Phosphorus Mustards. II. The Synthesis and Biological Evaluation of Bis(2-chloroethyl)methylphosphine Hydrochloride[1]

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Bis(2-chloroethyl)methylphosphine, isolated as the hydrochloride, has been synthesized. Biological evaluation of the hydrochloride, in comparison with that of the nitrogen analog (HN2), has shown that the phosphorus compound exhibits no "mustard-like" activity as assessed by its lack of effect on bone marrow in mice at doses up to 500 μg/g. Preliminary results suggest that the lack of biological activity of the phosphine hydrochloride is due to transformations of the latter, which occur extremely rapidly in aqueous systems, even under oxygen-free conditions.

The useful biological properties of nitrogen and sulfur mustards as tumor growth inhibitors have prompted studies leading to the synthesis of phosphorus analogs of such compounds.[2] Since bis(2-chloroethyl)phenylphosphine oxide did not exhibit "mustard-like" activity,[2a] it appeared highly relevant (for a direct biological comparison of phosphorus mustards with their nitrogen counterparts) to prepare the phosphorus analog of the highly potent bis(2-chloroethyl)methylamine (HN2). This paper reports the first synthesis and initial biological evaluation of bis(2-chloroethyl)methylphosphine, isolated as the hydrochloride.

Discussion and Results

In the first paper of this series[2a] describing the synthesis of bis(2-chloroethyl)phenylphosphine, the key compound in the reaction sequence was bis(2-*t*-butoxyethyl)phenylphosphine prepared from phenylphosphine. An analogous reaction scheme for the preparation of the methyl analog would involve the initial synthesis of the highly reactive and volatile methyl phosphine. In view of its high toxicity and the lack of success in preparing metallo derivatives of the phosphines from phosphorous halides,[2a] an alternate route was considered for the preparation of the compound, bis(2-*t*-butoxyethyl)methylphosphine.

Conductimetric titration of sodium in liquid ammonia with phosphine at −64° has shown that monosodium phosphide is formed quantitatively, the reaction end point being depicted by a color change from deep blue to pale green.[3] This observation, together with the commercial availability of phosphine (PH_3), prompted its use in the synthesis of 2-*t*-butoxyethylphosphine (II). This was accomplished by the interaction of monosodium phosphide in liquid ammonia with an equivalent of 2-*t*-butoxyethyl bromide (Ia). Initial experiments, designed to yield bis(2-*t*-butoxyethyl)-phosphine (III) in a two-step, single-flask reaction using the sodium–liquid ammonia technique,[2a] proved unsatisfactory with only very low yields of III being obtained. In contrast, almost quantitative conversion of phosphine to II was achieved. On this basis, an alternate route to III was devised.

Metalation[4] of II with *n*-butyllithium, followed by reaction with 2-*t*-butoxyethyl chloride (Ib),[2a] afforded moderate yields of bis(2-*t*-butoxyethyl)phosphine (III) and appreciable amounts of tris(2-*t*-butoxyethyl)-phosphine (IV). Formation of IV is undoubtedly due to the presence of two "active" hydrogen atoms in the primary phosphine (II). Subsequent metalation of III and reaction with methyliodide gave bis(2-*t*-butoxyethyl)methylphosphine (V) in high yield. The phosphines (II–V) were distillable at reduced pressure, under nitrogen, without decomposition (see Scheme I).

Cleavage of both *t*-butoxy groups in V by concentrated hydrochloric acid[5] gave a quantitative yield of bis(2-hydroxyethyl)methylphosphine hydrochloride (VI) as a colorless syrup. Isolation and purification of the parent phosphine of VI was not attempted in view of the reported instability of the corresponding phenyl derivative.[2a] Reaction of the hydrochloride salt (VI) with 2 equiv of thionyl chloride in chloroform gave bis(2-chloroethyl)methylphosphine hydrochloride (VII) in good yield, as a highly labile white solid. Its properties prevented further purification for, on exposure to air at normal temperatures, it changes immediately to an oil. It can, however, be stored indefinitely under dry nitrogen at temperatures below 0°.

