[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, UNIVERSITY OF ILLINOIS COLLEGE OF MEDICINE]

A Series of ω-Trimethylammoniumalkylphosphonic Acids and Their Diethyl Ester Iodides[1]

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A series of ω-trimethylammoniumalkylphosphonic acids, $(CH_3)_3N^+(CH_2)_nPO(OH)O^-$ (n = 1, 2, and 3), and of the iodides of their diethyl esters $I^-(CH_3)_3N^+(CH_2)_nPO(OEt)_2$ have been prepared. These compounds are analogs of naturally occurring choline esters and trimethylammoniumalkylcarboxylic acids. As such they may serve as alternate substrates or as inhibitors involving the natural metabolites.

A number of substituted trimethylammonium compounds play important metabolic roles in the living organism. Prominent among these substances are certain betaines of trimethylammoniumalkylcarboxylic acids, choline, and certain of its esters. The structures of some well known illustrative members of these classes of compounds are given in Table I together with a brief statement as to their physiological function.

TABLE I

BETAINES OF TRIMETHYLAMMONIUMALKYLCARBOXYLIC ACIDS

Common Name	Structure	Physiological Function
Betaine	$(CH_3)_3N^+CH_2COO^-$	Transmethylation in the living organism[2]
Carnitine (Vitamin B_T)	$(CH_3)_3N^+CH_2CHOHCH_2COO^-$	A growth factor for certain lower organisms.[3] Present in higher animals but metabolic function not established
	ESTERS OF CHOLINE	
Acetylcholine	$(CH_3)_3N^+CH_2CH_2CH_2O\overset{\overset{O}{\|}}{C}CH_3$	Functions in transmission of nerve impulse in the living organism[2]
Phosphorylcholine	$(CH_3)_3N^+CH_2CH_2OPO(OH)_2$	An intermediate in the biosynthesis of lecithin in the living organism[4]

A great many trialkylammonium derivatives, which may be viewed as analogs of compounds of this type, have been shown to possess physiological and pharmacological activity. Trialkylammonium analogs of acetylcholine have been therapeutically useful in *myasthemia gravis*, as miotics and as ganglionic blocking agents.[5] The sulfonic acid analog and the triethylammonium analog of carnitine have weak carnitine-like activity while the deoxy analog is an antagonist of the parent metabolite.[3]

The present paper describes the preparation of a series of ω-trimethylammoniumalkylphosphonic

$(CH_3)_3N^+(CH_2)_nPO(OH)O^-$ (I) $\quad I^-(CH_3)_3N^+(CH_2)_nPO(OEt)_2$ (II)

acid betaines (I, n = 1, 2, and 3) and of the iodides of their diethyl esters (II, n = 1, 2 and 3).[7,8]

The betaines are designed as phosphonic acid analogs of the natural trimethylammoniumalkylcarboxylic acid betaines and of the natural phosphoric acid esters of choline. The series of esters

(1) Supported by a grant from the Graduate Research Committee, University of Illinois Professional Colleges.

(2) M. R. Everett, *Medical Biochemistry*, Paul B. Hoeber, Inc., New York, N. Y., 1948.

(3) P. K. Bhattacharyya, S. Friedman, and G. Fraenkel, *Arch. Biochem. and Biophys.*, **54**, 424 (1955).

(4) E. P. Kennedy and S. B. Weiss, *J. Am. Chem. Soc.*, **77**, 250 (1955).

(5) L. S. Goodman and A. Gilman, *The Pharmacological Basis of Therapeutics*, 2nd ed., The Macmillan Co., New York, N. Y., 1955.

(6) Phosphonic acids have been suggested as analogs of naturally occurring phosphates [G. M. Kosolapoff, *Organophosphorous Compounds*, John Wiley and Sons, Inc., New York, N. Y., 1950, pp. 145–146; S. Preis, T. C. Myers and E. V. Jensen, Abstracts of papers, 123rd meeting, AMERICAN CHEMICAL SOCIETY, (1953)]. Recently B. S. Griffen and A. Burger [*J. Am. Chem. Soc.*, **78**, 2336 (1956)] have prepared D-glucopyranose 6-deoxy-6-phosphonic acid as an analog of the metabolite glucose-6-phosphoric acid.

