2,2′-Phthaloyl-, 2,2′-Isophthaloyl-, and 2,2′-Terephthaloylbis[1,1,1-trimethylhydrazinium] Dihydroxide, Bis(Inner Salts): Synthesis, Partition Coefficients, Toxicity and Effect on Ganglionic Transmission

LINDLEY A. CATES*[x], VEN-SHUN LI*, JYOTI P. BASRUR*, AND KARIM A. ALKADHI[+]

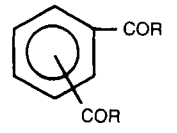
Received September 4, 1985, from the *Department of Medicinal Chemistry and the [+]Department of Pharmacology, College of Pharmacy, University of Houston–University Park, Houston, TX 77004. Accepted for publication January 9, 1986.

Abstract □ 2,2′-Phthaloyl-, 2,2′-isophthaloyl, and 2,2′-terephthaloylbis[1,1,1-trimethylhydrazinium] dihydroxide, bis(inner salts) **7**, **8**, and **9** and their hydrazide and hydrazinium diiodide precursors were synthesized and tested for toxicity and their ability to block sympathetic ganglionic transmission. Only the 2,2′-phthaloyl and isophthaloylhydrazinium diiodides **4** and **5** produced weak inhibition of nerve transmission (35% at 2.15×10^{-3} M). The inner salts were appreciably less toxic than the hydrazinium diiodides in brine shrimp testing. The log P ($\log_{10}$, chloroform pH 7 buffer system) values of all compounds were determined and those of the inner salts and hydrazinium diiodides were in the range of −3.03 to −3.60.

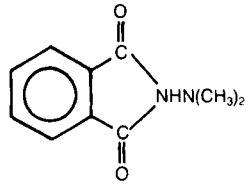
Hydrazinium inner salts (aminimides) possess a quaternary nitrogen atom attached to an anionic nitrogen atom, the latter being a part of a carboxamide group or related system. This class of compounds has been the subject of three reviews.[1–3] The effect on ganglionic transmission and on toxicities of cyclic[4] and acyclic[5] phosphoraminimides, in which a phosphinyl (P=O) group replaces a carbonyl group, have previously been investigated in this laboratory. Similarly, the present study involves 2,2′-phthaloyl-, 2,2′-isophthaloyl-, and 2,2′-terephthaloylhydrazinium dihydroxide, bis(inner salts) (**7–9**), their hydrazinium diiodide (**4–6**) and hydrazide precursors (**1–3**), and includes the determination of partition coefficient values of aminimides.

Phthaloylhydraziaium dihydroxide bis(inner salts) and their hydrazinium diiodide precursors possess structural features that suggest an ability to block ganglionic transmission. The phthaloyl, isophthaloyl and terephthaloyl isomers each contain two quaternary amino groups ("onium heads") separated by six, seven, and eight atoms, respectively. Many ganglionic blocking agents are "bis-onium" compounds in which the positive charges are separated by two (e.g., chlorisondamine) to six (e.g., hexamethonium) carbon atoms or an equivalent distance, while curare-like effects occur principally in compounds with a separation of seven to twelve carbon atoms or an equivalent distance. We, therefore, studied the quaternary phthaloyl derivatives to ascertain the extent to which the isomers produce ganglionic blockade and to compare the hydrazinium dihydroxide bis(inner salts) with the hydrazinium diiodides.

In addition, the inner salts would be expected to possess solubility characteristics that differ from those of their hydrazinium diiodide precursors, i.e. a greater degree of lipophilicity may exist. The log P ($\log_{10}$ of the partition coefficient in chloroform pH 7 buffer) values of the products and intermediates were, therefore, determined. If aminimides do block ganglionic transmission an increased lipophilicity would be desirable since the highly ionized and hydrophilic bis-quaternary drugs now used for this purpose are erratically and poorly absorbed from the alimentary tract. Also, the effects of the inner salts and hydrazinium diiodides on brine shrimp were compared in order to determine if the former type of compound is less toxic as were the phosphaminimides.[4,5]



1, 1,2- R = $NHN(CH_3)_2$
2, 1,3- R = $NHN(CH_3)_2$
3, 1,4- R = $NHN(CH_3)_2$
4, 1,2- R = $NHN^+(CH_3)_3$, I^-
5, 1,3- R = $NHN^+(CH_3)_3$, I^-
6, 1,4- R = $NHN^+(CH_3)_3$, I^-
7, 1,2- R = $N{-}N^+(CH_3)_3$
8, 1,3- R = $N{-}N^+(CH_3)_3$
9, 1,4- R = $N{-}N^+(CH_3)_3$



10

Results and Discussion

The inner salts **7–9** were prepared by the common synthetic route involving dehydroiodination of hydrazinium salts **4–6** which were obtained by the quaternization of the corresponding hydrazides **1–3**.

