Phosphorus–Nitrogen Compounds. 26. Phosphaminimides. 2.[1] 2,2′-Phosphinylidenebis(1,1,1-trimethylhydrazinium) Iodide Inner Salts as Agents Affecting Ganglionic Transmission

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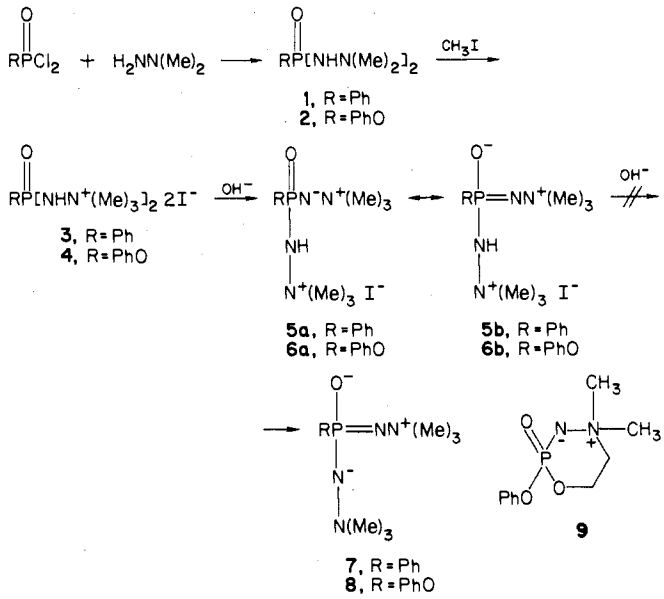
The N-2 atoms of phosphorus 2,2-dimethylhydrazides, contrary to a previous report, can be methylated by iodomethane. Treatment of the resulting dihydrazinium iodides with aqueous sodium hydroxide results in mono- instead of didehydroiodination, apparently due to resonance stabilization of the inner salt form. The phosphaminimide products and their hydrazinium iodide precursors blocked sympathetic ganglionic transmission while one dihydrazide intermediate produced potentiation. Brine shrimp testing indicated that conversion of a hydrazinium iodide to an aminimide moiety results in decreased toxicity.

Aminimides, also termed hydrazinium inner salts, are a type of compound in which a quaternary nitrogen is bonded to an anion derived from a carboxamide or related system.[2-4] The replacement of the carbon in an acyl group with a phosphorus atom results in a subclass of agent, which can be designated as phosphaminimides, with a general formula of $R_{1-3}P(O)(N^-N^+R_3)_{1-3}$. Being pentavalent, phosphorus should permit the attachment of two or three hydrazinium inner salt moieties and, in the first case, allow a modification of physiochemical properties by variation of a third substituent. The few phosphaminimides that have been reported are confined to $Ph_2P(O)N^-N^+Me_3$[5] and the type $R_2P(O)N^-N^+R^1R^2CH_2CH(OH)R^3$.[6-7] Our initial effort in the area of phosphaminimides resulted in the preparation and bioevaluation of 2-phenoxy-4,4-dimethyltetrahydro-2*H*-1,3,4,2-oxadiazaphosphorinium 2-oxide inner salt (**9**), a novel cyclic hydrazinium inner salt.[1]

Aminimides possess a quaternary amine grouping which has been associated with numerous biological properties as concerns nerve impulse transmission processes. Of particular interest to this study is their possible involvement in sympathetic ganglionic blockade, and an attempt to incorporate quaternary nitrogens into a more lipophilic form was a prime consideration in the investigation of phospha(di)aminimides for this activity. As inner salts, the solubility characteristics of these agents would be expected to differ from their hydrazinium halide intermediates. The apparent increased lipophilicity of aminimides is exemplified by a general method of their synthesis whereby the products are extracted from aqueous solutions of quaternary hydrazide intermediates by chloroform.[8] It was anticipated that aminimides could provide a hydrophilic–lipophilic balance that results in a more rapid and complete absorption from the gastrointestinal tract and a proper in vivo distribution.[9] A second reason for examining phosphaminimides is their approximation of the structural features required by bis(quaternary amines) to produce ganglionic blockade. This type of compound has its onium centers separated by two (e.g., chlorisondamine) to six (e.g., hexamethonium) carbon atoms or an equivalent distance. Phosphadiaminimides meet these criteria by having a three-atom group, $^-N–P(O)–N^-$, as an intervening moiety.

In this present study we investigated a possible route for the synthesis of phosphadiaminimides, $RP(O)(N^-N^+R_3)_2$, a new class of aminimides. The resulting products of monodehydroiodination, $RP(O)(N^-N^+Me_3)(NHN^+Me_3I^-)$, and its precursors were tested for effects

Scheme I



on sympathetic ganglionic nerve transmission and for toxicity.

