Synthesis and Biological Activity of Some Diphenylphosphine Derivatives

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As part of a program designed to evaluate pharmacological activity among phosphines, 3-dimethylaminopropyldiphenylphosphine (III) and the corresponding oxide (IV) and sulfide (V) were prepared. Activity cage studies revealed that these substances caused a marked reduction in spontaneous activity in mice at dose levels which do not show generalized toxicity.

In a program aimed at synthesis and biological evaluation of phosphorus analogs of classic nitrogen-containing drugs, the phosphine (III), as well as its oxide (IV) and sulfide (V), have been prepared as intermediates. These materials were evaluated pharmacologically in a preliminary manner and found to induce marked depression of spontaneous activity in mice at dose levels far below the LD_{50}.

Synthesis of the desired materials was first attempted *via* the route shown below. The hydride reduction was carried out according to the general

$$Ph_2PCl \xrightarrow{LiAlH_4} \underset{\text{I}}{Ph_2PH} \xrightarrow[2.\ RX]{1,\ NaH} \underset{\text{II}}{Ph_2PR}$$

procedure of Horner *et al.* (1). It was found, however, that when the reactions were worked up in the usual manner, much diphenylphosphine (I) was lost due to its appreciable water solubility. A dramatic increase in yield could be obtained by cooling the reaction mixture in ice after the heating period, removing all inorganic materials by filtration through a sintered glass disk, washing the reaction vessel and the precipitate with chloroform, and evaporating the organic solvents. The reaction of diphenylphosphine with sodium hydride was sluggish, requiring about 4 hr. for completion, but the reaction did proceed, as evidenced by the fact that a model reaction using ethyl iodide gave ethyldiphenylphosphine (II, R = ethyl) in good yield. Ethyldiphenylphosphine was identified by its boiling point and by its infrared spectrum, in which the P—H stretching band at 2300 $cm.^{-1}$ was absent, and in which bands characteristic of $—CH_2—$ and $—CH_3$ were observed at 2920, 2850, 1460, 1450, and 1380 $cm.^{-1}$. However, no reaction could be induced between the diphenylphosphide anion and 3-chloropropyldimethylamine to give the desired III under these conditions.

The required phosphine (III) was finally obtained in good yield according to the scheme shown below, in which lithium diphenylphosphide, as well as phenyllithium, are produced according to the method of Aguir *et al.* (2). The phenyllithium is then destroyed by *tert*-butyl chloride, after which the lithium diphenylphosphide is allowed to react with the appropriate chloramine to produce III. Attempted conversion to the oxide (IV) with hydrogen peroxide under a variety of conditions failed, apparently due to solubility problems, but was easily effected with 3-chloroperbenzoic acid. Sulfur in toluene, using iodine as catalyst, effected the transformation of III into the sulfide (V).

$$Ph_3P + Li \rightarrow Ph_2P^{\ominus}Li^{\oplus} + ClCH_2CH_2CH_2N(CH_3)_2 \rightarrow \underset{\text{III}}{Ph_2PCH_2CH_2CH_2N(CH_3)_2}$$

BIOLOGICAL ACTIVITY[1]

To ascertain the gross biological activity which might be associated with these substances, exploratory pharmacological determinations were conducted in our laboratories. The results of acute toxicity studies are presented in Table I. Despite the small number of animals employed, it is apparent that a dose-related response is obtained with all three compounds. Observation of the animals indicated that death, where obtained, was due to CNS depression. All animals showed profound reduction in the level of spontaneous activity, accompanied in some cases by tremors and/or convulsions.

TABLE I.—ACUTE TOXICITY

Compd.	Dose, mg./Kg.	No. of Survivors/ Total No. Animals	Approx. Time to Death, min.
III	500	0/2	3
	100	0/2	7
	50	1/2	12
IV	500	0/2	10
	100	2/2	
	50	2/2	
V	500	0/2	4–5
	100	2/2	
	50	2/2	

To determine whether reduction in spontaneous activity could be elicited at lower dose levels, small-scale studies were carried out in activity cages, using groups of two animals per cage. The cages were constructed such that an infrared beam passed twice diagonally through the cage. When an animal broke the light beam, a photoelectric cell activated a cumulative recorder. The design and construction of the cages will be reported (3). All animals survived the test and were observed for at least 1 hr. The results are shown in Table II. The small number of animals again prevented statistical verification of the significance of these preliminary results, but to compensate partially for this we have paired the results as shown. It is

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TABLE II.—EFFECT ON SPONTANEOUS ACTIVITY

Compd.	Dose, mg./Kg.	Activity, Counts/min. Before Drug	Activity, Counts/min. After Drug
III	10	3.3	0.34
	25	2.4	0.6
IV	100	4.7	1.1
V	25	4.6	8.0
	75	4.9	3.4

clear even without the statistical precaution that the phosphine (III) possesses significant activity at both dose levels employed. Results with the other substances are not quite so clear, but the oxide (IV) definitely appears active at the dose level employed, and while activity is not conclusively demonstrated for the sulfide (V), this is probably a matter of dose level and/or the number of animals employed. These compounds, then, are the first phosphines shown to possess significant biological activity at nontoxic dose levels. Pharmacological tests are continuing, particularly with a view toward elucidating the nature of the observed CNS depression, and toward relating this effect to other possible actions, particularly on the cardiovascular system.