(7) A single trialkylammoniumphosphonic acid betaine (trimethylammoniumethylphosphonic acid) has been described in the literature [T. Ya. Medved and M. I. Kabachnik, *Bull. acad. sci. U.R.S.S. Classe sci. chim.*, 999–1001, (1953)] and the methiodide of diethyl β-diethylaminoethylphosphonate has been prepared as a derivative of the free amine [G. M. Kosolapoff, *J. Am. Chem. Soc.*, **70**, 1971 (1948)].

represents a new type of analog of acetyl choline.[8,9]

The ester methiodides were prepared from the corresponding amines by the action of methyl iodide in ether solution and were converted to the betaines by hydrolysis with concentrated hydrochloric acid followed by treatment with silver oxide and then with hydrogen sulfide.

The free amines were synthesized as follows: diethyl β-dimethylaminoethylphosphonate and diethyl γ-dimethylaminopropylphosphonate were produced by the action of aqueous dimethylamine on the halides, diethyl β-bromoethylphosphonate[10] and diethyl γ-chloropropylphosphonate (following the general procedure of Kosolapoff for the preparation of diethyl β-diethylaminoethylphosphonate[12]); diethyl dimethylaminomethylphosphonate was obtained from dimethylamine, formaldehyde, and diethyl phosphite by a modification of the method of Fields.[13]

The ester iodides were obtained as hygroscopic, water soluble, crystalline solids; the betaines as crystalline, high melting, hygroscopic solids which titrated as monobasic acids with approximate *p*K's of 5.0, 6.4, and 6.8 for the series in order of increasing molecular weight.

Studies relating to the biological properties of these compounds are in progress.

EXPERIMENTAL[14]

Diethyl dimethylaminomethylphosphonate. To liquid dimethylamine (22.5 g., 0.5 mole) in a flask cooled to 0° in an ice bath, and fitted with a stirrer, a dropping funnel, and an efficient condenser cooled to 0°, there was rapidly added, with stirring, freshly distilled diethyl phosphite (60.0 g., 0.5 mole). No rise in temperature was noted during this addition. To the resulting solution, still in the ice bath, there was added with stirring, 36% formaldehyde (415 g., 0.5 mole) at such a rate that controlled spontaneous refluxing occurred. After addition was complete, stirring was continued for an additional 30 min. in the ice bath and then at room temperature for 1 hr. The resulting solution was concentrated at reduced pressure to a viscous yellow liquid which was distilled from a modified Claisen flask with a low take-off arm, at a bath temperature of 200°, and the fraction distilling between 43°/2mm. and 120°/15 mm. was collected. Considerable decomposition occurred during this distillation and considerable high boiling, highly viscous residue remained in the flask. On redistillation, after a low boiling forerun, the product was collected at 72–77°/0.4 mm. (reported,[13] 88°/3.0 mm.) as a water white liquid; $n_D^{24.5}$ 1.4280; yield 18.1 g. (18.5%). This product was redistilled and the fraction of b.p. 70°/0.25 mm. and $n_D^{24.5}$ 1.4281 was analyzed.

Anal. Calcd. for $C_7H_{18}NO_3P$: N, 7.17. Found: N, 7.35.

Diethyl trimethylammoniummethylphosphonate iodide (II, $n = 1$). To a solution of 1.1 g. of diethyl dimethylaminomethylphosphonate in 7 ml. of anhydrous ether, there was added 2.3 g. of methyl iodide in 8 ml. of anhydrous ether, drop by drop, with efficient stirring and careful exclusion of moisture, at 0°. A white solid began to appear after 4 ml. of solution had been added and the mixture, after complete addition, was a thick white slurry. Stirring was continued at room temperature for 1.5 hr. The solid was filtered with careful protection from moisture, washed with anhydrous ether, and dried under vacuum to yield 1.5 g. (70%) of product, m.p. 126–128°; m.p. 128–129° after recrystallization from methanol-ether.

