dose of 1 g./kg. produced a 33% increase in the number of animals falling asleep and likewise doubled the duration of the sleep, and the same results were achieved when 6-methyl-2-pyridylurethane was given in a dose of 500 mg./kg. The three urethanes did not interfere with the potentiating activity of serotonin on the hypnotic properties of thiopental.

(4) Tests for Antagonistic Activity Toward Agitation Induced by 1,4-Dipyrrolidino-2-butyne (Tremorine) and Amphetamine.—With oral doses of 100 mg./kg., none of the three urethanes displayed an antagonistic effect toward agitation induced in mice by an intraperitoneal injection of 25 mg./kg. of 1,4-dipyrrolidino-2-butyne. In the amphetamine test,[8] 4-methyl- and 5-methyl-2-pyridylurethane showed a slight, and 6-methyl-2-pyridylurethane a more pronounced, protective effect against agitation induced in mice by an oral dose of 25 mg./kg. given 30 min. after ingestion of 100 mg./kg. of the urethane.

(5) Tests for Neuroleptic Activity.—Mice were used. In the jiggle cage test,[9] 4-methyl-2-pyridylurethane produced a reduction of spontaneous motility of the mice lasting several hours after the oral administration of 100 mg./kg. and higher. The other two urethanes were inactive in this respect.

In the Julou and Courvoisier hoist test in mice,[9] only a slight sedative effect was observed with a dose of 1 g./kg. given orally. In this test, a horizontal wire is presented to the forepaws of the mouse; in normal conditions, the mouse will grasp the wire and, in under 5 sec., hoist itself up so that at least one of its hindpaws gets on the wire. This hoist effect is suppressed by neuroleptics, including drugs of the meprobamate type.

(6) Test for Antipyretic Activity.—This test, based on observation of the thermoregulation in rabbits submitted to the hyperthermia-inducing action (increase in rectal temperature) of a standardized antigonococcal vaccine given intravenously, was performed only with 4-methyl-2-pyridylurethane. An oral dose of 100 mg./kg. reduced by about 0.5–0.9° the central hyperthermia induced by a dose of 100 × 10^6 germs/kg. of vaccine.

Acknowledgment.—This work was carried out at the Institut d'Anesthésiologie of the Paris Medical Faculty (Director, Prof. J. Baumann), and financially supported by the Institut National d'Hygiène (Director, Prof. L. Bugnard); the authors thank the authorities concerned, and also Prof. O. Blanpin (Medical School of Tours, France) for the pharmacological assays.

(9) *Cf.* J. R. Boissier, *Acta Neurophysiologica*, **2**, 253 (1960).

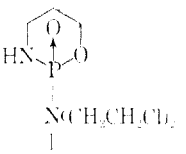
1,3,2-Diazaphosphorinane 2-Oxides. 1. Synthesis of Some 2-(N-Arylamino)-1,3,2-diazaphosphorinane 2-Oxides[1]

JOHN H. BILLMAN, JOHN L. MEISENHEIMER, AND RICHARD A. AWL

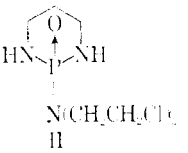
Department of Chemistry, Indiana University, Bloomington, Indiana

Received September 12, 1963

Cyclophosphamide (I),[2] prepared by Arnold, *et al.*,[3] is an inhibitor of many animal and human tumors. Its action is assumed to be through the enzymatic liberation of the bis(2-chloroethyl)amine moiety. The similar triamido compound (II) has been prepared, but unlike I, it has not displayed antitumor activity.[4,5]



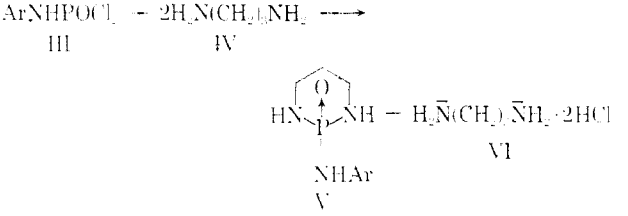
I



II

This paper reports on the preparation of a series of compounds of the general structure V. Early antitumor testing of the first member of this series to be prepared (IX, Table I) was suggestive of inhibitory activity. Therefore, additional members of the series were prepared to explore this structural area for possible active antitumor compounds. Since these compounds possess an arylamino group on the phosphorus atom instead of a bis(2-chloroethyl)amino substituent, any biological activity exhibited would result from some factor other than the potential alkylating action of the latter grouping.

