble the ionic terminals of the phospholipids so closely that they

(7) J. C. Watkins, *J. Theoret. Biol.*, in press.

(8) G. Toennies and J. E. Lavine, *J. Biol. Chem.*, **113**, 571 (1934).

would not be expected to form protein complexes of sufficiently higher stability, compared with the membrane lipid-protein complexes, to be able to overcome the spatial advantage held by the membrane components in the formation of such complexes.

Experimental Section

Melting points are uncorrected and were determined using an electrically heated copper block. The method of crystallization of compounds from mixtures of water, alcohol, and ether have been described earlier.⁹

N,N'-Dimethyl-L-cystine.—L-4-Thiazolidinecarboxylic acid was prepared according to the method of Ratner and Clarke⁹ and converted into the cystine derivative as described by Keller-Schierlein, *et al.*¹⁰

N,N'-Dimethyl-DL (and meso)-cystine.—This mixture of the optically inactive forms was prepared in an analogous way to the L form from DL-4-thiazolidinecarboxylic acid; yield 63%, m.p. 206° (from water). The sample for analysis was dried at 60° (0.1 mm.) for 6 hr. over P₂O₅.

Anal. Calcd. for C₈H₁₆N₂O₄S₂: C, 35.80; H, 6.01, N, 10.44; S, 23.90. Found: C, 36.09; H, 6.09; N, 10.36; S, 23.93.

N,N'-Dimethyl-DL (and meso)-homocystine.—(±)-Tetrahydro-(1,3-thiazine)-4-carboxylic acid monohydrate¹¹ (7 g.) was dissolved in liquid ammonia (220 ml.), and water (0.77 ml.) was added. Small pieces of sodium were added with stirring until the blue color persisted (3.8 g. of Na required). The mixture was treated with solid NH₄Cl (6.8 g.), and the NH₃ was allowed to evaporate overnight. After the removal of final traces of ammonia in a vacuum desiccator containing concentrated H₂SO₄, the white residue was dissolved in water (100 ml.) and the solution was evaporated to about half its volume under reduced pressure at 40°. The mixture was strongly acidified with 6 N HCl, causing a white precipitate, and the suspension was evaporated to dryness *in vacuo*. The residue was repeatedly extracted with alcohol (800 ml. total) until paper electrophoresis [2 hr., 10 v./cm., 0.1 M pyridinium acetate buffer (pH 4.0)] showed the absence of ninhydrin-staining material in the alcohol-insoluble residue, and the alcoholic extracts were combined and evaporated *in vacuo* yielding a white residue.¹² This was taken up in water (400 ml.), the solution was made strongly alkaline with concentrated NH₃ solution (sp. gr. 0.91), 10 mg. of FeSO₄ was added, and a stream of air was passed through the solution until a negative nitroprusside test¹³ was obtained. The resulting solution was filtered to remove a small amount of suspended material, the filtrate was acidified with 25% acetic acid solution to pH 3, and 600 ml. of alcohol was added. The white precipitate which formed on standing at 0° was filtered and recrystallized twice from water, yielding 4.3 g. (64%) of N,N'-dimethylhomocystine, presumably as a mixture of the DL and meso forms; m.p. 243–244° [lit.¹⁴ 257–260° (cor.)].

Anal. Calcd. for C₁₀H₂₀N₂O₄S₂: C, 40.50; H, 6.80; N, 9.45; S, 21.64. Found: C, 40.31; H, 6.88; N, 9.48; S, 21.43.

Preparation of Sulfinic Acids.—These substances were all prepared from the appropriate cystine derivatives by the general methods previously described.⁹

DL-Cysteinesulfinic acid (III) was prepared from DL-cystine; needles from water-alcohol-ether, m.p. 159° dec. The sample for analysis was dried (P₂O₅) at 60° (0.05 mm.) for 7.5 hr.

Anal. Calcd. for C₆H₁₁NO₃S·0.5H₂O: C, 22.22; H, 4.98; N, 8.64; S, 19.77; equiv. wt., 162.2. Found: C, 22.59; H, 4.90; N, 8.72; S, 19.90; equiv. wt., 161.4.

The water of crystallization could not be removed by more vigorous drying procedures [90° (0.05 mm.) for 4 hr.], which re-

sulted in extensive decomposition, as demonstrated by paper chromatography.

N-Methyl-DL-cysteinesulfinic acid (IV) was prepared from N,N'-dimethyl-DL-cystine; needles from water-alcohol-ether, m.p. 146° dec. The sample for analysis was dried at 60° (0.1 mm.) for 8 hr. over P₂O₅.