Compound **8** was previously synthesized by McKillip et al.[6] who prepared the requisite hydrazide **2** in 75% yield by a laborious and lengthy process involving extraction and water evaporation. We obtained this material in 82% yield through an improved process whereby triethylamine was used as the HCl-scavenger and the resulting salt was removed by methylene chloride washing. A competing reaction occurs in the synthesis of hydrazide **1** when *o*-phthaloyl chloride is added to 1,1-dimethylhydrazine. *N*-(Dimethylamino)phthalimide **10** was produced in increased amounts upon reverse addition of reactants and in less quantities when the reaction temperature was carefully regulated.

The determination of the log P values for hydrazinium salts, a type of quaternary amine, is difficult. The inner salts **7–9** would be expected to cause similar difficulties, since they are hydrophilic and also act as surfactants. The latter proper-

0022-3549/86/0400-0407$01.00/0

ty causes emulsion formation between partitioning phases which, in turn, produces false high absorbance readings when spectrophotometry is used for quantitation. Attempts were initially made to determine the log P values in the octanol/water system. The emulsification problems could not, however, be overcome using several different approaches. The addition of sodium chloride, as used by Raynard for quaternary amines,[7] permitted more rapid separation of phases but this treatment and centrifugation at 15,000 rpm and 0–5°C for one hour were ineffective in preventing emulsion formation. The use of deemulsifiers was also unsuccessful as was the use of a reference prepared by shaking the sample solutions with a small amount of octanol. In contrast, these difficulties were not encountered in the subsequent determinations using the chloroform:water system in which separation of phases was achieved by allowing the mixture to stand or by brief centrifugation.

The high degree of hydrophilicity of quaternary amines has presented difficulties in the accurate measurement of their partition coefficients. The hydrophobic additivity rule does not pertain to any three carbon atoms adjacent to a highly charged group (e.g., onium head).[8] According to two studies of quaternary amine compounds, this exception applies to 1–3 carbon atoms further removed.[9,10] A determination of the log P values of a homologous series of bisquaternary amines, differing by separations of two to 10 methylene groups, showed an average increase in log P of only 0.17, and by an average of only 0.12 for those carbons four to six atoms distant from the onium head, instead of the usual 0.50 produced by the addition of a methylene moiety.[9] Taking this factor into account, Eldefrawi and O'Brien,[10] achieved poor results despite the use of radiolabeled compounds. These workers reported log P values of 0.40, 0.77, 0.76, and 0.46 for trimethylammonium compounds which were consecutively increased by two carbon units. These values show that experimental log P values for quaternary amines can vary appreciably even when determined by a precise quantitative method.

When spectrophotometry is employed as the quantitation method, a high ratio of organic to aqueous solvent volumes must be employed. If a log P value of about −3, the approximate value for aminimides and hydrazinium iodides, is to be measured, a ratio of at least 100:1 is required to provide a significant difference in absorbance readings. In the case of the hydrazinium iodides **4–6** and aminimides **7–9**, one mL of concentrated solution was partitioned against 100 mL of chloroform, and 100 μL of this solution was diluted for absorbance reading. Although good separation of aqueous and chloroform layers was apparently achieved, any absorbance due to emulsification produced by the compounds was nullified by use of a reference consisting of 10 mL of each sample shaken with a negligible amount (0.3 mL) of chloroform. The log P values of **7**, **8**, and **9** were −3.03, −3.33, and −3.56, respectively. The values obtained for the hydrazinium diiodides **4** (−3.22), **5** (−3.60), and **6** (−3.39) were within experimental error of their corresponding inner salts, **7**, **8** and **9**. These results indicate that there is no appreciable difference in lipophilic character between these two types of phthaloyl derivatives. As expected, the hydrazides **1**, **2**, and **3** were considerably more lipophilic with log P values of −1.62, −1.28, and −1.58, respectively.