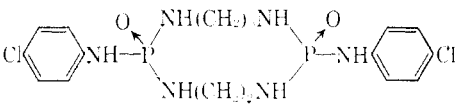
The syntheses were accomplished by reacting a phosphoramidic dichloride (III) with 2 moles of 1,3-diaminopropane (IV) in benzene to give the 2-substituted 1,3,2-diazaphosphorinane 2-oxide (V) and 1 mole of the diamine dihydrochloride (VI). The compounds V were



III IV V VI

quite polar in nature and separated from benzene along with the diamine dihydrochloride (VI). Fortunately the phosphorus–nitrogen bonds in these compounds were found to be quite stable with respect to alkaline hydrolysis so that they could be separated from VI in a basic solution by converting VI to the free diamine.

The calculated formula weight for X (Table I) is 246. A molecular weight determination for this compound by the Rast camphor method indicated a value of 510.[6] Therefore, there was the possibility that the compound might have the structure VII. A molecular weight determination was then made by a vapor pressure lowering method using methanol as a solvent.[7] This method indicated a molecular weight of 259, thus ruling out a structure such as VII. A determination



VII

of the molecular weight of compound IX by this latter method gave a value of 255. Its calculated formula weight is 241. It may be that these 2-substituted 1,3,2-diazaphosphorinane 2-oxides are dimeric in the solid state due to hydrogen bonding and are dissociated in the polar solvent, methanol.

Biological Results.—The compounds in Table I have been screened against Sarcoma 180, Carcinoma 755, Leukemia 1210, and a cell culture system.[8] It is

(1) This investigation was supported in part by a Public Health Service Fellowship (GF-13,650), National Institutes of Health, Public Health Service.

(2) Cytoxan®.

(3) H. Arnold, F. Bourseaux, and N. Brock, *Nature*, **181**, 131 (1958).

(4) H. Arnold, F. Bourseaux, and N. Brock, *Arzneimittel-Forsch.*, **2a**, 143 (1961).

(5) O. M. Friedman, E. Boger, V. Grubliauskas, and H. Sommer, *J. Med. Chem.*, **6**, 50 (1963).

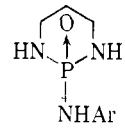
(6) Bernhardt Laboratories, Mülheim, Germany.

(7) A Mechrolab Osmometer was employed.

(8) Antitumor screening was accomplished by the Cancer Chemotherapy National Service Center, Bethesda, Md.

TABLE I

2-(N-ARYLAMINO)-1,3,2-DIAZAPHOSPHORINANE 2-OXIDES



	Ar	Yield, % crude	M.p., °C.	Yield, % pure	M.p., °C.	% nitrogen Calcd.	% nitrogen Found
VIII	Phenyl	53	217–219	24	217–219	19.92	19.94
IX	*p*-Methoxyphenyl[a]	59	181.5–183	14	190–190.5	17.45	17.51
X	*p*-Chlorophenyl[b]	53	205–215	17	214–215	17.12	17.13
XI	*p*-Tolyl[c]	27	...	14	224–225	18.68	18.84
XII	*m*-Tolyl	85	228–233	30	233–234	18.68	18.63
XIII	*o*-Chlorophenyl[d]	...	...	18	165–166	17.12	17.31

[a] Recrystallized from absolute ethanol. [b] Recrystallized from benzene–methanol (4:1). [c] Recrystallized from ethanol–water (2:1). [d] Recrystallized from water.

interesting that the first compound of this series to undergo the screening (IX, Table I) exhibited 66% and 78% inhibition in the first two trials against Carcinoma 755 even though this compound does not contain the nitrogen mustard moiety. However, no activity was indicated for the compound in further screening against Carcinoma 755 or the other tumor systems. The other compounds listed in Table I were also found to be inactive.

Experimental

1,3-Diaminopropane was obtained from the Union Carbide Chemicals Company.