Chemistry. Aminimides are commonly synthesized by dehydrohalogenation of hydrazinium salts, and this method was employed in this present study (Scheme I) and in the preparation of **9**. In this latter investigation, the 2-phenoxy derivative gave the expected product whereas none was formed from the 2-phenylhydrazinium precursor, and this phenomenon was attributed to resonance stabilization. Originally we expected the formation of phosphadiaminimides **7** and **8** from the treatment of the corresponding hydrazinium iodides **3** and **4** with aqueous

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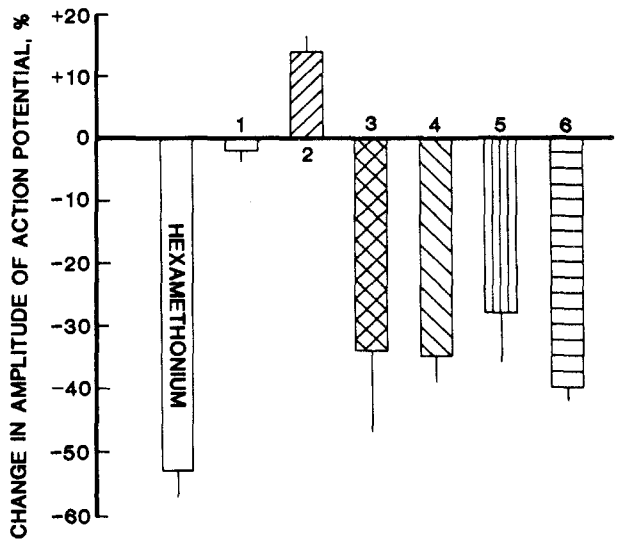

Figure 1. Effect of 1–6 (1.65 mM) on the amplitude of the compound action potential recorded from superior cervical ganglia of rats. The effect of hexamethonium (0.2 mM) is shown for comparison. Each bar and vertical line represents the mean ± SE of three to five experiments.

sodium hydroxide. This process, however, led to bis(hydrazinium) iodide inner salt compounds **5** and **6** in which only one molecule of HI was extracted. Resonance is thought to influence the course of this reaction as it did the stability of **9**. The resonance forms **5b** and **6b** apparently make a major contribution to the actual phosphaminimide structure, a phenomenon that has been observed in acylaminimides[3] and in *N*-(trimethylammonio)diphenylphosphinamidate, the only phosphaminimide prior to this series to be studied for its chemical properties.[5] Without a phosphinyl (P=O) group available for conversion to a P–O⁻ form, the remaining hydrazinium iodide substituent is thought to be unable to undergo resonating stabilization and, hence, resists dehydroiodination to the inner salt.

Despite a previous report to the contrary, hydrazides **1** and **2** underwent methylation to yield hydrazinium iodides **3** and **4**. The quaternization of phosphinylhydrazides presented difficulties to previous investigators, with Nielson et al. reporting that no reaction occurred when **1** was treated with iodomethane in ether, benzene, or toluene at 25, 50, and 110 °C, respectively.[10] They attributed these negative results to deactivation by the phosphinyl group of not only the N-1 but also the N-2 nitrogen of the hydrazide moiety such that neither serve as an electron donor. Using acetonitrile, in lieu of nonpolar media, we were, however, able to methylate **1** and **2** with the formation of **3** and **4** in yields of 80% and 88%, respectively. It should be noted that not all polar solvents are operative in this reaction since methanol caused cleavage of the hydrazino bond with formation of a material identified, on the basis of its NMR spectrum and melting point, as 1,1,1-trimethylhydrazinium iodide.

Biological Activity. The abilities of **1–6** to modify nerve impulse transmission in sympathetic ganglia as compared to hexamethonium, a well-established ganglionic blocking agent, are shown in Figure 1. The iodide inner salt compounds **5** and **6** fulfill the structure–activity requirements as concerns separation of onium centers, but they do not completely satisfy the original goal of eliminating an external salt form. Both the hydrazinium iodides **3** and **4** and the aminimides **5** and **6**, in contrast to **9**, which inexplicably caused potentiation, blocked ganglionic transmission, but this effect required 8–10 times the concentration required by hexamethonium to produce the same magnitude of interference. The blocking abilities were approximately equal for both the iodide and iodide inner salt types and 2–3 times more potent than the monohydrazide and monohydrazinium iodide precursors of **9**.[1] Thus, these agents appear to adhere to the general rule that greater blocking effect resides in those agents having two, appropriately separated onium centers rather than one. The phenyl-substituted hydrazide **1** was essentially void of activity while the phenoxy derivative **2** produced an increase in amplitude and a prolongation of the duration of action potential. The increase in amplitude and duration of action potential resulting from the administration of **2** and compared to the block produced by **5** is shown in Figure 2. Although requiring further study, this interesting effect produced by **2**, as well as by **9**, may indicate inhibition of acetylcholinesterase.