Attempts to convert the phosphines (II–IV) into crystalline methiodide derivatives for characterization purposes gave oils, but in the case of bis(2-*t*-butoxyethyl)methylphosphine (V) a solid methiodide was obtained. Highly crystalline, stable derivatives, however, were obtained in all cases by the addition of sodium tetraphenylboron to the respective methiodide products.[6] The formation of solid phosphonium tetraphenylborate salts proved to be extremely useful even in the characterization of the phosphine hydrochlorides (VI and VII) themselves.

Initial biological evaluation of bis(2-chloroethyl)-methylphosphine hydrochloride (VII) was carried out by a study of its effect on bone marrow in white Swiss Albino mice in comparison with the hydrochloride of the nitrogen analog (HN2). This biological test has been used to evaluate the potency of alkylating agents.[7] Within 3 days following the intravenous injection of the nitrogen mustard hydrochloride (in saline solution) at doses ranging from 3–7

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(2) (a) Paper I: T. P. Abbiss, A. H. Soloway, and V. H. Mark, *J. Med. Chem.*, **7**, 763 (1964); (b) R. W. Turner and A. H. Soloway, *J. Org. Chem.*, **30**, 4031 (1965).

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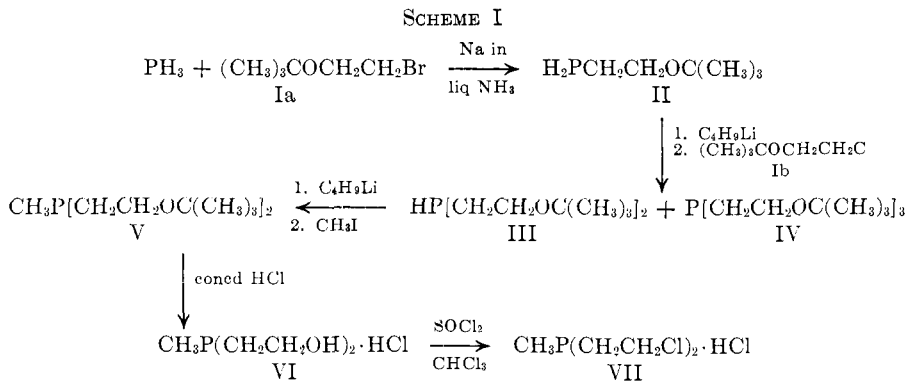
SCHEME I



μg/g, almost complete destruction of the marrow elements was observed with only red cells and an occasional altered stem cell remaining. In marked contrast, no significant destruction of marrow elements was observed following intravenous or intraperitoneal injection of the phosphorous compound (dissolved in oxygen-free water or as a suspension in mineral oil) at doses up to 500 μg/g. Higher doses proved lethal within 3 days but with no observable bone marrow depression.

All attempts to isolate the free bis(2-chloroethyl)-methylphosphine from its hydrochloride salt (VII), either by neutralization in aqueous, oxygen-free systems or by reaction with oxygen-free water alone, have failed. In the case of other bis(β-substituted ethyl)phosphines, liberation of the free phosphine from its hydrochloride salt by the action of oxygen-free water has been readily accomplished.[8] Structural changes, however, do occur very rapidly when oxygen-free water is added to the phosphine hydrochloride (VII) as evidenced by the immediate changes seen in the pmr spectrum.[8] The occurrence of such facile transformations, even in oxygen-free systems, would help to explain the lack of biological activity of VII.

Experiments designed to elaborate on the transformations undergone by the hydrochloride salt (VII) in oxygen-free, aqueous systems together with studies on the stability of other alkyl β-substituted ethylphosphines are under investigation.

Experimental Section[9]

Phosphine was obtained from Rocky Mountain Research Inc., Denver, Colo., as a liquified gas. All apparatus used with phosphine was dried thoroughly and purged with nitrogen immediately prior to and during use, and all reactions were performed in a well-ventilated hood. ***n*-Butyllithium** was obtained from the Lithium Corp. of America, Inc., New York, N. Y., as a 22% solution in *n*-hexane.