Anal. Calcd. for $C_8H_{21}IN_3OP$: C, 28.49; H, 6.27; I, 37.64; N, 4.15; P, 9.18. Found: C, 28.78; H, 6.27; I, 37.5; N, 4.16; P, 9.13.

Trimethylammoniummethylphosphonic acid betaine (I, ($n = 1$). A solution of 4.0 g. of diethyl trimethylammoniummethylphosphonate iodide (crude) in 20 ml. of concentrated hydrochloric acid was refluxed for 12 hr. and the resulting solution was concentrated at reduced pressure to a yellow solid. Following the procedure of Medved and Kabachnik[7] an excess of freshly prepared silver oxide suspension was added to a solution of the solid in 50 ml. of distilled water and the mixture was allowed to stand at room temperature for 3 hr. with intermittent shaking. The mixture was filtered with suction (Super Cel filter aid), refiltered until clear, and the filtrate was treated with excess hydrogen sulfide. The resulting suspension was concentrated to one third volume on the steam bath at reduced pressure and filtered to give a clear colorless filtrate which was concentrated to a colorless solid, 1.4 g. (76.5%); m.p., after recrystallization from ethanol, 270–272° (reported[4] 267°).

Anal. Calcd. for $C_4H_{12}NO_3P$: Neut. equiv., 153. Found: Neut. equiv., 154 (*p*K *ca.* 5.0).

Diethyl β-dimethylaminoethylphosphonate. A solution of 8.2 g. of diethyl β-bromoethylphosphonate[10] and 32 g. of 25% aqueous dimethylamine was refluxed for 2 hr. and allowed to stand overnight at room temperature. The colorless solution was treated with 20 ml. of 25% aqueous sodium hydroxide and extracted with 100 ml. of benzene. The benzene was dried over sodium sulfate and concentrated at reduced pressure to a yellow oil which on distillation gave 4.1 g. (58.6%) of the ester with almost no forerun or residue; b.p. 75–77°/0.6 mm.; n_D^{25} 1.4331 (reported,[10] b.p. 108–109°/6.0 mm.; n_D^{20} 1.4340).

Anal. Calcd. for $C_8H_{20}NO_3P$: N, 6.69. Found N, 6.87.

Diethyl β-trimethylammoniumethylphosphonate iodide (II, $n = 2$). The free amine (1.8 g.) and methyl iodide (4.0 g.) were allowed to react in ether solution in a manner similar to that described for the preparation of II, $n = 1$. The yield was 2.5 g. (80.6%); m.p. 155–156°, raised to 156–157° on recrystallization from methanol–ethyl acetate.

Anal. Calcd. for $C_9H_{23}INO_3P$: C, 30.77; H, 6.60; I, 36.14; N, 3.98; P, 8.82. Found: C, 30.77; H, 6.74; I, 36.05; N, 3.82; P, 8.5.

β-Trimethylammoniumethylphosphonic acid betaine (I, $n = 2$). Two grams of the ester was hydrolyzed with concentrated hydrochloric acid and the product was isolated in a manner described for the preparation of I, $n = 1$.

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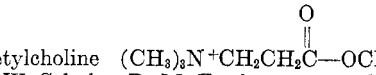
(8) B. E. Smith and A. Burger [*J. Am. Chem. Soc.*, **75**, 5891 (1953)] have prepared a number of dialkylaminoalkyl esters of some phosphonic and phosphinic acids and have demonstrated parasympatholytic activity by the methiodide of β-dimethylaminoethyl diphenylphosphinate.