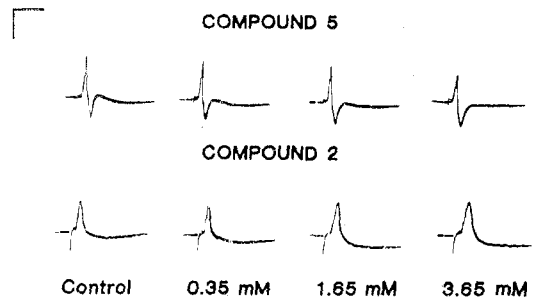

Figure 2. Effect of various concentration of **5** (upper traces) and **2** (lower traces) on the compound action potential recorded from two different isolated superior cervical ganglia of the rat. Note the marked increase in the duration of action potential in the ganglion treated with **2**. Vertical calibration bar 0.6 mV (upper traces) and 0.3 mV (lower traces); horizontal bar 60 ms in all traces.

The effects of **3–6** on brine shrimp was investigated as a simple and rapid means of comparing the toxicities of hydrazinium iodides and phosphaminimides. The same general toxicity profile was obtained with these compounds as with **9** and its hydrazinium iodide. The iodide salts **3** and **4** have LD_{50} values of 2.15 and 2.37 μg/mL, respectively, while the iodide inner salts **5** and **6** gave LD_{50} values of approximately 15 μg/mL. The effect of iodide ion in the same concentration as found in **3** and **4** (~32%) and in **5** and **6** (~48%) was examined and found to account for 37–55% of the toxicity displayed by these compounds. Even taking into consideration this correction factor, these findings substantiate the previous results shown with **9** that conversion of hydrazinium iodides to aminimide forms results in decreased toxicity.

Experimental Section

Melting points were taken on a Thomas-Hoover apparatus and are corrected to reference standards. ^1H NMR spectra were determined on a Varian FT-80A or T-60 spectrometer using tetramethylsilane as the internal standard and deuterated dimethyl sulfoxide (**2–5**) or deuterium oxide (**6**) as the solvents. Infrared spectra (KBr) were recorded on a Perkin-Elmer 283 spectrophotometer. Elemental analyses for C, H, and N were performed by Atlantic Microlab, Inc., Atlanta, GA, and the results are within ±0.4% of the theoretical values. Silica gel 60 (70–230 mesh) and a 25 × 500 mm column was used for chromatography.

Bis(2,2-dimethylhydrazino)phenyl- and -phenoxyphosphine Oxide (1 and 2). These compounds were prepared by a modification of the method described by Nielsen and Sisler.[11]

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With use of methylene chloride as the reaction solvent and column chromatography with 5% MeOH in $CHCl_3$ as the eluent for purification, a yield of 80% (lit.[11] 61%) and a melting point of 169–170 °C (lit.[11] mp 161–164 °C) were achieved for known compound 1. For compound 2, phenylphosphorodichloridate (42.2 g, 0.2 mol) in CH_2Cl_2 (50 mL) was added dropwise under N_2 and with stirring to 1,1-dimethylhydrazine (60.1 g, 1.0 mol) in CH_2Cl_2 (100 mL) at 0–10 °C. The reaction mixture was refluxed for 18 h, cooled to 25 °C, and filtered, and the residue was washed with CH_2Cl_2. The filtrate and washing were evaporated in vacuo to give a residue, which was chromatographed with 5% MeOH in $CHCl_3$ as the eluent to yield 42.3 g (82%) of 2 ($C_{10}H_{19}N_4O_2P$): mp 118–120 °C; IR 3180 (NH), 1600 (C=C), 1200, 1230 (P=O) cm^{-1}; NMR δ 2.5 (s, 12 H, 4 CH_3), 5.75 (s, 1 H, NH), 6.25 (s, 1 H, NH), 7.30 (m, 5 H, arom).

2,2′-(Phenyl- and -phenoxyphosphinylidene)bis(1,1,1-trimethylhydrazinium) Diiodide (3 and 4). An excess of iodomethane (17.0 g, 60 mmol) was added to 30 mmol of 1 (suspension) or 2 (solution) in CH_3CN (120 mL) at 25 °C. The reaction mixtures were heated to 70 °C (1 went into solution) for 2 h and then to 60 °C for 18 h. The mixtures were cooled to 25 °C and filtered and the residues washed with CH_3CN and Et_2O to yield pure products. For 3 ($C_{12}H_{25}N_4I_2OP$): 12.7 g, 80%; mp 180–182 °C dec; IR 3170 (NH), 1590 (C=C), 1230 (P=O) cm^{-1}; NMR δ 3.61 (s, 18 H, 6 CH_3), 7.63–8.12 (m, 5 H, arom). For 4 ($C_{12}H_{25}N_4I_2O_2P$): 14.4 g, 88%; mp 174–175 °C dec; IR 3030 (NH), 1590, 1600 (C=C), 1170, 1200 (P=O) cm^{-1}; NMR δ 3.68 (s, 18 H, 6 CH_3), 7.43 (m, 5 H, arom).