N-Arylphosphoramidic Dichlorides.—These intermediates were prepared by refluxing the arylamine hydrochloride in phosphorus oxychloride, a method described by Michaelis and Schulze.[9]

2-(N-Arylamino)-1,3,2-diazaphosphorinane 2-Oxides (Table I).—The general preparative method was to add approximately 0.1 mole of the phosphoramidic dichloride in 500 ml. of benzene to a stirred solution of approximately 0.2 mole of the 1,3-diaminopropane in 500 ml. of benzene. The addition took about 2 hr., with the reaction mixture remaining near 35°. The mixture was stirred for an additional 1 hr. and then the white solids which had precipitated during the reaction were collected on a Buchner funnel. This residue was allowed to dry thoroughly in the air. The separation of the product from the 1,3-diaminopropane dihydrochloride was accomplished either by method A or method B.

A.—This method was used in obtaining compounds VIII and IX. For example, with compound VIII there was 37 g. of dry solid collected from the reaction. This was boiled with 400 ml. of water until almost all of the material had dissolved. The hot solution was filtered through a fluted filter and then allowed to cool to room temperature. A total of 20 g. of sodium carbonate was slowly added to the solution, with stirring, and 3.5 g. of white solid separated. Cooling the solution overnight furnished an additional 3.5 g. of this material. A final 4.1-g. portion of crude product was obtained by distilling one-half of the solvent and cooling the remaining solution. Three recrystallizations from methanol gave 5.1 g. (24%) of crystals, m.p. 217–219°.

B.—This general method was used for compounds X–XIII and is given for compound XII, prepared in a reaction with a theoretical yield of 0.135 moles of product. The dried solids from this reaction weighed 51.4 g. They were powdered and stirred into 300 ml. of water containing 17.5 g. of sodium carbonate. The mixture was stirred for 0.5 hr. and then the suspended material was collected and dried in a vacuum desiccator. It was again powdered and stirred for 0.5 hr. in 150 ml. of warm water containing 3.5 g. of sodium carbonate. The suspended solid, after collecting and drying, weighed 25.5 g. (85%) with a 228–233° m.p. range.

An 8-g. portion of the crude material was stirred in 120 ml. of refluxing ethanol and 20 ml. of 5% sodium carbonate solution was added. Then 40 ml. of ethanol was gradually added to the mixture. The resulting cloudy solution was filtered while hot. The clear filtrate was cooled and 4.8 g. of colorless crystals, m.p. 230–231°, separated. A final recrystallization was accomplished from 80 ml. of 90% ethanol which contained 0.15 g. of sodium carbonate. There was obtained 2.9 g. (30% of theory, by proportion) of solid, m.p. 233–234°.

The recrystallization solvents for other compounds are listed under Table I.

Acknowledgment.—Mr. Stephen Johnson for preparing some of the intermediates, Drs. H. W. Bond, R. B. Ross, and J. E. Leiter of the CCNSC for their cooperation and for making screening data available. We also wish to thank the Union Carbide Chemicals Company for supplying some of the 1,3-diaminopropane used in this research.

(9) A. Michaelis and G. Schulze, *Ber.*, **26**, 2937 (1893).

Monoamine Oxidase Inhibitors. Hydrazine Derivatives

DANIEL KAMINSKY, BERNARD DUBNICK, AND FLOYD E. ANDERSON

Warner-Lambert Research Institute, Morris Plains, New Jersey

Received November 20, 1963

The monoamine oxidase (MAO) inhibitory activity in an earlier series of acylated and carbalkoxylated aralkyl hydrazines appeared to be dependent upon hydrolysis of the blocking groups to the free aralkyl hydrazines.[1] Since the relative degree of MAO inhibition in the brain and liver caused by some inhibitors of the hydrazine type depends on the route of drug administration,[2] the possibility was considered that specificity of inhibition, independent of route of administration, could be achieved if the hydrolysis of a blocked hydrazine were catalyzed by an enzyme, esterase or amidase, specific to a particular organ, for example, the brain. Table I shows the results of a study of brain *vs.* liver localization of the inhibition with several new MAO inhibitors injected subcutaneously or given orally.

One of the compounds, VIII in Table I, the least potent of the group, exhibited greater inhibition in the

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(2) A. Horita, *Toxicol. Appl. Pharmacol.*, **3**, 474 (1961).