Compounds **6–9** had no marked effect (<10% inhibition) on ganglionic transmission even at the highest concentration tested (4.15×10^{-3} M). Compound **1**, the 1,2-hydrazide derivative, produced a small degree of inhibition (~20%) which may have resulted from the 10% ethanol required for solubilization (Fig. 1). Aqueous solutions of the 1,2-**2** and the 1,3-**5** hydrazinium diiodides caused about 35% inhibition at 2.15×10^{-3} M (Fig. 1). Even at this high concentration,

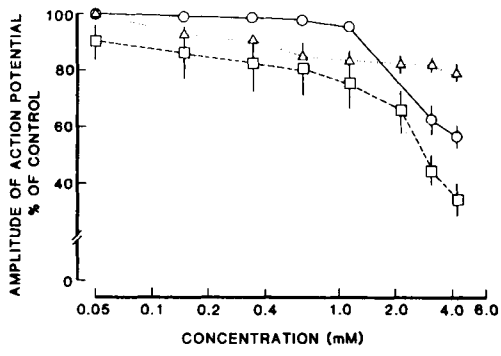


Figure 1—*Concentration–response relationships for* ***1*** (△), ***2*** (□), *and* ***5*** (○) *on transmission in the isolated superior cervical ganglion of the rat. Each point represents the mean ± SEM of three experiments.*

however, these two compounds are about 40 times less effective than hexamethonium, a classic ganglionic blocking drug which produced an inhibition of 37% at 5×10^{-5} M in three experiments. In accordance with structure-activity relationships, the order of ganglionic blockade for the hydrazinium diiodide isomers was 1,2-> 1,3-> 1,4- at all concentrations studied. These results indicate that members of this class of compounds are ineffective or weak blocking agents. In comparison, semiphosphaminimides (hydrazinium iodide inner salts) are approximately four times as potent in their ganglionic blocking effect.[5]

None of the hydrazinium dihydroxide bis-inner salts produced toxicity in brine shrimp at concentrations of 10, 100, and 1000 μg/mL. The presence of the hydrazinium iodides, corrected for iodide ion effect, resulted in complete mortality with the exception of compound **4** which was nontoxic at 10 and 100 μg/mL. These results show that isomeric phthaloylbistrimethylhydrazinium dihydroxide bis-inner salts are less toxic than the phthaloylhydrazinium diiodides and confirm the similar, previous findings with regard to phosphaminimides and their iodide precursors.[5]

Experimental Section

Melting points were taken on a Thomas-Hoover apparatus and corrected to reference standards. IR spectra were determined on a Perkins–Elmer 283 spectrophotometer and showed the expected absorption bands. All of the compounds are hygroscopic and their OH absorption bands are attributed to water. UV spectra were obtained using a Perkin–Elmer 200 spectrophotometer. ^{1}H NMR spectra were in agreement with the assigned structures and were recorded on a Varian T–60A or FT–80A spectrometer using Me_4Si as the internal standard and Me_2SO-d_6 (**1–5**, and **7**), D_2O (**6**, **8**, and **9**) or $CDCl_3$ (**10**) as the solvents. Elemental analyses were performed by Atlantic Microlab., Inc., Atlanta, GA. New compounds **1**, **3**, **4**, **6**, **7**, and **9**, and known compound **10** were analyzed for C, H, and N, the values of which were within +0.3% of the theoretical values. Silica gel 60 (70–230 mesh) for **1** and **10**, neutral alumina (Brockman activity 1, 80–200 mesh) for **7–9**, and a 25 × 500 mm column were used for chromatography. Reagent grade chloroform (MCB Omnisolv) was used for partitioning. The isomeric phthaloylbistrimethylhydrazinium dihydroxide bis-inner salts **7–9** were prepared by the method of McKillip et al.[6] However, the use of 5% (for **7**), 10% (for **8**), or 20% (for **9**) MeOH in $CHCl_3$, instead of C_6H_6 permitted facile packing of the column and more rapid elution during the purification procedures.

