miosis. For example, fumaric acid isolated from *A. muscaria* produced hyperpyrexia but neither mydriasis nor miosis in experimental animals. Therefore, in the biological screening of various fractions from *A. muscaria*, a material which produced mydriasis accompanied by hyperpyrexia was considered tentatively to possess LSD-like behavior.

Through fractionation of the aqueous concentrate (III) with judicious mixtures of methanol, ethanol, ethyl acetate, and acetone an hygroscopic crystalline material, provisionally designated "z" was isolated (307 mg., m.p. 135–138° dec).

Anal. Calcd. for $C_{15}H_{30}K_4O_{15}$: C, 29.7; H, 4.98; K, 25.77. Found: C, 30.17, 30.30; H, 4.83, 4.60; K, 25.23; mol. wt., 615; equiv. wt., 218 (potentiometric titration).

The analytical data are consistent with a compound having four COOK and three COOH groups. The n.m.r. spectrum showed only two peaks; one characteristic of methyl-keto functions, the other of labile hydrogens. Integration of these peaks suggests a ratio of three methyl groups to two labile hydrogens.

The very small amount of "z" left after pharmacological and analytical experiments was methylated with diazomethane. Its n.m.r. spectrum was complicated and showed no expected relationship to that of the original compound other than to confirm the methylation.

"z" was tested intravenously in rabbits at two-dose levels. A low level dose of "z," 1.0 mg./kg., resulted in mydriasis, 30 min. after administration, which persisted for 30 min. Pupil dilation was 2 mm. above normal. Three hr. after administration, there was a temperature rise of 0.9° which lasted less than 1 hr. At the high level dose of 6.25 mg./kg., mydriasis was immediate, persisting for 30 min. Pupil dilation was initially 1.5 mm. above normal, increasing to 2 mm. later. Thirty min. after administration, temperature rose 0.7° and persisted for 30 min. Fifteen min. after administration, there was rapid respiration persisting for 2 hr. There were no other symptoms.

By means of thin-layer chromatography minute quantities of indolic compounds have been isolated from the aqueous concentrate. From fractions I and II potassium gluconate, a glucoside m.p. 106° dec and two minor crystalline substances, m.p. 172–174° and 160–165°, respectively, were isolated as hitherto unreported constituents. Mannitol, fumaric acid, a fixed oil fraction, and a considerable amount of KCl also were isolated.

The fixed oil was fractionated on alumina columns and subjected to chemical and biological testing. All fractions were found to be mainly esters of oleic acid and devoid of insecticidal properties.[13]

Acknowledgment.—We wish to thank Dr. Edward Pelikan and his colleagues for carrying out the pharmacological tests, Varian Associates for n.m.r. data and Abbott Laboratories for large scale extractions.

(13) *A. muscaria* is commonly known as fly-agaric due to its reputed property as an insecticide.

Potential Carcinolytic Agents. I. Derivatives of P,P-Bis(1-aziridinyl)phosphinic Amide[1]

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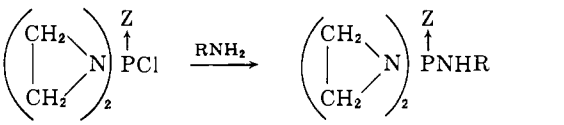
Received July 11, 1962; revised manuscript received February 2, 1963

The alkylating ability of aziridine is known to be enhanced by an electron-withdrawing N-substituent, and there are indications that molecules with several activated aziridine groups are likely to display antitumor activity.[3] However, a favorable therapeutic index depends on the entire molecular structure rather than simply on the cytotoxic moieties. A degree of selectivity of action has been achieved with certain P,P-bis(1-aziridinyl)phosphinic derivatives, notably the "dual antagonists."[4]

Further work along these lines has led to the preparation of the derivatives (II) reported in the present paper.