EXPERIMENTAL[2]

3-Chloropropyldimethylamine.—This was liberated from the commercial hydrochloride by dissolving 30.0 Gm. of the salt in 50 ml. of water, covering the aqueous solution with 90 ml. of toluene, and adding 7.60 Gm. of solid NaOH. The layers were shaken and separated, and the aqueous layer extracted twice with 30-ml. portions of toluene. The combined toluene solutions were dried (Na_2SO_4), the drying agent washed with 40 ml. of toluene, and the combined solutions stored over molecular sieves. The concentration of the amine was determined by adding a 1-ml. portion of the toluene solution to excess water and quickly titrating the mixture with 0.1 *N* HCl on a Sargent titrator.

3-Dimethylaminopropyldiphenylphosphine (III).—These operations must be carried out in a completely oxygen-free atmosphere, which was attained by assembling the necessary glassware, evacuating by means of a vacuum pump, and allowing nitrogen to enter. This process was repeated several times, after which a small positive pressure of nitrogen was maintained by connecting the gas outlet to a glass U-tube partially filled with mercury. A mixture of 2.10 Gm. of lithium ribbon, 26.8 Gm. of triphenylphosphine, and 200 ml. of tetrahydrofuran was allowed to react in a nitrogen atmosphere at room temperature for 3 hr. After cautious dropwise addition of 11.8 Gm. of *tert*-butyl chloride, the mixture was heated to boiling for 10 min. The resulting red solution was then cooled and transferred by means of a large syringe to a second nitrogen-filled apparatus, and 122 ml. of a 0.83 *N* solution of 3-chloropropyldimethylamine in toluene was added dropwise. The resulting mixture was heated under reflux for 2 hr. After cooling, the mixture was filtered through a sintered-glass disk, and the solvents evaporated at reduced pressure. Fifty milliliters of benzene was then added, and the resulting solution extracted with three 50-ml. portions of 5% aqueous HCl. The combined aqueous solutions were placed in a beaker, covered with 50 ml. of benzene, and carefully basified with concentrated aqueous sodium hydroxide. The layers were then shaken and separated, and the aqueous layer extracted twice more with 50-ml. portions of benzene. The combined benzene solutions were dried (Na_2SO_4) and the benzene evaporated, affording 20.0 Gm. (74%) of a yellow oil. Distillation of a portion of the material gave a fraction boiling at 175–178° (1.2 mm.) which was homogeneous to thin-layer chromatography, but was attended with much decomposition. In later experiments the product was therefore purified by chromatography on alumina (Merck 71707); near quantitative recoveries were obtained.

Anal.—Calcd. for $C_{17}H_{22}NP$: C, 75.24; H, 8.19; N, 5.16. Found: C, 74.62; H, 8.00; N, 4.76.

3-Dimethylaminopropylphosphine Oxide (IV).—To a solution of 6.0 Gm. of the phosphine (I) in 50 ml. of chloroform was added dropwise 4.5 Gm. of 3-chloroperbenzoic acid dissolved in 50 ml. of chloroform. The mixture was then stirred at room temperature for 2 hr. Acids were extracted with 3 vol. of aqueous sodium hydroxide, after which the reaction products were extracted with 3 vol. of 5% aqueous hydrochloric acid. The HCl extracts were combined, covered with 50 ml. of benzene, and carefully basified with concentrated aqueous sodium hydroxide. After shaking, the benzene was separated, and the aqueous layer washed with two additional 50-ml. portions of benzene. Benzene was evaporated at reduced pressure, affording a yellow oil, which was taken up in skellysolve B. On cooling, the solution deposited 3.5 Gm. (56%) of white crystals, m.p. 87.5° (in an evacuated sealed capillary). This material was shown to be homogeneous by thin-layer chromatography; additionally it was very hygroscopic, and repeated recrystallization attempts from a variety of solvents resulted in melting point depression. Biological testing and microanalysis were therefore carried out using the initial skellysolve precipitate.

Anal.—Calcd. for $C_{17}H_{22}NOP$: C, 71.06; H, 7.72; N, 4.88; P, 10.78. Found: C, 71.19; H, 7.80; N, 4.70; P, 10.40.