Phthalic acid, bis(2,2-dimethylhydrazide) (1) Isophthalic acid, bis(2,2-dimethylhydrazide (2), Terephthalic acid, bis(2,2-dimethylhydrazide (3)—A procedure described by McKillip et al.[6] was modified whereby o-phthaloyl, isophthaloyl or terephthaloyl chloride (10.15 g, 0.05 mol) in CH_2Cl_2 (50 mL) was added in a dropwise manner at 0–5°C with stirring to 1,1-dimethylhydrazine (15 g, 0.25 mol) for compound **1** or 1,1-dimethylhydrazine (7.5 g, 0.125 mol), and triethylamine (12.65 g, 0.125 mol) were used for **2** and **3**. The

mixture was stirred for 2 h at 0°C, and then at 25°C for 18 h, and filtered. The residue was washed with CH_2Cl_2 and dried under reduced pressure. This process gave **2** (82% yield) with the same physical properties as previously reported.[6] Compound **1** (66% yield) was purified by column chromatography using 2%, 5%, and 10% MeOH in CH_3Cl as successive eluants while compound **3** (74% yield) was recrystallized from MeOH. Compound **1**: mp 159–160°C; IR: ν_{max} (KBr): 3420 and 3510 (OH), 3510 and 3200 (NH), 1600 (C=C), and 1645 and 1665(C=O) cm^{-1}; ^1H NMR: δ 2.46 (d, J = 11 Hz, 12, 4-CH_3), 7.41 (s, 4, ArH). Compound **3**: mp 275–277°C; IR: ν_{max} (KBr): 3440 (OH), 3180 (NH), 1640 (C=O), and 1600 (C=C) cm^{-1}; ^1H NMR: δ 2.59 (s, 12, 4-CH_3), 7.60–8.15 (m, 4, ArH), and 9.48 ppm (s, 2, 2-NH).

2,2'-Phthaloylbis[1,1,1-trimethylhydrazinium] diiodide (4), 2,2'-Isophthaloylbis[1,1,1-trimethylhydrazinium] diiodide (5), 2,2'-Terephthaloylbis[1,1,1-trimethylhydrazinium] diiodide (6)—These derivatives were synthesized by the method of McKillip et al.[6] in which the nature of solvent, a reactant quantity, reaction time, temperature conditions and purification procedure were modified. Iodomethane (5.7 g, 0.04 mol) was added to **1**, **2**, or **3** (5 g, 0.02 mol), suspended in CH_3CN (100 mL), and the mixture was heated at 70°C for 1 h. During this time a solution resulted, and then a precipitate formed. After cooling to 40°C, iodomethane (5.7 g, 0.04 mol) was added and the mixture heated at 60°C for 18 h. The mixture was cooled to 25°C, filtered, and the residue washed with CH_3CN (**4** and **5**) or MeOH (**6**) and then dried under reduced pressure to yield the products as pale yellow powders. Compound **5** (94% yield) had the same physical properties as previously reported.[6] Compound **4**: mp 174–176°C; 69% yield: IR: ν_{max} (KBr): 3440 (OH), 3120 (NH), 1695 (C=O), and 1595 (C=C) cm^{-1}; ^1H NMR: δ 3.73 (s, 18, 6-CH_3), and 7.85 ppm (s, 4, ArH). Compound **6**: mp 243–245°C (dec.): 97% yield; IR: ν_{max} (KBr): 3440 (OH), 3200 (NH), 1700 (C=O), and 1610 (C=C) cm^{-1}; ^1H NMR: δ 3.84 (s, 18, 6-CH_3), and 7.92 ppm (s, 4, ArH).

2,2'-Phthaloylbis[1,1,1-trimethylhydrazinium] dihydroxide, bis(inner salt) (7), 2,2'-Isophthaloylbis[1,1,1-trimethylhydrazinium] dihydroxide, bis(inner salt) (8), 2,2'-Terephthaloylbis[1,1,1-trimethylhydrazinium] dihydroxide, bis(inner salt) (9)—The method of McKillip et al.[6] was modified whereby a solution of **4**, **5**, or **6** (4.8 g, 9 mmol) in water (5 mL) was treated with 1.6 M NaOH solution (11.2 mL). The mixture was stirred for 90 min, filtered, and the filtrate evaporated to dryness under reduced pressure. The residue was washed with acetone and chromatographed on neutral aluminum oxide 90 (activity stage 1, 70–230 mesh) using 10% MeOH in $CHCl_3$ as the eluant to yield **7**, **8** and **9**. McKillip et al.[6] employed 10% MeOH in benzene as the eluant. The use of $CHCl_3$ in lieu of benzene results in facile column packing and more rapid elution. Compound **8** (86% yield) has the same physical properties as previously described.[6] Compound **7**: mp 201–202°C (dec.); 71% yield; IR: ν_{max} (KBr): 3450 (OH), 1610 (C=C), and 1570 (C=N) cm^{-1}; ^1H NMR: δ 3.29 (s, 18, 6-CH_3), 7.71 (m, 4, ArH). Compound **9**: mp > 300°C; 95% yield; IR: ν_{max} (KBr): 3400 (OH), 1660 (C=C), and 1570 (C=N) cm^{-1}; ^1H NMR: δ 3.46 (s, 18, 6-CH_3) and 7.71 ppm (s, 4, ArH).