(9) The phosphonic acid esters (II, $n = 1$, 2, and 3) bear a strong structural resemblance to the "reversed carboxyl"



analog of acetylcholine $(CH_3)_3N^+CH_2CH_2C(=O)OCH_3$ of W. B. Bass, F. W. Schuler, R. M. Festherstone, and E. H. Gross [*J. Pharmacol. Exptl. Therap.*, **100**, 465 (1950)] which possesses a high order of muscarinic activity.

(10) A reaction reported by A. N. Pudovic and G. M. Denisova, *Zhur. Obschei. Khim.*, **23**, 263 (1953).

(11) Prepared by the action of sodium diethyl phosphite on trimethylene chlorobromide; with R. Harvey, H. Jacobson, and E. V. Jensen, to be published.

(12) G. M. Kosolapoff, *J. Am. Chem. Soc.*, **70**, 1971 (1948).

(13) E. K. Fields, U. S. Patent **2,635,112** (1953).

(14) Analyses of the free amines were conducted by The Microtech Laboratories of Skokie, Ill.; of the betaines and ester iodides by G. Weiler and F. B. Strauss, Microanalytical Laboratory, Oxford, England, and by William Saschek of Chicago, Ill.

Recrystallization from ethanol gave 800 mg. (80%) of the product, which analyzed as the hemihydrate, m.p. 250–252°.

Anal. Calcd. for $C_5H_{14}NO_3P \cdot 1/2\ H_2O$: C, 34.09; H, 8.58; N, 7.95; P, 17.58; Neut. equiv., 176. Found: C, 33.7; H, 8.8; N, 8.19; P, 17.6; Neut. equiv., 174 (*p*K, *ca.* 6.4).

Diethyl γ-trimethylammoniumpropylphosphonate. This compound was prepared as described for the analog II, $n = 2$. The yield from 6.5 g. of diethyl γ-chloropropylphosphonate[11] and 50 ml. of 25% aqueous dimethylamine was 2.2 g. (33%); b.p. 82–84°/0.25 mm., with almost no forerun or residue; $n_D^{25.5}$ 1.4327–1.4325. The product was redistilled and the fraction of b.p. 83°/0.16 mm., and n_D^{26} 1.4340 was analyzed.

Anal. Calcd. for $C_9H_{22}NO_3P$: N, 6.27. Found: N, 6.27.

Diethyl γ-trimethylammoniumpropylphosphonate iodide (II, $n = 3$). This compound was prepared by the procedure described for the analogs II, $n = 1$ and 2, except that special techniques were employed for its isolation because of its extremely hygroscopic nature.

After the reaction (1.8 g. of the free amine and 4.0 g. of methyl iodide in ether solution), the reaction mixture was concentrated under vacuum to a white solid which was washed in the flask by decantation with anhydrous ether with careful protection from moisture. The solid was then dissolved in the reaction vessel in 30 ml. of ethyl acetate from which it precipitated as colorless needles on standing overnight at 0°. This product was centrifuged, washed with anhydrous ether, and dried at high vacuum at room temperature in the centrifuge tube. The yield of colorless extremely hygroscopic needles was 1.8 g. (61%); m.p. 106–110°, raised to 109–111° on recrystallization from ethyl acetate-acetone.

Anal. Calcd. for $C_{10}H_{25}INO_3P$: C, 32.88; H, 6.90; I, 34.75; N, 3.83; P, 8.48. Found: C, 32.5; H, 7.07; I, 34.5; N, 3.40; P, 8.3.

γ-Trimethylammoniumpropylphosphonic acid betaine (I, $n = 3$). The above ester (3.5 g.) was hydrolyzed and the product was isolated in a manner described for the preparation of the analogs I, $n = 1$ and 2, to yield 1.4 g. (80%); m.p. 273–278°, raised to 277–278° on recrystallization from ethanol.

Anal. Calcd. for $C_6H_{16}NO_3P$: C, 39.77; H, 8.90; N, 7.73; P, 17.09; neut. equiv. 181. Found: C, 39.3; H, 8.98; N, 7.74; P, 16.9; neut. equiv. 184 (*p*K, *ca.* 6.8).