Anal. Calcd. for C₄H₉NO₃S: C, 28.74; H, 5.43; N, 8.38; S, 19.18. Found: C, 28.60; H, 5.19; N, 8.31; S, 19.01.

N-Methyl-L-cysteinesulfinic acid (V) was prepared from N,N'-dimethyl-L-cystine; needles from water-alcohol-ether, m.p. 145° dec. The sample for analysis was dried under the same conditions as the DL form (IV).

Anal. Calcd. for C₄H₉NO₃S: C, 28.74; H, 5.43; N, 8.38; S, 19.18. Found: C, 28.67; H, 5.45; N, 8.27; S, 19.12.

DL-2-Methylamino-4-sulfinobutyric acid (VII) was prepared from N,N'-dimethyl-DL (and meso)-homocystine; needles from water-alcohol-ether, m.p. 158–159° dec. The sample for analysis was dried (P₂O₅) at 62° (0.05 mm.) for 14 hr.

Anal. Calcd. for C₈H₁₁NO₃S: C, 33.14; H, 6.12; N, 7.73; S, 17.69. Found: C, 33.14; H, 6.24; N, 7.69; S, 17.77.

Barium N-Phenyl-DL-aspartate.—A solution of maleic anhydride (28 g.) in 100 ml. of dry methanol was refluxed for 30 min. on a water bath. The excess methanol was removed by distillation, and the light yellow reaction mixture, after cooling in ice, was treated dropwise, with stirring, with 50 ml. of ice-cooled triethylamine. Aniline (23.3 g.) was added to the reaction mixture, which was then refluxed on a boiling water bath for 1 hr. with stirring. Excess triethylamine and unreacted aniline were removed by distillation *in vacuo* and the yellow gummy residue was treated with hot ethanol. Light yellow prisms of maleanilic acid (total 6.2 g., m.p. 196°) crystallized on standing, and further crops were obtained by concentration of the mother liquors.

Anal. Calcd. for C₁₀H₉NO₃: C, 62.85; H, 4.75; N, 7.33. Found: C, 63.18; H, 4.56; N, 7.30.

The final mother liquor was evaporated to dryness at 40° *in vacuo* giving 40 g. of yellow gum. This was dissolved in 475 ml. of 1 N NaOH solution, and the solution was heated at 90° on a water bath for 45 min., cooled to room temperature, and treated with concentrated acetic acid to pH 4.0. The resulting solution was kept overnight at 0°, filtered from the deposited sodium acetate, and evaporated, yielding 88 g. of yellow gum. This residue (22 g.) was taken up in 25 ml. of warm water and the solution was filtered and passed through a column of 158 ml. of Dowex 50 W (H⁺), 200–400 mesh. The column was washed with water until the eluate was neutral and then eluted with 1 M aqueous pyridine solution. The pH 2 eluate which just preceded pyridine breakthrough was evaporated to dryness *in vacuo*, and the resulting viscous residue was taken up in 10 ml. of water. To this solution was added 25 ml. of saturated Ba(OH)₂ solution followed by ethanol until precipitation of the barium salt was complete. The resulting suspension was centrifuged and the salt was purified by repeated precipitation with ethanol from hot aqueous solution, giving a total yield of 1.1 g. of colorless product; λ_{max}^{H₂O} 288 mμ (log ε 3.10), 242 mμ (log ε 4.00). For analysis, the sample was dried at 56° (0.1 mm.) for 7 hr. over P₂O₅.

Anal. Calcd. for C₁₀H₉BaNO₄: C, 34.84; H, 2.63; N, 4.06. Found: C, 34.96; H, 2.63; N, 3.95.

The remainder of the viscous residue (66 g.) was dissolved in 200 ml. of water, filtered to remove a few milligrams of insoluble material, and treated with a solution of 25 g. of barium acetate in 100 ml. of water. Ethanol (80 ml.) was added to the solution and the precipitated barium salt was collected by centrifugation. After repeated precipitation from aqueous solution by the addition of ethanol, the yield of colorless barium salt was 8.7 g. This product was only 80% pure on the basis of its ultraviolet absorption at 242 mμ.

N-Phenyl-DL-aspartic Acid (VIII).—The bulk of the 80% pure barium N-phenyl-DL-aspartate (8.7 g.), obtained above, was dissolved in 450 ml. of hot water, and the solution was treated with 50.6 ml. of 1 N H₂SO₄. The precipitated BaSO₄ was removed by centrifugation and the supernatant solution was evaporated to dryness *in vacuo* at 40°. Repeated extraction of the light yellow residue with boiling ethylene dichloride yielded a total of 1.6 g. of VIII, m.p. 150° *in vacuo*, which crystallized from the extracts as colorless needles; λ_{max}^{H₂O} 286 mμ (log ε 2.93), 240 mμ (log ε 3.81). Recrystallization for analysis was effected from ethylene dichloride containing 5% (v./v.) of ethanol. For analysis, the sample was dried (P₂O₅) at 51° (0.1 mm.) for 9 hr.