2-*t*-Butoxyethyl bromide (**Ia**) was prepared from 2-bromoethanol (62.5 g, 0.5 mole) and isobutylene (238 ml, 2.5 moles) in the presence of concentrated H_2SO_4 (1 ml) in an analogous manner to 2-*t*-butoxyethyl chloride (Ib).[2a] However, due to the similarity in boiling points of the bromo alcohol and Ia, it was necessary to remove unreacted alcohol prior to distillation by shaking the crude product with an equal volume of 50% aqueous H_2SO_4. The nonaqueous portion was then washed with 5% aqueous $NaHCO_3$ and dried over K_2CO_3. Distillation afforded Ia (57.0 g, 63%), bp 45–48° (10 mm).

Anal. Calcd for $C_6H_{13}BrO$: C, 39.8; H, 7.2; Br, 44.2. Found: C, 40.1; H, 7.2; Br, 44.3.

2-*t*-Butoxyethylphosphine (II).—To liquid NH_3 (400 ml) stirred at −80°, small pieces of freshly cut sodium (23.0 g, 1.0 g-atom) were added cautiously followed by dry ethyl ether (200 ml). Phosphine gas was then bubbled through the vigorously stirred mixture for 1 hr until a color change from dark blue to pale green occurred. The system was purged of excess phosphine with a stream of nitrogen for 20 min, and then the bromo ether (Ia, 181 g, 1.0 mole) in ethyl ether (200 ml) was added dropwise over 90 min. The stirred mixture was maintained at −80°, and after 2 hr, a heavy white solid had precipitated. The NH_3 was then permitted to boil off and oxygen-free water (200 ml) was added cautiously to dissolve the precipitated NaBr. After separation of the ether layer and drying over K_2CO_3, ethyl ether was removed by evacuation, and the product (II, 62.7 g, 94%), bp 52–55° (28 mm), was obtained as a colorless oil which could be oxidized readily in air.

Anal. Calcd for $C_6H_{15}OP$: C, 53.7; H, 11.3. Found: C, 53.5; H, 11.1.

The phosphine (II) was converted to its methiodide (an oil). Reaction of this with sodium tetraphenylboron in absolute methanol afforded (2-*t*-butoxyethyl)methylphosphonium tetraphenylborate, mp 133–135°, colorless prisms from methanol.

Anal. Calcd for $C_{31}H_{38}BOP$: C, 79.5; H, 8.2; B, 2.3; P, 6.6. Found: C, 79.7; H, 7.9; B, 2.5; P, 6.5.

Compound II was also characterized by oxidation with H_2O_2 in ethanol at 0° and then reaction with benzaldehyde in hydrochloric acid solution[10] to give bis(α-hydroxybenzyl)-2-*t*-butoxyethylphosphine oxide, mp 150–152°, needles from methanol.

Anal. Calcd for $C_{20}H_{27}O_4P$: C, 66.3; H, 7.5; P, 8.6. Found: C, 66.4; H, 7.6; P, 8.4.

Bis- (III) and Tris(2-*t*-butoxyethyl)phosphine (**IV**).—To the phosphine (II, 89.4 g, 0.67 mole) dissolved in dry ether (400 ml) was added dropwise with stirring 308 ml of a 21.3% solution of *n*-butyllithium in *n*-hexane (equivalent to 0.67 mole of C_4H_9Li). The temperature of addition was maintained at 17–20° by cooling the reaction mixture in an ice–acetone bath; the clear solution became bright yellow. During this time negligible condensate appeared in a cold trap (−80°) incorporated in the system to measure *n*-butane evolution. Dropwise addition of the chloro ether (Ib, 91.1 g, 0.67 mole) in ethyl ether (150 ml) over 1 hr caused a color change to dark, olive green which slowly paled with the precipitation of a white solid over a 4-hr period. During this time 35.5 g (92%) of *n*-butane was collected in the cold trap. After the addition of oxygen-free water (200 ml) to the reaction mixture, the ether was separated and dried over anhydrous K_2CO_3. Removal of solvent and distillation of the product afforded bis(2-*t*-butoxyethyl)phosphine (III, 72.8 g, 47% based on II), bp 138–141° (22 mm), tris(2-*t*-butoxyethyl)phosphine (IV, 22.3 g, 10% based on II), bp 98–101° (0.08 mm), and a brown viscous residue.