D-Dimethylaminopropyldiphenylphosphine Sulfide (V).—A mixture of 1.5 Gm. of the phosphine (I), 0.53 Gm. of sulfur, 0.053 Gm. of iodine, and 10 ml. of toluene was heated under reflux by means of an oil bath (bath temperature 150°) for 4 hr. Evolution of H_2S was verified by holding a filter paper moistened with lead acetate solution over the reflux condenser. H_2S vapors were still observed after 4 hr., but it was found that the yield was the same when the reaction time was reduced to 1 hr. The hot reaction mixture was poured on a mixture of charcoal and Celite and allowed to cool. The mixture was then filtered through a Celite pad, which was washed with a small quantity of toluene. The combined toluene solutions were extracted with three 25-ml. portions of 5% aqueous HCl. The combined HCl solutions were placed in a large beaker, covered with 50 ml. of benzene, and carefully basified with concentrated aqueous NaOH. After shaking, the layers were separated, and the aqueous fraction was extracted with three additional 50-ml. portions of benzene. The benzene was evaporated

[2] Melting points were taken in a Thomas-Hoover unimelt apparatus and are corrected. Boiling points are uncorrected. Microanalyses by Drs. Weiler and Strauss, Oxford, England, and by Huffman Laboratories, Wheatridge, Colo.

TABLE III.—R_f DIFFERENCES AND INTENSITY OF COMPOUNDS

	R_f	Intensity
3-Chloropropyldimethylamine	0.84	Light blue
Phosphine (III)	0.88	Deep blue
Oxide (IV)	0.26	Light blue
Sulfide (V)	0.83	Very deep blue

at reduced pressure, affording an oil which was taken up in 30 ml. of skellysolve B. On cooling, 800 mg. (49%) of light yellow crystals were obtained, m.p. 78.5°.

Anal.—Calcd. for $C_{17}H_{22}NPS$: C, 67.28; H, 7.32; N, 4.62. Found: C, 67.06; H, 7.24; N, 4.58.

Thin-Layer Chromatography.—All materials in this series could very conveniently be checked for homogeneity and identity by thin-layer chromatography, carried out as follows. A 0.25-mm. layer of aluminum oxide G (Merck) was deposited on glass plates, which were activated by heating at 125° for 30 min. and stored in a desiccator. Spots representing 5 μl. of 10% solutions in acetone were applied, and the chromatograms were developed with a 1:1 mixture of acetone–chloroform. The spots were visualized by spraying with bromthymol blue. Aside from R_f differences, characteristic gradations in the intensity of the spot produced by the bromthymol blue reaction were observed.

Infrared Spectra.—In addition to the expected fundamental bands, these substances exhibited what appear to be highly characteristic infrared absorptions, which aided greatly in their characterization. All three substances, III, IV, and V, show a strong, sharp peak near 1430 cm.$^{-1}$. Additionally, the oxide possesses a very intense, broad band near 1180 cm.$^{-1}$, probably the P=O stretching frequency, and the sulfide shows a characteristic complex of seven intense bands between 690 and 810 cm.$^{-1}$.

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N-Alkylsulfamate Salts of Lincomycin

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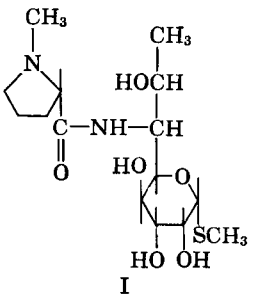
A number of *N*-alkylsulfamic acids and the corresponding lincomycin *N*-alkylsulfamates were prepared. In contrast to most salts of the antibiotic, lincomycin, higher *N*-alkylsulfamate salts possessed low water solubility.

LINCOMYCIN[1] is an orally effective, water soluble antibiotic whose isolation (1), physical properties (2), and structure (3) were recently announced. This antibiotic possesses a medium antibacterial spectrum and is orally effective in the treatment of bacterial infections in experimental animals (4) and in humans (5).

Lincomycin (I) possesses one tertiary amine group which permits salt formation. Many of the common salts of lincomycin, such as the hydrochloride, though crystalline, are highly water soluble. Salts of lincomycin formed with the higher *N*-alkylsulfamic acids were found to be relatively water insoluble and therefore of interest for certain types of pharmaceutical formulation. This report describes the preparation and properties of certain *N*-alkylsulfamic acids and *N*-alkylsulfamate salts of lincomycin. One of these salts, namely, lincomycin *N*-*n*-hexadecylsulfamate (LHS), is reported to show slightly greater antibacterial properties than lincomycin hydrochloride in an experimental infected-mouse system (6).



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N-Alkylsulfamic acids may be prepared by a variety of procedures which have been tabulated in two reviews (7, 8). *N*-Cyclohexylsulfamic acid (9), the most widely known member of this class of compounds, is commercially available.[2]

Initially, the preparation of the *N*-alkylsulfamic acids by the action of chlorosulfonic acid on an excess of amine was investigated (7–9). While this method was satisfactory for the preparation of lower *N*-alkylsulfamic acids, the isolation and purification of the higher homologs proved difficult due to the extreme insolubility of the alkylamine and alkali metal salts of the *N*-alkylsulfamic acids. To obviate these difficulties, the authors studied the cleavage of *N*-alkylsulfonamides with chlorosulfonic acid (10) as shown in the following equation. The *N*-alkylsulfamic acids prepared by this method are listed in Table I.

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[2] Marketed as Hexamic Acid by Abbott Laboratories, North Chicago, Ill.