2,2′-(Phenyl- and -phenoxyphosphinylidene)bis(1,1,1-trimethylhydrazinium) Iodide Inner Salt (5 and 6). Compound 3 or 4 (3.8 mmol) was dissolved in 10% NaOH (1.7 mL) at 25 °C to yield neutral solutions. An additional 1.7 mL of base gave alkaline solutions, an indication that a second molecule of HI was not neutralized. The reaction mixtures were stirred at 25 °C for 1 h, neutralized with 1 N HCl at 5–10 °C, and evaporated in vacuo to dryness at 25 °C. For 5 the residue was extracted several times with CH_2Cl_2, and the extracts were evaporated in vacuo to yield 1.7 g (98%) of 5 ($C_{12}H_{24}N_4IOP \cdot H_2O$): mp 126–128 °C dec; IR 3450 (OH), 3210 (NH), 1590 (C=C), 1200 (P=O) cm^{-1}; NMR δ 3.37 (s, 18 H, 6 CH_3), 5.75 (s, 1 H, NH), 7.39–7.88 (m, 5 H, arom). For 6 the residue was dissolved in acetone and filtered. A solid formed in the filtrate that was collected on a filter, washed with acetone, and dried to yield 2.7 g (86%) of 6 ($C_{12}H_{24}N_4IO_2P$): mp 202–203 °C dec; IR 3200 (NH), 1595 (C=C), 1200, 1230 (P=O) cm^{-1}; NMR δ 3.53 (s, 18 H, 6 CH_3), 7.35 (m, 5 H, arom).

Sympathetic Ganglionic Transmission Testing. Superior cervical ganglia were quickly excised from Sprague–Dawley rats. The ganglia were carefully desheathed while maintained in cold oxygenated (5% CO_2, 95% O_2) Locke's solution (pH 7.4). The ganglia were immersed in this solution in a constant temperature chamber with the pre- and postganglionic (internal carotid) nerves drawn into stimulating and recording suction electrodes. The preganglionic nerve was stimulated supramaximally at 0.3 Hz. Postganglionic compound action potentials were recorded with a capacity-coupled preamplifier and the amplified potentials were displayed on an oscilloscope and permanent records were made on photographic paper or film. Ganglia were selected for study only when the postganglionic action potential was stable for at least 30 min. Solutions of test compounds were added to the perfusion fluid and changes in amplitude of the action potential used as indices of effect on transmission. After the steady-state condition was attained, 8–10 "control" consecutive action potentials were recorded. Dose–response relationships were obtained by testing increasing, cumulative concentrations of the compounds on the action potential. Each new concentration was left in the bath for 5–10 min before recording of the action potential. Change in ganglionic transmission is expressed as the percent change in amplitude in the action potential.

Toxicity Testing. The brine shrimp assay procedure reported by Meyer et al.[12] was employed with two modifications. Filter paper was not used in the sample preparation; instead the sample of test solutions were pipetted directly into the sample vials prior to drying in vacuo and the percent mortalities were determined after 12 h, instead of 6 and 24 h. LD_{50} values were calculated by using the probit analysis method described by Finney.[13] Correlation coefficients (R^2) values of 0.998 and 0.994 were obtained for 5 and 6, respectively, by linear regression analysis.

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3-Alkyl-3-hydroxyglutaric Acids: A New Class of Hypocholesterolemic HMG CoA Reductase Inhibitors. 1[1]

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Derivatives of 3-hydroxy-3-methylglutaric acid (HMG), a portion of the substrate for HMG CoA reductase, were prepared and tested for their inhibitory action against rat liver HMG CoA reductase and for their hypocholesterolemic activity. Structure-dependent competitive inhibition was observed. Optimal structures had a free dicarboxylic acid with an alkyl group of 13–16 carbons at position 3. 3-*n*-Pentadecyl-3-hydroxyglutaric acid (**3j**) (IC_{50} = 50 μM) reduced serum cholesterol in the Triton-treated rat and HMG CoA reductase activity in the 20,25-diazacholesterol-treated rat.

The loss of feedback regulation of HMG CoA reductase (HMGR) by a deficiency of function in the LDL receptor leads to an inefficient disposal of plasma cholesterol and premature atherosclerosis in man.[3] Compactin and mevinolin, inhibitors of HMG CoA reductase, lower serum total cholesterol levels in normal and familial hypercholesterolemic patients.[4] Hypolipidemic properties of 3-hydroxy-3-methylglutaric acid (HMG) have been reported for rats, rabbits, and man.[5] HMG, as the coenzyme A

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