Anal. Calcd for $C_{12}H_{27}O_2P$ (III): C, 61.5; H, 11.6; P, 13.2. Found: C, 61.4; H, 11.6; P, 13.2.

Anal. Calcd for $C_{18}H_{39}O_3P$ (IV): C, 64.7; H, 11.7; P, 9.3. Found: C, 64.8; H, 11.7; P, 9.5.

The phosphines (III and IV) were characterized by conversion in methanol *via* the methiodides (oils) to their respective phosphonium tetraphenylborate derivatives IIIa, mp 129–130° (prisms from methanol), and IVa, mp 168–169° (prisms from benzene).

Anal. Calcd for $C_{37}H_{50}BO_2P$ (IIIa): C, 78.1; H, 8.9; B, 1.9; P, 5.5. Found: C, 78.6; H, 9.0; B, 2.1; P, 5.5.

(8) D. C. Smith and A. H. Soloway, unpublished results.

(9) All analyses were performed by Schwarzkopf Microanalytical Laboratories, New York, N. Y. All manipulations were carried out under nitrogen, except for the purification of the tetraphenylboron salts. All melting points were taken on a Fisher-Johns melting point apparatus and are corrected.

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Anal. Calcd for $C_{43}H_{62}BO_3P$ (IVa): C, 77.2; H, 9.4; B, 1.6; P, 4.6. Found: C, 77.2; H, 9.7; B, 1.9; P, 4.7.

Bis(2-*t*-butoxyethyl)methylphosphine (V).—To III (78.1 g, 0.33 mole) in ethyl ether (300 ml) was added 154 ml of a 21.3% solution of *n*-butyllithium in *n*-hexane (equivalent to 0.33 mole of C_4H_9Li) over a 30-min period. The temperature was maintained at 17–20° during the addition. Precipitation of a white solid occurred, but no condensate collected in the −80° cold trap. However, upon the dropwise addition of methyl iodide (47.3 g, 0.33 mole) in ether (100 ml) over 45 min with continued stirring for 3 hr, this solid dissolved with the evolution of *n*-butane (17.8 g, 92%). A bright yellow clear solution remained. After cooling to −40° to precipitate the lithium salts, oxygen-free water (200 ml) was added. The ether layer was separated and dried with K_2CO_3. Removal of solvent and distillation yielded V (72.4 g, 88%), bp 146–147° (22 mm), as a colorless oil.

Anal. Calcd for $C_{13}H_{29}O_2P$: C, 62.8; H, 11.8; P, 12.5. Found: C, 62.9; H, 11.8; P, 12.3.

The phosphine was characterized by conversion in methanol first to the methiodide (Va), mp 84–85° (acetone–cyclohexane), and then to the phosphonium tetraphenylborate salt (Vb), mp 185–187° (needles from methanol–benzene).

Anal. Calcd for $C_{14}H_{32}IO_2P$ (Va): C, 43.1; H, 8.3; I, 32.5; P, 7.9. Found: C, 43.1; H, 8.3; I, 32.4; P, 7.9.

Anal. Calcd for $C_{38}H_{52}BO_2P$ (Vb): C, 78.3; H, 9.0; B, 1.9; P, 5.3. Found: C, 78.2; H, 8.9; B, 1.9; P, 5.4.