I

Ia, Z = O
Ib, Z = S

II

IIa, R = C_6H_{11}; Z = O
IIb, R = C_6H_{11}; Z = S
IIc, R = $(CH_2)_3OH$; Z = O
IId, R = $(CH_2)_3OH$; Z = S
IIe, R = $(CH_2)_3OCH_3$; Z = S

The usual procedure (excepting one instance)[5] for preparing P,P-bis(1-aziridinyl)phosphinic amides has been to attach the sensitive aziridine groups in the last step.[6] This approach was not used in our work because a number of the intermediates would contain reactive functional groups so situated that unwanted cyclizations could occur prior to the final step. The desired compounds were prepared from the intermediate P,P-bis(1-aziridinyl)phosphinic chloride (Ia) and the thiono analog (Ib), both of which were used directly without isolation. The phosphinic chloride was so unstable that it was used immediately upon preparation, whereas the thiophosphinic chloride could be kept at least overnight in solution at low temperatures. All reactions were conducted in the cold, and the products isolated under the mildest possible conditions because of their inherent instability. Simple amines such as cyclohexylamine, used in excess to serve as condensing agents, gave fairly good yields of nearly pure products (generally the yields decreased rapidly with attempts at purification). With other amines where triethylamine was used as the condensing agent the products were less easily purified. The oily products in particular were difficult to purify because they decomposed when subjected to most standard purification techniques, including molecular distillation at 10^{-6} mm. pressure or chromatography.

Pure derivatives could not be obtained from 1,3-propanediamine, 3-mercapto-1-propylamine, 1,3-dimercaptopropane, 3-mercapto-1-propanol, 1,3-propanediol, and ethyl glycinate. The use of sodium or magnesium salts of the alcohols and mercaptans was unsuccessful as was the triethylamine technique. In addition to triethylamine, various other hindered and unhindered amines (N-methylpiperidine, N-methylmorpholine, tri-

(1) This work was sponsored by the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. SA-43-ph-4360.

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(3) J. A. Hendry, R. F. Homer, F. L. Rose, and A. L. Walpole, *Brit. J. Pharmacol.*, **6**, 357 (1951); S. M. Buckley, *et al.*, *Proc. Soc. Exptl. Biol. Med.*, **78**, 299 (1951).

(4) Z. B. Papanastassiou and T. J. Bardos, *J. Med. Pharm. Chem.*, **5**, 1000 (1962); D. R. Seeger and A. S. Tomcufcik, *J. Org. Chem.*, **26**, 3566 (1961).

(5) Z. B. Papanastassiou and T. J. Bardos, Belgian Patent 577,883 (1959); journal manuscripts in preparation.

(6) E. Kuh and D. R. Seeger, U. S. Patent 2,670,347 (1954).

ethylamine, 2,6-dimethylpyridine, and triethylenediamine) were tried as condensing agents without improvement. The bicyclic triethylenediamine reacted with P,P-bis(1-aziridinyl)phosphinic chloride to produce a white complex of unknown structure which did not react further with primary amines at room temperature.

Preliminary screening in the Walker 256 test system[7] revealed that the compounds prepared possess the following approximate therapeutic indices, LD_{10}/ED_{90} = TI[8]: IIa, 1.2/0.43 = 3; IIb, 12.5/2.7 = 5; IIc, 4.5/1.6 = 3; IIe, 3.8/0.62 = 6. IId is the most toxic compound in this series (LD_{10} = 0.6) and it is inactive against Walker 256. The difference in pharmacological activity between IId and its methyl ether IIe provides for another example of the importance of the so-called "carrier" moiety[9] in biological alkylating agents.

Experimental[10]

P,P-Bis(1-aziridinyl)phosphinic Chloride (Ia).—A solution of 46 g. (0.3 mole) of phosphorus oxychloride in 700 ml. of dry 1,2-dimethoxyethane was cooled to −20° and stirred vigorously under completely anhydrous conditions. A solution of 60.0 g. (0.6 mole) of triethylamine in 470 ml. of 1,2-dimethoxyethane was added during 0.5 hr., followed by a solution of 25.8 g. (0.6 mole) of ethylenimine in 470 ml. of 1,2-dimethoxyethane which was added during 1 hr. After 10 additional min., the precipitated triethylamine hydrochloride was removed by filtration (98% of theory). The filtrate containing the product was used immediately for subsequent reactions.