(9) S. Ratner and H. T. Clarke, *J. Am. Chem. Soc.*, **59**, 200 (1937).

(10) W. Keller-Schierlein, M. Lj. Mihailović, and V. Prelog, *Helv. Chim. Acta*, **42**, 305 (1959).

(11) This substance, m.p. 215–216°, was obtained purer than before.⁶ *Anal.* Calcd. for C₆H₉NO₃S·H₂O: C, 36.36; H, 6.71; N, 8.48; S, 19.41. Found: C, 36.15; H, 6.59; N, 8.47; S, 19.26.

(12) This alcohol extraction step was laborious and did not effect much separation of inorganic material from the homocysteine hydrochloride. It could probably be omitted to advantage.

(13) G. Toennies and J. J. Kolb, *Anal. Chem.*, **23**, 823 (1951).

(14) W. I. Patterson, H. M. Dyer, and V. du Vigneud, *J. Biol. Chem.*, **116**, 277 (1936).

Anal. Calcd. for $C_{10}H_{11}NO_4$: C, 57.41; H, 5.30; N, 6.70. Found: C, 57.61; H, 5.41; N, 6.69.

Phosphoric Acid Diesters. Ethanolamine Methyl Phosphate (IX).—Ethanolamine phosphate (1 g.) was dissolved in 0.126 *M* tetramethylammonium hydroxide solution (56 ml.) and the solution was evaporated at 40° *in vacuo* to a colorless gum, which was further dried for 2 hr. at room temperature and 0.1-mm. pressure. The gum was taken up in dry methanol (20 ml.) and the solution was cooled in ice and treated with a cold (0°) solution of diazomethane¹⁵ (generated from 2.92 g. of nitrosomethylurea) in ether (27 ml.). The yellow reaction mixture was allowed to stand at 0° for 20 hr. and was then evaporated *in vacuo* to a colorless semicrystalline mass. A solution of this material in water (12 ml.) was passed through a column containing 50 ml. of Dowex 50 W (H^+), 200–400 mesh, and the column was eluted with water. 10-ml. fractions were collected and samples from each were applied to paper and tested with ninhydrin. Fractions 7–14 showed a positive reaction. Paper electrophoresis for 1 hr., 20 v./cm., in KH_2PO_4 –NaOH buffer (0.05 *M*, pH 7.0) indicated that the ninhydrin-reacting material of these fractions was the same in each case, had zero charge, and was free from other ninhydrin-staining contaminants. These fractions were combined, evaporated to dryness under reduced pressure, and the residue was dried (P_2O_5) for 3 hr. at 0.1-mm. pressure, yielding 1.06 g. of a white crystalline solid. For analysis the product was recrystallized from a water–alcohol–ether mixture⁶ and dried at 56° (0.05 mm.) for 8 hr.; m.p. 196–197°.

Anal. Calcd. for $C_4H_{10}NO_4P$: C, 23.24; H, 6.50; N, 9.03; P, 19.97. Found: C, 23.45; H, 6.57; N, 9.03; P, 19.76.

Choline Methyl Phosphate (X).—To a solution of 3 g. of the hydrated calcium salt of choline phosphate ($C_5H_{13}ClNO_4P \cdot Ca \cdot 3.5H_2O$, supplied by Calbiochem) in 24 ml. of water was added a solution of 1.18 g. of oxalic acid dihydrate in 8 ml. of water. The resulting mixture was centrifuged and the clear supernatant fluid, which was free from Ca^{+2} , was decanted and evaporated at 40° *in vacuo*. The resulting gum was further dried at room temperature for 2 hr. at 0.1-mm. pressure and dissolved in dry methanol (130 ml.), and the solution was cooled in ice. An ice-cold ethereal solution (80 ml.) of diazomethane (generated from 9.6 g. of nitrosomethylurea) was added, and the reaction mixture was kept overnight at 0° in a lightly stoppered flask. It was then evaporated to dryness, and the viscous residue was taken up in 30 ml. of water and freed from a small amount of suspended matter by centrifugation. The supernatant solution was passed through a column of 120 ml. of Dowex 50 W (H^+), 200–400 mesh, and the column was eluted with water. Fractions (10-ml.) were collected, and samples from each were tested on paper with Hanes–Isherwood reagent¹⁶ for the presence of phosphorus-containing substances. Fractions 26–45 contained phosphorus. Paper electrophoresis for 1 hr. at 20 v./cm. in tris-(hydroxymethyl)aminomethane–HCl buffer (0.05 *M*, pH 7.2) demonstrated that the phosphorus-containing substance in all the fractions was the same, and that this substance had zero charge. Evaporation of the combined fractions at 40° *in vacuo* yielded a colorless gum which crystallized on standing at 0°. A sample of this material was dried at room temperature and pressure over P_2O_5 for 3 days. This sample had m.p. 112–114°

and showed a correct analysis for choline methyl phosphate monohydrate; yield 1.76 g. (87%).