Bis(2-hydroxyethyl)methylphosphine Hydrochloride (VI).—To the phosphine (V, 45.5 g, 0.18 mole) stirred at 0°, concentrated HCl (168 g, *ca.* 1.7 moles) was added dropwise during 20 min. An exothermic reaction occurred with the formation of a white emulsion. The mixture was stirred for 6.5 hr at 35° and in the cold trap (−80°) *t*-butyl chloride (32.3 g, 95%), bp 50.5°, was obtained. The residual, colorless aqueous phase was concentrated under reduced pressure at 60° to give a quantitative yield of the bis alcohol hydrochloride (VI, colorless syrup) which was characterized by direct conversion in methanol to bis(2-hydroxyethyl)methylphosphonium tetraphenylborate, mp 158–160°, prisms from methanol.

Anal. Calcd for $C_{29}H_{34}BO_2P$: C, 76.3; H, 7.5; B, 2.4; P, 6.8. Found: C, 76.5; H, 7.5; B, 2.4; P, 6.8.

Bis(2-chloroethyl)methylphosphine Hydrochloride (VII).—Compound VI (31.5 g, 0.18 mole) was suspended in dry $CHCl_3$ (300 ml) and cooled to −10° in an ice–acetone bath. Thionyl chloride (87.0 g, 0.73 mole) in $CHCl_3$ (200 ml) was added dropwise to the stirred mixture over 70 min at such a rate that the temperature did not rise above −5°. After the addition was complete, the mixture was stirred for 1 hr during which time it attained room temperature. A yellow insoluble paste separated from solution. Gentle heating at 40–43° for 3 hr caused solution of this paste (clear yellow solution) with the separation of a small amount of colloidal sulfur. Filtration and concentration of the filtrate at 20° (10 mm) gave the phosphine hydrochloride (VII) as a white solid (27.0 g, 71%). This was washed well with dry petroleum ether and dried *in vacuo* (0.05 mm) at room temperature.

Anal. Calcd for $C_5H_{12}Cl_3P$: Cl, 50.9. Found: Cl, 49.9, 52.7.

The hydrochloride salt (VII) is rapidly transformed to an oil at normal temperatures, but it can be kept for indefinite periods below 0° either under a dry nitrogen atmosphere or *in vacuo*. It was characterized by direct conversion with sodium tetraphenylboron in methanol to the corresponding phosphonium salt, mp 134–135°, colorless prisms from methanol.

Anal. Calcd for $C_{29}H_{32}BCl_2P$: C, 70.6; H, 6.5; B, 2.2; Cl, 14.4; P, 6.3. Found: C, 71.4, 69.3; H, 6.5; B, 2.1; Cl, 13.5; P, 5.9.

Results of analysis for carbon, chlorine, and phosphorus, on the above phosphonium salt, were subject to great variation, even on the same analytical sample. The chlorine content was consistently low, and the reason may be ascribed to the instability of the tetraphenylboron derivative. Repeated attempts at recrystallization gave only dark brown oily products.

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Attempted Synthesis of 2,4-Dihydroxy-4,3-borazaropyridine. Preparation of Aminoalkylboronic Acids[1]

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Attempts directed toward the synthesis to 2,4-dihydroxy-4,3-borazaropyridine ("4-borauracil"), a possible antimetabolite of uracil, for use in boron-10 neutron-capture irradiation of brain tumors are described. The preparation and properties of 2-aminoethyl- and 3-aminopropylboronic acids, intermediates in the synthesis, are reported.

As a continuation of the program designed to synthesize boron compounds which could be used for the treatment of cancer by neutron-capture irradiation,[3] the preparation of 2,4-dihydroxy-4,3-borazaropyridine (I) (4-borauracil) was undertaken. The biological rationale for preparing such potential antimetabolites is that a twofold attack on tumors may become feasible, (1) by direct inhibition of the neoplasm itself, and (2) by incorporation of the compound into the nucleic acids of the neoplasm as a consequence of tumor metabolism. A nuclear site for a thermal neutron absorber, such as a boron-10 atom, may permit effective disruption of tumor chromosomes by the radiation procedure.

The chemical basis for the synthesis of such a structure is the fact that two cyclic derivatives IIa[4] and IIb[5] containing this ring system have been described as well as the preparation now of a third one, IIc. Though a definitive proof of structure for IIa–c has as yet not

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