P,P-Bis(1-aziridinyl)phosphinothioic Chloride (Ib).[5]—This compound was prepared in a similar manner to Ia, using either 1,2-dimethoxyethane or tetrahydrofuran as a solvent. After all of the ethylenimine had been added, the mixture was allowed to warm to room temperature and was stirred overnight. After removal of the amine salt (95% of theory), the filtrate was used directly for further reactions.

P,P-Bis(1-aziridinyl)-N-cyclohexylphosphinic Amide (IIa).—A solution of 29.7 g. (0.30 mole) of cyclohexylamine in 700 ml. of 1,2-dimethoxyethane was added during 2 hr. to a solution of 0.15 mole of Ia in 900 ml. of 1,2-dimethoxyethane which was cooled to 0° and stirred vigorously. After stirring overnight at room temperature, the suspension was filtered to remove the cyclohexylamine hydrochloride (77%) and the filtrate was evaporated *in vacuo*. The residue was dissolved in 200 ml. of hot 1,2-dimethoxyethane, decolorized, cooled slowly, and finally stored in the freezer. The precipitated white, crystalline product weighed 8.3 g. (24%); m.p. 100–102°; ethylenimine assay[11] 96% of theory. ν_{max}^{KBr} 3210, 2930, 1460, 1270, 1190, 1110, 935, and 705 cm.$^{-1}$.

Anal. Calcd. for $C_{10}H_{20}N_3OP$: C, 52.39; H, 8.79; N, 18.33. Found: C, 52.42; H, 8.72; N, 17.98.

P,P-Bis(1-aziridinyl)-N-cyclohexylphosphinothioic Amide (IIb).[5]—A solution of 17.8 g. (0.18 mole) of cyclohexylamine in 500 ml. of tetrahydrofuran was added during 2.5 hr. to a tetrahydrofuran solution (570 ml.) containing 0.09 mole of Ib which was cooled to 0°and stirred vigorously. After stirring overnight at room temperature, the mixture was filtered to remove the amine hydrochloride (70%). The filtrate was concentrated to dryness in a rotary evaporator, taken up in 400 ml. of benzene, decolorized, filtered, and evaporated to dryness. The residue (60% of theory) was stirred with 300 ml. of warm "Skellysolve B," decanted from a gummy, insoluble residue, then decolorized and cooled slowly to −12°. The precipitated crystalline product was recrystallized by the same procedure, yielding 7.3 g. (33%) of white needles; m.p. 96–97°; ethylenimine assay[11] 99.8% of theory. ν_{max}^{KBr} 3350, 2930, 1420, 1260, 1150, 1100, 930, 892, 828 and 720 cm.$^{-1}$.

Anal. Calcd. for $C_{10}H_{20}N_3PS$: C, 48.95; H, 8.22; N, 17.13; P, 12.63. Found: C, 48.20; H, 8.28; N, 16.94; P, 12.52.

P,P-Bis(1-aziridinyl)-N-(3-hydroxypropyl)phosphinic Amide (IIc).—This compound was prepared by the same technique used for the corresponding cyclohexyl derivative (IIb). The amine hydrochloride was produced in nearly theoretical yield. Evaporation of the filtrate afforded an oil which was dissolved in benzene and decolorized. Because the solution gave a positive chloride test with alcoholic silver nitrate, it was stirred with anhydrous sodium carbonate for 2 hr. The chloride-free filtrate was evaporated, then dried at 1 mm. pressure. The residue ($n^{25}D$ 1.5028, ethylenimine assay[11] 96%) was dissolved in absolute ethanol, decanted from a small quantity of insoluble oil, decolorized, and evaporated. The final oily product was stripped of volatiles at 10^{-3} mm.; it weighed 21 g. (70%); $n^{25}D$ 1.5030. The product deteriorated slowly when stored in the refrigerator. $\nu_{max}^{CHCl_3}$ 3400, 2990, 1409, 1269, 1170, 1110, 1070, and 940 cm.$^{-1}$.

Anal. Calcd. for $C_7H_{16}N_3O_2P$: C, 40.97; H, 7.86; N, 20.48; P, 15.10. Found: C, 40.76; H, 7.84; N, 19.87; P, 14.46.