***N*-(Dimethylamino)phthalimide (10)**—This compound was obtained as the principal product during an attempted synthesis of **1**. Compound **10** was previously reported, but only the melting point was given.[11] The addition of hydrazine (15 g, 0.25 mol) to phthaloyl chloride (10.15 g, 0.05 mol) in CH_2Cl_2 (50 mL) at 25°C resulted in a temperature increase to 60°C. The mixture was refluxed for 4 h, cooled, and filtered and the filtrate evaporated to dryness under reduced pressure. The yellow residue was chromatographed using 10% MeOH in $CHCl_3$ as the eluant to give **10** as pale yellow needles. Compound **10**: mp 121–122°C; 62% yield; IR: ν_{max} (KBr): 3480 (OH); 1710 and 1730 (C=O), and 1615 (C=C); ^1H NMR: δ 3.10 (s, 6, 2-CH_3) and 7.85 ppm (s, 4, ArH).

Partition Coefficient Determinations—Solutions of all compounds obeyed Beer's Law when measured at their λ_{max} and at concentrations giving absorbance readings between 0.6 and 0.9. CH_3Cl-saturated phosphate buffer (pH 7.0) was used as the solvent and separatory funnels as the partitioning vessels. A 5:50 mL (**1–3**) or a 0.5:100 mL (**4–9**) ratio of solvent:buffer (saturated with CH_3Cl) was used for the partitioning. Ten mL of the buffer solution was similarly partitioned with 0.3 mL of CH_3Cl to serve as a reference. Equilibrium between phases was achieved by rotating the funnels on a motor driven, 35-cm diameter disk to provide ~1000 inversions. The phases were allowed to remain undisturbed for 30 min or the aqueous layer was centrifuged at 6000 rpm for 10 min. After being appropriately diluted with the buffer (to give absorbance readings between 0.6 and 0.9) the λ_{max} of the materials in the aqueous phase was determined in duplicate.

Sympathetic Ganglionic Transmission Testing—A previously reported method involving rat ganglia and percent change in amplitude of action potential was used.[5]

Brine Shrimp Toxicity Testing—The procedure of Meyer et al.,[12] as modified by Cates et al.,[5] was employed. This method has the advantages of being rapid, inexpensive, and simple.

References and Notes

1. Timpe, H. J. *Z. Chem.* 1972, *12*, 250.
2. McKillip, W. J.; Sedor, E. A.; Culbertson, B. M.; Wawzonek, S. *Chem. Rev.* 1973, *73*, 255.
3. Wawzonek, S. *Ind. Eng. Chem. Prod. Res. Dev.* 1980, *19*, 338.
4. Cates, L. A.; Li, V.-S.; Basrur, J. P.; Saddawi, B. H.; Alkadhi, K. A. *J. Heterocyclic Chem.* 1984, *22*, 183.
5. Cates, L. A.; Li, V.-S.; Saddawi, B. H.; Alkadhi, K. A. *J. Med. Chem.* 1985, *28*, 595.
6. McKillip, W. J.; Clemens, L. M.; Haugland, R. *Can. J. Chem.* 1967, *45*, 2613.
7. Raynard, A. M. *J. Pharmacol. Exp. Ther.* 1968, *163*, 461.
8. Franken, R. "Theoretical Drug Design Methods", vol. 7; Elsevier: New York, 1984; p 36.
9. Wiley, R. A.; Faraj, R. A.; Jantz, A. *J. Med. Chem.* 1972, *15*, 374.
10. Eldefrawi, M. E.; O'Brien, R. D. *J. Exp. Biol.* 1967, *46*, 1.
11. Drew, H. D. K.; Hatt, H. H.; Hobart, F. A. *J. Chem. Soc.* 1937, 33.
12. Meyer, B. M.; Ferrigni, N. R.; Putnam, J. E.; Jacobson, L. B.; Nichols, D. E.; McLaughlin, J. L. *J. Plant. Res.* 1982, *45*, 31.

Acknowledgments

This work was supported by the Robert A. Welch Foundation, Houston, TX (Grant E-920).