CHICAGO 12, ILL.

[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY[1]]

Steroidal Sapogenins. XXXV. Gentrogenin (Botogenin) and Correllogenin, New Sapogenins from *Dioscorea spiculiflora*[2,3,4]

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Two new ketonic sapogenins, gentrogenin (botogenin) and correllogenin, have been isolated from the tubers of *Dioscorea spiculiflora*. Gentrogenin was converted to diosgenin and hecogenin; and correllogenin, to yamogenin and sisalagenin. Accordingly, gentrogenin must be 20α, 22α, 25D-, and correllogenin 20α, 22α, 25L-spirost-5-en-3β-ol-12-one.

Side chain degradation of gentrogenin gave 5,16-pregnadien-3β-ol-12,20-dione which was converted to 5-pregnen-3β-ol-12,20-dione, allopregnan-3β-ol-12,20-dione, and allopregnan-3,12,20-trione. The properties of gentrogenin, correllogenin and the various pregnene and allopregnane derivatives, differed markedly from values previously presented by Marker for botogenin, neobotogenin, and various side chain degradation product.

Some years ago Marker and Lopez reported the isolation of a new sapogenin from *Dioscorea mexicana* which they called botogenin.[5] It was characterized as 12 keto-diosgenin by conversion to diosgenin and hecogenin. Since such a sapogenin would have been a desirable cortisone precursor, we were alert for it during the screening of a large number of *Dioscorea* species,[6a,b,c] but with negative results. Recently we isolated two isomeric 12 ketonic sapogenins which corresponded in structure to botogenin and neobotogenin.[7] As shown in Table I, the melting points of the new sapogenins and their derivatives were decidedly different from those of the incompletely characterized "botogenin" series. Because of these differences we named the sapogenins gentrogenin (botogenin) and correllogenin.[8a,b]

Gentrogenin and correllogenin were isolated by means of Girard's Reagent T from a crude sapogenin mixture also containing diosgenin and yamogenin. The isomers were best separated by fractional crystallization of their acetates from ethyl

(1) A laboratory of the Eastern Utilization Research Branch, Agricultural Research Service, United States Department of Agriculture. Article not copyrighted.

(2) Paper XXXIV, *J. Am. Chem. Soc.*, **78**, 1747 (1956).

(3) A preliminary report has appeared in *J. Am. Chem. Soc.*, **77**, 5196 (1955).

(4) Presented at Delaware Valley Regional meeting, AMERICAN CHEMICAL SOCIETY, Philadelphia, Pa., Feb. 16, 1956; and 129th National Meeting, AMERICAN CHEMICAL SOCIETY, Dallas, Tex., April 8–13, 1956.

(5) R. E. Marker and J. Lopez, *J. Am. Chem. Soc.*, **69**, 2397 (1947).

(6) (a) M. E. Wall *et al.*, *J. Am. Pharm. Assoc.*, **43**, 1 (1954). (b) M. E. Wall *et al.*, *J. Am. Pharm. Assoc.*, **43**, 503 (1954). (c) M. E. Wall *et al.*, *J. Am. Pharm. Assoc.*, **44**, 438 (1955).

(7) R. E. Marker, *J. Am. Chem. Soc.*, **71**, 2656 (1949).

(8) (a) These sapogenins were named in honor of Doctors H. S. Gentry and D. S. Correll, Horticultural Crops Research Branch, Agricultural Research Service, United States Department of Agriculture, Beltsville, Md., who obtained the *Dioscorea* samples from which the new sapogenins were obtained. (b) One of the reviewers feels that it was improper to change the earlier name botogenin to gentrogenin since the two sapogenins apparently have the same structure. The other reviewer feels that the renaming was justified. At present we are retaining both names gentrogenin (botogenin) until this issue can be further resolved.