P,P-Bis(1-aziridinyl)-N-(3-hydroxypropyl)phosphinothioic Amide (IId).—A solution of 800 ml. of tetrahydrofuran containing 0.126 mole of Ib was added during 2.5 hr. to a solution of 9.4 g. (0.126 mole) of 3-aminopropanol and 25.5 g. (0.252 mole) of triethylamine in 500 ml. of tetrahydrofuran which was cooled to 0° and stirred vigorously. After stirring at room temperature overnight, the mixture contained a semisolid precipitate indicated by infrared spectra to be a mixture of the two amine hydrochlorides. The supernatant solution was evaporated to dryness in a rotary evaporator. The residue was dissolved in 400 ml. of benzene, decolorized, and concentrated to about 150 ml. On standing overnight, the solution deposited large, colorless prisms weighing 6.1 g.; m.p. 75–77°; ethylenimine assay[11] 99.8%. The mother liquor afforded an additional 2.5 g. (31% total yield) of product of the same quality. ν_{max}^{KBr} 3250, 2910, 1425, 1250, 1109, 950, 930, 892, 825, and 738 cm.$^{-1}$.

Anal. Calcd. for $C_7H_{16}N_3OPS$: C, 38.00; H, 7.29; N, 18.99; P, 14.00. Found: C, 38.11; H, 7.07; N, 18.90; P, 13.87.

P,P-Bis(1-aziridinyl)-N-(3-methoxypropyl)phosphinothioic Amide (IIe).—This preparation was carried out exactly as the preceding one, except that 1,2-dimethoxyethane was used as a solvent. After the amine hydrochlorides had been removed, the filtrate was evaporated to dryness and the residue was dissolved in 500 ml. of benzene. It was decolorized and evaporated to dryness again. The residual oil was taken up in 500 ml. of ether, decolorized, and concentrated in a small volume, then boiling "Skellysolve B" was added until the solution became turbid. After cooling to room temperature the suspension was refrigerated overnight. Several crops of colorless plates were obtained in this way. They were combined and recrystallized (twice from a mixture of ether and "Skellysolve B," yielding 3.06 g. (10%) of product; m.p. 65–67°; ethylenimine assay[11] 97%. ν_{max}^{KBr} 3260, 2910, 1440, 1260, 1110, 930 and 725 cm.$^{-1}$.

Anal. Calcd. for $C_8H_{18}N_3OPS$: C, 40.84; H, 7.71; N, 17.86. Found: C, 40.39; H, 7.30; N, 17.06.

(7) The compounds are being evaluated by the Cancer Chemotherapy National Service Center, and complete data will be published in a future *Cancer Chemotherapy Screening Data* supplement to *Cancer Research*.

(8) The therapeutic index (TI) is defined as the ratio of the lethal dose producing 10% deaths in non-tumor bearing rats (LD_{10}) to the dose producing a 90% inhibition of tumor weight (ED_{90}). The LD_{10} and ED_{90} are expressed in mg./kg./day and the therapeutic index is determined graphically. Also, consult: H. E. Skipper and L. H. Schmidt, *Cancer Chemotherapy Reports*, **17**, 1 (1962).

(9) L. F. Larionov, "Biological Approaches to Cancer Chemotherapy," R. J. C. Harris, Ed., Academic Press, London, 1961, p. 139.

(10) All starting materials and solvents were carefully purified before use. All reactions and most other manipulations were conducted in a nitrogen atmosphere. Some of the final products were prepared, purified, and analyzed many times before acceptable analytical values could be obtained. Melting points are corrected.

(11) E. Allen and W. Seaman, *Anal. Chem.*, **27**, 540 (1955).

Thiopegan Derivatives. XXIII. Synthesis of 5H-Thiazolo[3,2-a]quinazolin-5-one and 5H-Thiazolo[2,3-b]quinazolin-5-one Derivatives Containing Phenolic, Alkoxy, and Alkyl Groups

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Received March 28, 1962; Revised manuscript received December 24, 1962

This paper constitutes an extension and continuation of previous work.[1b] As the incorporation of a hydroxyl