Anal. Calcd. for $C_5H_{13}NO_4P \cdot H_2O$: C, 33.49; H, 8.43; N, 6.51; P, 14.40. Found: C, 33.55; H, 8.35; N, 6.41; P, 14.27.

The water of crystallization was removed by drying the sample at 56° (0.1 mm.) for 20 hr. (P_2O_5), after which the melting point was 192–193°.

The substance could be recrystallized by addition of ether to an alcohol solution of the diester to the point of initial faint turbidity and cooling the mixture gradually to –20°. Because of its hygroscopic nature, the crystallized material was sometimes difficult to filter, and in these cases it was preferable to decant the mother liquor, wash the crystals rapidly with alcohol–ether and then with ether, and allow the excess ether retained in the flask after the final decanting to evaporate through a $CaCl_2$ tube. Recrystallized choline methyl phosphate, after drying at 56° (0.1 mm.) for 20 hr. (P_2O_5), had m.p. 193–194°, and was extremely hygroscopic.

Anal. Calcd. for $C_6H_{16}NO_4P$: C, 36.55; H, 8.18; N, 7.10; P, 15.71. Found: C, 36.78; H, 8.27; N, 7.06; P, 15.77.

Methylation of DL-Serine Phosphate.—DL-Serine phosphate (1 g.) was dissolved in 0.126 *M* tetramethylammonium hydroxide solution (86 ml.), and the solution was evaporated to dryness *in vacuo*. The resulting gum was dissolved in dry methanol (40 ml.) and treated at 0° with a solution of CH_3N_2 (generated from 2.79 g. of nitrosomethylurea) in ether (38 ml.). After the reaction mixture had been allowed to stand at 0° for 18 hr. the product was isolated as described for ethanolamine methyl phosphate, using 75 ml. of Dowex 50 W (H^+). Ninhydrin-reacting material was detected in fractions 6–15 (each 10 ml.). Paper electrophoresis demonstrated that the ninhydrin-staining material in fractions 6–10 was electrophoretically homogeneous and that it was less anionic than the starting material, small amounts of which were present, together with a little more of the main product and trace quantities of by-products, in fractions 11–15. Fractions 6–10 were combined and evaporated to dryness yielding a colorless gum (800 mg.). This was taken up in water (3 ml.) and ethanol (27 ml.) was added, followed by ether to the point of faint turbidity. The mixture was allowed to stand at room temperature for 1.5 hr., and thereafter at 0° for 18 hr., yielding 450 mg. of a white crystalline solid. For analysis the product was recrystallized once more under similar conditions and dried at 56° (0.1 mm.) (P_2O_5) for 8 hr. The dried material had no definite melting point, decomposing with frothing at about 125°.

Anal. Calcd. for $C_4H_{10}NO_6P$: C, 24.12; H, 5.06; N, 7.94; P, 15.56. Found: C, 26.57; H, 5.72; N, 6.64; P, 14.84.

An examination of the n.m.r. spectrum of the product (in D_2O , TMS as external standard) revealed a singlet peak at $\tau = 7.13$ p.p.m., consistent with the presence of an N-methylated by-product, and a small triplet at $\tau = 8.80$ p.p.m., ascribed to the presence of a trace of solvent of crystallization (presumably alcohol). Reanalysis of a sample which had been dissolved in water and freeze dried gave figures consistent with the presence of about 55% of DL-serine methyl phosphate and about 45% of N-methyl-DL-serine methyl phosphate.

Anal. Calcd. for 55% $C_4H_{10}NO_6P$ + 45% $C_5H_{12}NO_6P$: C, 25.95; H, 5.34; N, 6.83. Found: C, 25.59; H, 5.69; N, 6.88.

The use of a lower excess of diazomethane and reduction of the reaction time resulted in substantially decreased yield and only slight improvement in the degree of purity of the product.

115) F. Arndt, "Organic Syntheses," Coll. Vol. II, A. H. Blatt, Ed., John Wiley and Sons, Inc., New York, N. Y., 1948, p. 165.

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